

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
22-363

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

7/31/09

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology
OND IO

NDA: 22-363

Submission date: October 3, 2008

Drug: pitavastatin (Livalo)

Sponsor: Kowa Research Institute

Indication: Adult patients with primary hypercholesterolemia

Reviewing Division: Division of Metabolism and Endocrine Products

Comments: The pharm/tox reviewer and supervisor found the nonclinical information submitted for pitavastatin to be sufficient to support approval for the indication as proposed above.

Developmental and Reproductive toxicity:

Embryofetal studies in rats and rabbits showed relatively minor fetal effects at doses exceeding human exposure by greater than 25 fold. Neonatal death was observed in a rat pre/postnatal study at all doses tested, which included doses producing exposures similar to those in humans. Use of pitavastatin is contraindicated in pregnancy and therefore a pregnancy category of X is recommended. This is based on the observed neonatal toxicity of pitavastatin, the known importance of cholesterol in fetal development and the relative lack of harm of discontinuing therapy during pregnancy. This is consistent with labeling of other HMG-CoA reductase inhibitors.

Carcinogenicity:

No drug-related tumors were observed in carcinogenicity studies in mice. An increase in thyroid follicular cell adenocarcinomas was observed in male rats but only at doses causing exposures that are much higher than human exposure (295 fold). These tumors are likely to be related to liver enzyme induction and increased metabolism of thyroid T₄ and so their relevance to humans is probably low.

Other issues:

CNS vascular lesions and optic nerve degeneration have been observed with other HMG-CoA reductase inhibitors. These findings are described in labeling of these other products. CNS and optic nerve lesions were not observed in animal studies with pitavastatin. Nevertheless, the sponsor and division recommended including wording about this toxicity similar to other HMG-CoA reductase inhibitors.

Cataracts and/or lens opacities were observed in pitavastatin treated dogs. Cataracts in animals were also observed with other HMG-CoA reductase inhibitors and other labeling includes a description of these findings. The sponsor and division recommended including a description of these findings in the labeling.

Conclusions:

I concur with the Division pharm/tox conclusion that the nonclinical data support approval of this NDA. I agree that pregnancy category X is acceptable and that the labeling may contain a brief description of the CNS lesions, optic nerve degeneration and lens opacities noted for this class of compounds.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22363	ORIG 1	KOWA RESEARCH INSTITUTE INC	LIVALO TABLETS

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/s/

PAUL C BROWN
07/31/2009

6/8/09



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-363
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	6/3/09
PRODUCT:	Livalo®
INTENDED CLINICAL POPULATION:	Adult patients with primary hypercholesterolemia
SPONSOR:	Kowa Research Institute, Inc.
DOCUMENTS REVIEWED:	eCTD submission
REVIEW DIVISION:	Division of Metabolism and Endocrinology Products (HFD-510)
PHARM/TOX REVIEWER:	C. Lee Elmore, Ph.D.
PHARM/TOX SUPERVISOR:	Karen Davis-Bruno, Ph.D.
DIVISION DIRECTOR:	Mary Parks, M.D.
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Date of review submission to Division File System (DFS): June 8, 2009

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability:

Approval (AP)

B. Recommendation for nonclinical studies:

The non-clinical studies reviewed are sufficient to support the safety of oral administration of pitavastatin to adult patients with primary hypercholesterolemia at the MRHD (1, 2 and 4 mg QD). No further studies are required.

C. Recommendations on labeling:

8.1 Pregnancy

Teratogenic effects: Pregnancy Category X

LIVALO is contraindicated in women who are or may become pregnant. Serum cholesterol and TG increase during normal pregnancy, and cholesterol products are essential for fetal development. Atherosclerosis is a chronic process and discontinuation of lipid-lowering drugs during pregnancy should have little impact on long-term outcomes of primary hyperlipidemia therapy [see *Contraindications (4)*].

There are no adequate and well-controlled studies of LIVALO in pregnant women, although there have been rare reports of congenital anomalies following intrauterine exposure to HMG-CoA reductase inhibitors. In a review of about 100 prospectively followed pregnancies in women exposed to other HMG-CoA reductase inhibitors, the incidences of congenital anomalies, spontaneous abortions, and fetal deaths/stillbirths did not exceed the rate expected in the general population. However, this study was only able to exclude a three-to-four-fold increased risk of congenital anomalies over background incidence. In 89% of these cases, drug treatment started before pregnancy and stopped during the first trimester when pregnancy was identified.

Reproductive toxicity studies have shown that pitavastatin crosses the placenta in rats and is found in fetal tissues at $\leq 36\%$ of maternal plasma concentrations following a single dose of 1 mg/kg/day during gestation.

Embryo-fetal developmental studies were conducted in pregnant rats treated with 3, 10, 30 mg/kg/day pitavastatin by oral gavage during organogenesis. No adverse effects were observed at 3 mg/kg/day, systemic exposures $\frac{(b)}{(4)}$ times human systemic exposure at 4 mg/day based on AUC.

Embryo-fetal developmental studies were conducted in pregnant rabbits treated with 0.1, 0.3, 1 mg/kg/day pitavastatin by oral gavage during the period of fetal organogenesis.

Maternal toxicity consisting of reduced body weight and abortion was observed at all doses tested ^(b)₍₄₎ times human systemic exposure at 4 mg/day based on AUC).

In perinatal/postnatal studies in pregnant rats given oral gavage doses of pitavastatin at 0.1, 0.3, 1, 3, 10, 30 mg/kg/day from organogenesis through weaning, maternal toxicity consisting of mortality and/or impaired lactation was observed at all doses, which contributes to the decreased survival of neonates in all dose groups (0.1 mg/kg/day represents ^(b)₍₄₎ times human systemic exposure at 4 mg/day dose based on AUC at 0.1 mg/kg/day).

LIVALO may cause fetal harm when administered to a pregnant woman. If the patient becomes pregnant while taking LIVALO, the patient should be apprised of the potential risks to the fetus and the lack of known clinical benefit with continued use during pregnancy.

8.3 Nursing Mothers

It is not known whether pitavastatin is excreted in human milk, however, it has been shown that a small amount of another drug in this class passes into human milk. Rat studies have shown that pitavastatin is excreted into breast milk. Because another drug in this class passes into human milk and HMG-Co A reductase inhibitors have a potential to cause serious adverse reactions in nursing infants, women who require LIVALO treatment should be advised not to nurse their infants [see *Contraindications* (4)].

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

In a 92-week carcinogenicity study in mice, at the maximum tolerated dose of 75 mg/kg/day with systemic maximum exposures (AUC) 26 times the clinical maximum exposure at 4 mg/day, there was an absence of drug-related tumors.

In a 92-week carcinogenicity study in rats given pitavastatin at 1, 5, 25 mg/kg/day by oral gavage ^(b)₍₄₎ (based on AUC) at the 4 mg/day maximum human dose of 25 mg/kg/day, there was a significant increase in the incidence of thyroid follicular cell tumors.

In a 26-week transgenic mouse (Tg rasH2) carcinogenicity study where animals were given pitavastatin at 30, 75, and 150 mg/kg/day by oral gavage, no ^(b)₍₄₎ clinically significant tumors were observed.

Pitavastatin had no adverse effects on male and female rat fertility at oral doses of 10 and 30 mg/kg/day respectively at systemic exposures 56-fold and 354-fold clinical exposure at 4 mg/day based on AUC.

Pitavastatin treatment in rabbits resulted in mortality in males and females given 1 mg/kg/day ^(b)₍₄₎ clinical systemic exposure) during a fertility study. Although cause

of death was not determined, rabbits had gross signs of renal toxicity (kidneys whitened) indicative of possible ischemia. Lower doses^{(b) (4)} human systemic exposure) did not show significant toxicity in adult males and females. However decreased implantations, increased resorptions, and decreased viability of fetuses was observed.

13.2 Animal Toxicology and/or Pharmacology

CNS vascular lesions, characterized by perivascular hemorrhages, edema, and mononuclear cell infiltration of perivascular spaces, have been observed in dogs treated with several other members of this drug class. A chemically similar drug in this class produced dose-dependent optic nerve degeneration (Wallerian degeneration of retinogeniculate fibers) in dogs, at a dose that produced plasma drug levels about 30 times higher than the mean drug level in humans taking the highest recommended dose. Wallerian degeneration has not been observed with pitavastatin^{(b) (4)}

Cataracts and opacities were seen in dogs treated for 52 weeks at a dose level of 0.3 mg/kg/day^{(b) (4)} times greater systemic exposures [AUC] than the clinical exposure at 4 mg/day^{(b) (4)}

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Acute, subchronic and chronic toxicology studies were performed in a variety of animal species including mice, rat, dog and monkey with pitavastatin. The chronic 6-month rat toxicity study establishes a NOAEL of 1 mg/kg/day (1,170 ng·h/ml) which based on AUC comparisons to the 4 mg (153 ng h/ml) maximum human dose (MRHD) of 8-fold. The chronic 12-month dog study establishes a NOEL of 0.3 mg/kg/day (630 ng h/ml) which based on AUC comparisons to the 4 mg (153 ng·h/ml) maximum human dose (MRHD) of 4-fold. Similarly a 6-month monkey toxicity study establishes a NOAEL of 3 mg/kg/day (850 ng·h/ml) which based on AUC comparisons to the 4 mg (153 ng·h/ml) maximum human dose (MRHD) of 6-fold. These studies would support chronic dosing at 4 mg/day in humans. Additional studies were performed to explore potential mechanisms of the identified target organ toxicities (lens, kidney, thyroid, liver, skeletal muscle, lung, forestomach) as well as qualification of various metabolites (lactone, 8-hydroxypitavastatin formed in humans) and impurities^{(b) (4)}

Lens toxicities

Cataracts were seen in dogs in repeat dose studies at ≥ 3 mg/kg/day in a 13 week study and at ≥ 1 mg/kg/day in the 12 month study (2-fold human exposure based on AUC at 4 mg/day). This finding has been associated with statin treated dogs. Distribution studies indicate that the dog lens has a higher level of pitavastatin than other species with a rank order of distribution in the lens-to-plasma ratio of: dog (0.6) > mouse (0.54) > monkey

(0.18) $\approx$ rat (0.17) > rabbit (0.06). The lens-to-plasma ratio in humans is 0.05-0.17. Although ocular opacities were observed in a mouse study at 13-weeks at ≥ 75 mg/kg/day, it was not seen at the same dose following lifetime exposure in a 92 week carcinogenicity study. Lens concentrations of pitavastatin at 4 mg/day were 7 ng/g, which is below the concentration range in dogs of 123-616 ng/g that led to the formation of cataracts after daily oral dosing at 3 or 12-months. These data, in addition to the known association of statin exposure and cataracts in dogs, suggest that the dog lens is particularly hypersensitive to the distribution of HMG-CoA reductase inhibitors.

Renal toxicities

Renal toxicities were seen in male and female monkeys in 1 month and 6 month toxicology studies. Findings consisted of mild swelling of the proximal tubule epithelium with slight desquamation of tubular epithelium at ≥ 6 mg/kg/day (≥ 8.7 -fold MRHD at a 4 mg/day dose) in conjunction with increased kidney weight. Lower doses showed this finding, but toxicities were recoverable by 2 months post-withdrawal. Creatinine and blood urea nitrogen or phenolsulphonethalein clearance were unremarkable. Higher doses of pitavastatin at shorter duration resulted in further exacerbation of renal toxicities, which suggests dose-dependency. Metabolism studies suggest that monkeys, more so than any other test species, form a greater proportion of 8-hydroxypitavastatin (active metabolite). While humans have a potential to form 8-hydroxypitavastatin, it was not considered a major human metabolite and was not measured in clinical studies. This metabolite is observed in monkey urine and feces. As a polar compound, 8-hydroxypitavastatin would be expected to be excreted in the urine along with the dihydroxypitavastatin and/or glucuronidated and excreted in the bile. A mechanistic study investigated a link between 8-hydroxypitavastatin and renal toxicity. In this study rats were administered 8-hydroxypitavastatin at 200 mg/kg BID for 2 weeks and 400 mg/kg/day BID for 1 week. All rats were found dead or sacrificed moribund before the study was scheduled for termination. Degeneration of the renal tubules, increased blood urea nitrogen and creatinine were also observed, suggesting a correlation. Fertility studies in rabbit showed mortality of treated males and females at 1 mg/kg/day (AUC=15,670 ng·h/ml, $C_{\max}$ =1184). Gross pathology (renal histopathology not performed) indicated whitening of kidneys, which may indicate ischemia, occurred at 100-fold clinical exposure at a 4 mg/day dose based on AUC. An explanation for the increased sensitivity of the rabbit to renal toxicity was not provided.

Thyroid toxicities

Pitavastatin increased thyroid follicular adenocarcinomas in male rats given ≥ 25 mg/kg/day in a lifetime carcinogenicity study (295-fold human exposure). This finding is attributed to excessive stimulation of thyroid follicular cells by elevated thyroid stimulating hormone (TSH). Pitavastatin appears to induce T_4 UDG-GT activity in liver which results in decreased plasma T_4 concentrations due to increased metabolism. Decreased T_4 leads to feedback-mediated increases in TSH release resulting in extensive follicular cell stimulation in the thyroid. This has been established for liver enzyme inducing agents, including statins. Follicular cell hypertrophy has been observed previously in subchronic toxicity testing. Also consistent with this hypothesis are potential mechanisms that address this finding. In short-term rat initiation/promotion

studies designed to evaluate the follicular cell-promoting effect, this effect of pitavastatin was eliminated upon concomitant administration of T₄. This finding supports that idea that the thyroid follicular cell tumors observed at high doses are induced by increased plasma TSH concentrations due to negative feedback regulation due to induced hepatic T₄ metabolism. Rats are known to be particularly sensitive to this induced metabolism, because they lack T₄-binding globulin, which results in increased turnover of T₄ relative to other thyroid hormone species.

Hepatic toxicities

Hepatic effects have been seen in mice, rats and dogs. In a 1-month rat toxicity study, increases in transaminases (AST, ALT) were observed in females given 50 mg/kg/day (894-fold human exposure at 4 mg/day). Elevated transaminase levels were recoverable following drug withdrawal. In the chronic 6-month rat toxicity study no increases in transaminases were observed at the highest dose studied 10 mg/kg/day (56-fold human exposure at 4 mg/day dose). In the 3-month dog toxicity study an increase in ALT was seen at 3 mg/kg/day (24-fold human exposure at 4 mg/day dose). In the 12 month toxicity study both AST and ALT activity was increased in male dogs given 3 mg/kg/day (24-fold human exposure at 4 mg/day dose). Elevated transaminases were not detected upon recovery following drug withdrawal; however, hepatic histopathologies were not observed. In a 3-month dog toxicity study with pitavastatin at 10 mg/kg/day centrilobular dilatation of liver sinusoids were observed, which resolved during recovery. Increased centrilobular hepatocytes hypertrophy was observed in male mice given ≥ 12 mg/kg/day in the carcinogenicity study. A 3-month dog toxicity study with co-administration of mevalonic acid (100-150 mg/kg/day) and pitavastatin (5 mg/kg/day) resulted in an absence of transaminase elevations at doses up to 5 mg/kg/day (42-fold human exposure at 4 mg/day based on AUC). Studies in mice up to 225 mg/kg/day (150-fold human exposure based on AUC) did show the liver histopathology; however, pitavastatin does not appear to induce drug metabolizing enzymes, nor is it associated with the severe necrosis and cellular atypia and cholestasis observed in animals following treatment with other statins. Further support of a lessened biliary toxicity is seen in guinea pigs (3 mg/kg/day for 15 days) and hamsters (1 mg/kg/day for 14 days) where pitavastatin did not show any change in biliary lipids.

Skeletal muscle toxicities

Degeneration and necrosis were observed in the 1- and 3-month rat toxicity study at 50 mg/kg/day (894-fold human exposure at the 4 mg/day dose). Myopathy was not observed in the chronic rat study at doses up to 10 mg/kg/day (56-fold MHRD based on AUC). Myopathy was observed in the mouse carcinogenicity study at the highest dose (75 mg/kg/day) and in male rats given 25 mg/kg/day in the carcinogenicity study. Doses associated with myopathy in the lifetime carcinogenicity studies in mice and rats represent exposure levels 26 and 295-fold MRHD, respectively, based on AUC.

Lung toxicities

Pulmonary lesions were seen at 3 mg/kg/day (24-fold MRHD based on AUC) in the 3 month and 12-month dog toxicity studies. The Sponsor described these lesions as lipid, pneumonia-like, composed of foam and inflammatory cells. No other organ or species

shows a similar lesion. This may be indicative of phospholipidosis, although transmission electron microscopy was not performed and it is uncertain if the lesions represented foamy macrophages that are the hallmark of phospholipidosis. Assessment in dogs given pitavastatin co-administered with mevalonic acid did not repeat the pulmonary findings suggesting an association with HMG-CoA reductase inhibition.

Forestomach toxicities

In mice and rats hyperkeratosis and acanthosis (hyperplasia) were observed with pitavastatin treatment. The thickening of the forestomach was not seen with subcutaneous administration, suggestive of a local, rather than systemic, effect. The Sponsor proposes a species-specific mechanism to explain this common finding in rodents given statins. Specifically, HMG-CoA reductase inhibition in keratinocytes of the mucosal epithelium of the forestomach results in decreased mevalonic acid and suppressed cholesterol synthesis by keratinocytes. This leads to an abnormal lipid composition in the presence of the statin, a result of accumulation of lipids in the junctional gaps of the keratinocyte layer. The results are structural changes at the mucosal epithelial surface of the forestomach, which may lead to detachment of keratin at the forestomach surface. Superficial cell proliferation, including basal cells of the epithelium, is induced to compensate for the detached keratin resulting in thickening of the mucosal epithelium through hyperplasia. Mevalonic acid has been shown to reverse this effect on the forestomach with other statins.

Testicular findings

Testicular changes (decreased testes weight, atrophy/degeneration of seminiferous tubules, impaired spermatocyte maturation and increased multinucleated giant cells) have been reported in dogs treated with statins. Histological changes in dog reproductive organs were not observed in the 12-month toxicity study at doses up to 3 mg/kg/day (>24-fold human systemic exposure at 4 mg/day dose) including sperm counts.

Metabolites and impurities

The principal circulating metabolites of pitavastatin in humans include the lactone, which the Sponsor considers pharmacologically inactive at the target, and small amounts of 8-hydroxypitavastatin which has pharmacologic activity slightly greater than that of the parent pitavastatin based on *in vitro* HMG-CoA reductase inhibition assays. Studies performed with the lactone included single dose toxicology study in the dog were augmented by negative results in the *in vitro* genetic toxicology battery, a bacterial gene mutation and chromosomal aberration test. Repeat-dose studies with pitavastatin lactone at 0, 1, 3, 10 mg/kg/day and 10 mg/kg/day pitavastatin showed similar toxicology findings. The similarity in toxicity profile is not surprising; pitavastatin undergoes glucuronidation and subsequent hydrolysis to form pitavastatin lactone, and pitavastatin lactone can further hydrolyze to reform pitavastatin), *in vivo*. The NOAEL of the lactone was 1 mg/kg/day. Qualification of the 8-hydroxypitavastatin has been discussed under “renal toxicities”.

Specific studies have been conducted on the 5-ketone metabolite of pitavastatin (M-3 metabolite) and the process impurities ^{(b) (4)}

(b) (4) Specifications for these impurities have been set for these compounds as follows: (b) (4) (b) (4)

The total impurities specification for pitavastatin, (b) (4) The (b) (4) impurity was evaluated in single-dose oral toxicity study in mice where the toxicities observed were comparably to pitavastatin. Five studies were performed with a mixture of the (b) (4) including a single-dose study, a 1-month repeat-dose rat study, and an evaluation of potential genotoxicity using an *in vitro* battery (including Ames and chromosomal aberrations analyses) and an *in vivo* mouse micronucleus assay. The (b) (4) were considered to have a weaker toxicity potential than pitavastatin and a NOAEL of 50 mg/kg/day with 1-month dosing supports this. An equivocal positive response was seen in the chromosome aberration test at concentrations of ≥ 150 $\mu\text{g/ml}$, which were associated with significant cytotoxicity. Other genetic toxicology tests in the completed battery were negative, so based on a weight of evidence approach, the (b) (4) were considered not to have appreciable genotoxic potential. Similar toxicity studies were conducted in the rat with the (b) (4). The (b) (4) showed less toxicity than pitavastatin and did not show a genotoxic potential in the bacterial gene mutation or micronucleus assays. The chromosomal aberration test in Chinese hamster lung cells showed an increase in aberrations at the highest concentration tested; slight increases in chromosomal aberrations were noted in the direct plate method (as opposed to preincubation method) within 24 hours (with metabolic activation) at 300 $\mu\text{g/ml}$. The same concentration for 48 hours was too cytotoxic for evaluation. Weak increases were seen with preincubation, without metabolic activation at 750 $\mu\text{g/ml}$, and with metabolic activation at 600-825 $\mu\text{g/ml}$. Cytotoxicity was not measured in the preincubation protocol, but similar plates in parallel cultures indicated acceptable survival (50%) suggesting a valid study. This would suggest that the positive response occurred under conditions expected to cause significant cytotoxicity, which is very likely to be at significantly higher exposure levels than those achieved *in vivo* with pitavastatin.

Pitavastatin absorbs light in the 290-700 nm range with a minor peak at 328 and major peak at 245 nm. Specific studies to address phototoxicity were not performed. Tissue distribution studies in pigmented and non-pigmented rats did not indicate any particular affinity for skin or eyes in either strain. These studies, in addition to animal and clinical studies, indicate that the likelihood of pitavastatin provoking phototoxic reactions is small.

Genetic Toxicology

Pitavastatin is not genotoxic, by weight of evidence. Pitavastatin was positive in a chromosomal aberration assay with metabolic activation in Chinese hamster lung (CHL) cells. The positive result was obtained at a concentration of pitavastatin that was close to that which caused 50% cytotoxicity with metabolic activation. Pitavastatin was negative for genotoxicity in a chromosomal aberration assay without metabolic activation in CHL cells. Pitavastatin was also negative in an Ames reverse mutation battery, *in vivo* mouse and rat micronucleus assays, an *in vivo/in vitro* single cell gel (Comet assay), and an *in vivo/in vitro* rat unscheduled DNA synthesis (UDS) assay.

Carcinogenicity

Initial carcinogenicity studies were performed prior to submission of the initial IND. In a 92-week mouse carcinogenicity study at doses of 1, 12, 30, 75 mg/kg/day of pitavastatin by oral gavage, survival was impaired in males by 37% and females by 47%. High incidences of liver and forestomach hypertrophy and hyperplasia and skeletal myofiber atrophy were observed. Executive Carcinogenicity Assessment Committee (ECAC) considered the dosing inadequate in males and requested further analysis to adjust for the excess deaths in the high dose group in the first year of the study as well as a peer review of the stomach histopathology to determine if the hyperplastic lesions may have progressed over time to a neoplastic response since carcinogenicity studies are typically 104 weeks duration.

In a 92-week rat carcinogenicity study at doses of 1, 5, 25 mg/kg/day by oral gavage, ECAC found the dose selection to be adequate. Survival in females at week 92 was 29%, 33%, 62%, 60% resulting in the early termination of the females. Male survival at week 104 was 35%, 38%, 38%, 56%. Survival in controls for each gender was similar to the lowest dose group. A high incidence of hypertrophy and hyperplasia of the liver and forestomach and skeletal myofiber atrophy were seen which might have progressed to neoplasia if the study were to have continued for the complete 104 week duration. An increased incidence of thyroid follicular cell adenocarcinomas was noted in males at 25 mg/kg/day. Pitavastatin increased thyroid follicular adenocarcinomas in male rats given ≥ 25 mg/kg/day in a lifetime carcinogenicity study (295-fold human exposure). This finding is attributed to excessive stimulation of thyroid follicular cells by elevated TSH. Pitavastatin appears to induce T₄ UDG-GT activity in male rat liver which results in decreased plasma T₄ based on increased metabolism. Decreased T₄ leads to a feedback-mediated increase in TSH release resulting in extensive follicular cell stimulation in the thyroid. This has been established for liver enzyme inducing agents, including statins. Rats are particularly sensitive to this induced metabolism because they lack T₄-binding globulin, which results in increased turnover of T₄ compared to other thyroid hormone species.

ECAC requested a transgenic mouse study to address these inadequacies. A 26-week carcinogenicity study was performed in Tg *rasH2* mice with administration of doses of 30, 75, 150 mg/kg pitavastatin by oral gavage. Males given 150 mg/kg/day had a higher frequency of alveolar/bronchiolar adenoma/carcinoma (2/25) and forestomach carcinoma (1/25). These tumors occurred at low frequency and are considered common tumors in Tg *rasH2* mice. This study was considered an adequate assessment of carcinogenicity by ECAC, without any statistically significant or clinically significant dose-related tumors.

A second Tg *rasH2* mouse carcinogenicity study was submitted using a 150 mg/kg/day pitavastatin dose with and without an initiating dose of urethane (250 mg/kg as a single IP dose on day 1) a known rodent carcinogen. Animals treated with urethane alone showed increased incidence of benign and malignant lung tumors that was unaffected by pitavastatin treatment. Thus, pitavastatin-treated animals did not show an increase in tumor incidence compared to urethane-treated controls. Urethane was used as an anesthetic during the carcinogenicity studies.

Overall, the carcinogenicity assessment suggests no tumorigenicity in rats at doses up to 25 mg/kg/day (295-fold human exposure at 4 mg/day based on AUC) after 104 weeks treatment and in Tg rasH2 mice up to 150 mg/kg/day (194-fold human exposure at 4 mg/day based on AUC) after 26 weeks.

Reproductive Toxicity – Fertility

Pitavastatin caused no apparent adverse effects on male and female rat fertility at oral doses of 10 and 30 mg/kg/day respectively at systemic exposures 56-fold and 354-fold clinical exposure at 4 mg/day based on AUC.

Pitavastatin treatment in rabbits resulted in mortality in males and females administered 1 mg/kg/day (102-fold clinical systemic exposure) during a fertility study. Although the cause of death was not determined, rabbits had gross signs of renal toxicity (kidneys whitened), perhaps indicating ischemia. Lower doses (28-fold human systemic exposure) did not show significant toxicity in adult males and females. However decreased implantations, increased resorptions as well as decreased viable fetuses were observed.

Reproductive Toxicity – Pregnancy

Pitavastatin crosses the placental barrier into the fetus. Rat fetus concentrations were $\leq 36\%$ of maternal plasma pitavastatin concentrations. Pitavastatin and 5-keto-pitavastatin are secreted by rats into milk at 7.2-fold greater pitavastatin levels than are present in maternal plasma. The predominant form of pitavastatin in the milk form is the unchanged parent molecule.

Embryo-fetal developmental studies were conducted in pregnant rats treated with 3, 10, 30 mg/kg/day pitavastatin by oral gavage during organogenesis. Evidence of maternal toxicity was indicated by decreased body weight gain and decreased food consumption at the 30 mg/kg/day level. The maternal NOAEL was established at 10 mg/kg/day (82-fold human systemic exposure at 4 mg/day dose by AUC). A neonatal malformation of agnathia (absence/partial lower jaw) 1/347 neonates, and irregular alignment of caudal vertebrae 1/347 neonates was observed at this dose. The developmental NOAEL is established at 3 mg/kg/day (>25 -fold human systemic exposure at 4 mg/day based on AUC).

Embryo-fetal developmental studies were conducted in pregnant rabbits treated with 0.1, 0.3, 1 mg/kg/day pitavastatin by oral gavage during organogenesis. Body weight loss was seen at all doses (6.7-fold human systemic exposure at 4 mg/day dose based on AUC). At maternal doses ≥ 0.3 mg/kg/day mortality and spontaneous abortions were seen (28-fold human systemic exposure). Significant maternal liver and renal toxicity appears to have contributed to the toxicity. A skeletal variation of 27 presacral vertebrae was seen with 0.1 mg/kg/day litters.

In peri-postnatal studies in pregnant rats given oral gavage doses of pitavastatin at 1, 3, 10, 30 mg/kg/day from organogenesis through weaning, mortality was observed at 1 mg/kg/day. As a result of the maternal mortality an additional peri-postnatal study was

performed in pregnant rats given oral gavage doses of pitavastatin at 0.1, 0.3 mg/kg/day from organogenesis through weaning. Insufficient lactation was noted at all doses which contributed to the decreased survival of the neonates in these dose groups. This occurs at 4-fold human systemic exposure at 4 mg/day dose based on AUC. Therefore, a developmental NOAEL could not be established even at the lowest dose tested of 0.1 mg/kg/day.

B. Pharmacologic activity

Pitavastatin is a new molecular entity of the statin class of drugs for cholesterol lowering therapy. Like other statins, pitavastatin is a selective HMG-CoA reductase inhibitor.

C. Nonclinical safety issues relevant to clinical use

None.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-363 (IND 60,492)

Review number: 1

Sequence number/date/type of submission: 000/October 1, 2008/commercial NDA

Information to Sponsor: Yes (X) No ()

Sponsor and/or agent: Kowa Research Institute, Inc., 430 Davis Drive, Suite 200, Morrisville, NC 27560, USA

Manufacturer for drug substance^{(b) (4)}

Reviewer name: C. Lee Elmore, Ph.D.

Division name: Division of Metabolism and Endocrinology Products

HFD #: 510

Review completion date: June 3, 2009

Drug:

Trade name: Livalo[®]

Generic name: pitavastatin

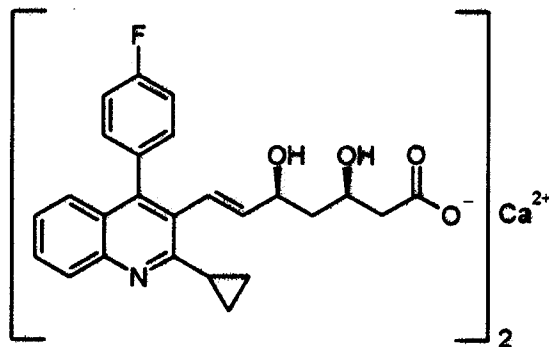
Code name: NK-104 (chiral)

Chemical name: (+)-monocalcium bis {(3R, 5S, 6E)-7-[2-cyclopropyl-4(4-fluorophenyl)-3-quinoliny] 3, 5-dihydroxy-6-heptenoate}

CAS registry number: 147526-32-7

Molecular formula/molecular weight: C₅₀H₄₆N₂O₈F₂Ca; 881 MW (molecular weight of pitavastatin base is 421)

Structure:



Relevant INDs/NDAs/DMFs: Lovastatin (NDA 19-643), Simvastatin (NDA 19-766), Pravastatin (NDA 19-898), Fluvastatin (NDA 20-261), Atorvastatin (NDA 20-702), Rosuvastatin (NDA 21-366), Cerivastatin (NDA 20-740), pitavastatin (IND 60,492)

Drug class: HMG-CoA reductase inhibitor

Intended clinical population: Statin for cholesterol-lowering in adult patients with primary hypercholesterolemia,^{(b) (4)} and combined (mixed) dyslipidemia, by decreasing total cholesterol, LDL-cholesterol, triglycerides, and apolipoprotein B. Pitavastatin increases HDL-cholesterol and apolipoprotein A1 concentrations.

Clinical formulation: 1, 2 and 4 mg tablets (immediate release), containing pitavastatin (calcium salt), lactose monohydrate, low substituted hydroxypropylcellulose, hypromellose, magnesium aluminometasilicate, magnesium stearate,^{(b) (4)} The tablets are film-coated with^{(b) (4)}, which contains hypromellose, titanium dioxide, triethyl citrate, colloidal anhydrous silica,^{(b) (4)}

Table 2.3.P.1-1 Target Composition of the 1 mg, 2 mg, and 4 mg Pitavastatin Tablets

Component	Reference	Function	Quantity		
			1 mg Tablet (mg)	2 mg Tablet (mg)	4 mg Tablet (mg)
(b) (4)					
Pitavastatin Calcium	DMF ^{(b) (4)}	Active			(b) (4)
Lactose Monohydrate	NF	(b) (4)			
Low Substituted Hydroxypropyl Cellulose	NF	(b) (4)			
Hypromellose ^{(b) (4)}	USP	(b) (4)			
Magnesium Aluminometasilicate ^{(b) (4)}	NF	(b) (4)			
Magnesium Stearate	NF	(b) (4)			(b) (4)
Total			83.00	165.00	329.00

¹Equivalent to 1.00 mg, 2.00 mg and 4.00 mg of pitavastatin.

(Sponsor, M2, Section 2.3.P, p1)

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission (listed as follows, as organized by Sponsor):

4.2.1.1 Primary Pharmacodynamics

4.2.1.1.1 Pharmacological effects of NK-104 chiral compound (1): Inhibitory effect on rat liver microsomal HMG-CoA reductase; Report No. [R92068]

4.2.1.1.2 Pharmacological effects of NK-104 chiral compound (3): Kinetic study of the HMG-CoA reductase inhibition by NK-104 chiral calcium salt; Report No. [R93007]

- 4.2.1.1.3 Inhibitory effect of NK-104 on cholesterol synthesis in HepG2 cells - Comparison among statins - ; Report No. [R98048]
- 4.2.1.1.4 Inhibition of cholesterol synthesis by NK-104 in HepG2 cells - Examination of specificity toward enzyme - ; Report No. [R99038]
- 4.2.1.1.5 Comparison of sterol synthesis inhibiting activity in human skeletal muscle cells among pitavastatin and other pharmaceuticals with similar pharmacological effects and/or indications; Report No. [R101130]
- 4.2.1.1.6 Pharmacological effects of NK-104 chiral compound (2): Inhibition of sterol synthesis in various tissues in rats - A potent and preferential effect in the liver - ; Report No. [R92069]
- 4.2.1.1.7 Inhibitory effect of NK-104 on sterol synthesis in rats; Report No. [R95078]
- 4.2.1.1.8a Effects of NK-104 on hepatic sterol synthesis in guinea pigs; Report No. [R98008]
- 4.2.1.1.8b Measurement of NK-104 concentration in plasma after single oral administration to guinea pigs; Report No. [R99058]
- 4.2.1.1.9 Plasma lipid lowering effect of NK-104 in guinea pigs; Report No. [R98022]
- 4.2.1.1.10 Plasma lipid lowering effect of NK-104 in guinea pigs - Effect of pravastatin - ; Report No. [R99041]
- 4.2.1.1.11 Plasma lipid lowering effect of NK-104 in guinea pigs - Effect of fluvastatin - ; Report No. [R99042]
- 4.2.1.1.12 Plasma lipid lowering effect of NK-104 in guinea pigs - Effect of cerivastatin - ; Report No. [R99043]
- 4.2.1.1.13 Plasma lipid lowering effect of NK-104 in guinea pigs - Effect of atorvastatin - ; Report No. [R99044]
- 4.2.1.1.14 Plasma lipid lowering effect of NK-104 in dogs; Report No. [R94012]
- 4.2.1.1.15 Hypolipidemic effect of NK-104 in Zucker rat plasma; Report No. [R94053]
- 4.2.1.1.16 Effect of NK-104 on postprandial hyperlipidemia in rats - Comparison with atorvastatin - ; Report No. [R98052]
- 4.2.1.1.17 Hypolipidemic effect of NK-104 in hyperlipidemic guinea pigs fed on laurate-rich diet; Report No. [R99031]
- 4.2.1.1.18a Hypolipidemic effect of NK-104 in WHHL rabbits (2): No. 2. Effects on plasma lipids and atherosclerosis; Report No. [R98011]
- 4.2.1.1.18b Hypolipidemic effect of NK-104 in WHHL rabbits (2): 1. Concentration of NK-104 in plasma, liver and lens after repeated oral administration of NK-104 with drinking water for six months to WHHL rabbits; Report No. [R97034]
- 4.2.1.1.19 Effect of NK-104 on lipid metabolism in HepG2 cells: Inhibition of cholesterol synthesis and enhancement of LDL receptor activity; Report No. [R99045]
- 4.2.1.1.20 LDL-receptor inducing effect of NK-104 in HepG2 cells at the mRNA levels; Report No. [R99037]
- 4.2.1.1.21 Effects of NK-104 on HMG-CoA reductase and LDL-receptor mRNA expression in HepG2 cells; Report No. [E-18]

- 4.2.1.1.22 Effects of NK-104 on expression of hepatic LDL-receptor in guinea pigs; Report No. [R98021]
- 4.2.1.1.23 Effects of NK-104 on LDL clearance in guinea pigs; Report No. [R97023]
- 4.2.1.1.24 Yanagita T, Hara E, Yotsumoto H, Rahman SM, Han S-Y, Cha J-Y *et al.* NK-104, a potent new 3-hydroxy-3-methylglutaryl Coenzyme A reductase inhibitor, enhances posttranslational catabolism of apolipoprotein B-100 and inhibits secretion of apolipoprotein B-100 and triacylglycerols from HepG2 cells; Current Therapeutic Research 1999; 60: 423-434 [Yanagita *et al.*, 1999]
- 4.2.1.1.25 Effect of NK-104 on VLDL secretion in guinea pig liver; Report No. [R97057]
- 4.2.1.1.26 NK-104 suppresses oxidized LDL-induced foam cell formation in RAW264.7 macrophages by inhibiting HMG-CoA reductase; Report No. [E-20]
- 4.2.1.1.27 NK-104 suppresses neointimal thickening and its fibronectin accumulation in balloon-injured rabbit carotid artery; Report No. [E-21]
- 4.2.1.1.28 Suzuki H, Kobayashi H, Sato F, Yonemitsu Y, Nakashima Y, Sueishi K. Plaque-stabilizing effect of pitavastatin in Watanabe Heritable Hyperlipidaemic (WHHL) rabbits; J Atheroscler Thromb 2003; 10: 109-116 [Suzuki *et al.*, 2003]
- 4.2.1.1.29 Inhibitory effect of NK-104-related compounds on HMG-CoA reductase; Report No. [R99032]
- 4.2.1.1.30 Effect of NK-104 lactone on HMG-CoA reductase activity; Report No. [R101030]
- 4.2.1.1.31 Pharmacological study on NK-104: Hypolipidemic effect of NK-104 orally administered to dogs for 14 days; Report No. [RF9118]

4.2.1.2 Secondary Pharmacodynamics

- 4.2.1.2.1 Effects of NK-104 on biliary lipids in hamsters; Report No. [R99028]
- 4.2.1.2.2 Effects of NK-104 on biliary lipids in guinea pigs; Report No. [R98051]
- 4.2.1.2.3 Effect of repeated administration of NK-104 on blood steroid hormones in rats; Report No. [R101018]
- 4.2.1.2.4 Effect of NK-104 on blood steroid hormones in guinea pigs; Report No. [R101021]

4.2.1.3 Safety Pharmacology

- 4.2.1.3.1 NK-104 general pharmacology study (1); Report No. [SR-9213]
- 4.2.1.3.2 NK-104 general pharmacology study (2); Report No. [SR-9214]
- 4.2.1.3.3 Effect of NK-104 on central nervous function in mice; Report No. [FBM 06-1114]
- 4.2.1.3.4 Effects of single oral dose administration of NK-104 on cardiovascular and respiratory function in conscious dogs; Report No. [FBM 06-4115]
- 4.2.1.3.5 Effects of NK-104 and its main metabolite, NK-104 lactone, on the hERG current; Report No. [KOW002HG]
- 4.2.1.3.6 Effects of NK-104 lactone on action potentials in isolated guinea pig papillary muscles; Report No. [KOW001MG]

4.2.1.4 Pharmacodynamic Drug Interactions

4.2.1.4.1 Drug interaction of NK-104 - Combination with warfarin - ; Report No [R99029]

4.2.2 Pharmacokinetics**4.2.2.1 Analytical Methods and Validation Reports**

4.2.2.1.1 Validation of a bioanalytical method for the measurement of NK-104 in mouse plasma; Report No. [KOW 14A/952389]

4.2.2.1.2 Validation of an analytical method for determination of NK-104 in mouse plasma by HPLC-UV; Report No. [PBC054-016]

4.2.2.1.3 Determination of storage stability of NK-104 in mouse plasma by HPLC-UV; Report No. [PBC054-017]

4.2.2.1.4 Validation of a bioanalytical method for the measurement of NK-104 in rat plasma; Report No. [KOW 12A/171094]

4.2.2.1.5 Validation of a method for measurement of the plasma concentration of NK-104 in rats; Report No. [RF9708]

4.2.2.1.6 Validation of the method for measuring plasma NK-104 hydroxide levels in rat plasma; Report No. [RF9965]

4.2.2.1.7 Validation of the determination methods of NK-104 optical isomers and pharmacokinetics of each optical isomer after oral administration of NK-104 in rats and dogs; Report No. [R98054]

4.2.2.1.8 Validation of the method for measuring NK-104^{(b) (4)} levels in rat plasma; Report No. [RF9936]

4.2.2.1.9 Validation of method to determine the impurity (NK-104^{(b) (4)}) concentrations in rat plasma; Report No. [RF9810]

4.2.2.1.10 Validation of the method of measuring NK-104 concentration in pregnant rabbit plasma; Report No. [RF2000/56]

4.2.2.1.11 Validation of the method of measuring NK-104 concentration in male rabbit plasma; Report No. [RF2001/19]

4.2.2.1.12 Investigation of sensitive method for the determination of hypolipidemic agent NK-104; Report No. [R92052]

4.2.2.1.13 Kojima J, Fujino H, Yosimura M, Morikawa H, Kimata H. Simultaneous determination of NK-104 and its lactone in biological samples by column-switching high-performance liquid chromatography with ultraviolet detection; J Chromatography B 1999; 724: 173-180 [Kojima *et al.*, 1999a]

4.2.2.1.14 Validation of method to determine concentrations of NK-104 and its lactone form in dog plasma; Report No. [RF9809]

4.2.2.1.15 Validation of column-switching high-performance liquid chromatographic methods for measuring NK-104 metabolites; Report No. [R98018]

4.2.2.1.16 Validation of HPLC method for measuring NK-104 and NK-104 lactone in plasma and bile of dogs; Report No. [R99046]

4.2.2.1.17 Validation of method for measurement of NK-104; Report No. [R95077]

4.2.2.1.18 Validation of a method for measurement of the plasma concentration of NK-104 in cynomolgus monkeys; Report No. [RF9714]

4.2.2.1.19 Analytical method validation for hydroxy NK-104 metabolite in plasma, urine and feces of humans and monkeys: No. 2. Measuring method for hydroxy NK-104 concentration in the plasma, urine and feces of monkeys by LC/MS; Report No. [R99051]

4.2.2.1.20 Validation of HPLC (UV) method for simultaneous measurement of human plasma NK-104 and NK-104 lactone; Report No. [RI105032]

4.2.2.2 Absorption

4.2.2.2.1 Plasma drug level in mice (Crj:CD-1) in single oral dose toxicology study of NK-104; Report No. [A95001]

4.2.2.2.2 Plasma concentration of NK-104 after single oral administration to rats; Report No. [RF9933]

4.2.2.2.3 Plasma drug level in rats (Crj:CD(SD)) in single oral dose toxicology study of NK-104; Report No. [AG25003]

4.2.2.2.4 Kimata H, Fujino H, Koide T, Yamada Y, Tsunenari Y, Yanagawa Y. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase (1): Absorption, distribution, metabolism and excretion in rats; *Xenobio Metabol Dispos* 1998; 13: 484-498 [Kimata *et al.*, 1998]

4.2.2.2.5 Fujino H, Kojima J, Yamada Y, Kanda H, Kimata H. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase (4): Interspecies variation in laboratory animals and humans; *Xenobio Metabol Dispos* 1999; 14: 79-91 [Fujino *et al.*, 1999a]

4.2.2.2.6 Fujino H, Tsunenari Y, Koide T, Yonemitsu M, Yanagawa Y, Kimata H. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase (2): Absorption, distribution, metabolism, excretion and accumulation following repeated oral administration of [¹⁴C]-NK-104 in rats; *Xenobio Metabol Dispos* 1998; 13: 499-507 [Fujino *et al.*, 1998a]

4.2.2.2.7 Plasma concentration of NK-104 after repeated oral administration to pregnant rats; Report No. [RF9932]

4.2.2.2.8 Pharmacokinetic of [¹⁴C]-NK-104 in guinea pigs; Report No. [R98042]

4.2.2.2.9 Investigation of the effect of NK-104 on blood cholesterol level of dams in oral administration in the fetal organogenesis period and estimation of the amount of exposure in rabbits; Report No. [G2526]

4.2.2.2.10 Pharmacokinetic study of NK-104 after oral administration to cynomolgus monkeys - Determination of NK-104 and 8-hydroxy-NK-104 concentrations in plasma, urine, and feces - ; Report No. [R99052]

4.2.2.2.11 Absorption, metabolism, and excretion after single oral and intravenous administration of NK-104 in Cynomolgus monkey; Report No. [9L804]

4.2.2.3 Distribution

4.2.2.3.1 Plasma concentration of NK-104 after single oral administration to mice; Report No. [RF9945]

4.2.2.3.2 Fujino H, Yamada I, Kojima J, Hirano M, Matsumoto H, Yoneda M. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase

- (5): *In vitro* metabolism and plasma protein binding in animals and human; Xenobio Metabol Dispos 1999; 14: 415-424 [Fujino *et al.*, 1999b]
- 4.2.2.3.3 Calculation of the human plasma protein binding ratio of NK-104 lactone by ultracentrifugation method; Report No. [R1107017]
- 4.2.2.3.4 Mechanism of uptake of NK-104 by human liver; Report No. [ATR-148-100]
- 4.2.2.3.5 Study on various transporters and NK-104; Report No. [R101068]
- 4.2.2.3.6 [¹⁴C]-NK-104 quantitative tissue distribution in the pigmented rat after single oral administration; Report No. [KOW 025/003656]
- 4.2.2.3.7 Fujino H, Morikawa S, Kanda H, Kimata H. Studies on the metabolic fate of NK-104, a new inhibitor of HMG-CoA reductase (3): Foeto-placental transfer and mammary excretion after oral administration in rats; Xeno Meta Disp 1998; 13: 508-515 [Fujino *et al.*, 1998b]
- 4.2.2.3.8 Tissue drug distribution in cynomolgus monkeys (*Macaca fascicularis*) following a single oral dose of NK-104; Report No. [9L805]

4.2.2.4 Metabolism

- 4.2.2.4.1 *In vitro* study of NK-104 metabolism; Report No. [AE-2544]
- 4.2.2.4.2 Metabolic study of NK-104 in *in vitro* - Examination of species difference - (First Report): *In vitro* study of NK-104 using hepatic microsomes obtained from animals and humans; Report No. [R99007]
- 4.2.2.4.3 *In vitro* studies on NK-104 using human metabolic enzyme system A novel mechanism of lactonization by UDP-glucuronosyltransferase; Report No. [R101029]
- 4.2.2.4.4 Kojima J, Fujino H, Abe H, Yoshimura M, Kanda H, Kimata H. Identification of metabolites of NK-104, an HMG-CoA reductase inhibitor, in rat, rabbit and dog bile; Biol Pharm Bull 1999; 22: 142-150 [Kojima *et al.*, 1999b]
- 4.2.2.4.5 An analytical investigation to determine the plasma and urinary drug concentrations in the pharmacokinetic study of NK-104 in elderly healthy volunteers (Second Report): Determination of NK-104 metabolites in non-elderly group; Report No. [R97047]

4.2.2.5 Excretion

- 4.2.2.5.1 Pharmacokinetic study of NK-104 by use of EHBR – Biliary excretion study; Report No. [R101066]
- 4.2.2.5.2 Biliary excretion of NK-104 after intravenous administration in dogs; Report No. [R99047]

4.2.2.6 Pharmacokinetic Drug Interactions

- 4.2.2.6.1 Enzyme-inhibitory study of NK-104 - Effects of NK-104 single administration on the drug-metabolizing enzyme system in female rats - ; Report No. [H-TB-9731]
- 4.2.2.6.2 Enzyme induction study of NK-104 - Effects of NK-104 on the drug-metabolizing enzyme system in rats - ; Report No. [H-TB-9607]
- 4.2.2.6.3 *In vitro* drug interaction between NK-104 and fibrate drugs; Report No. [R101113]

4.2.2.6.4 An *in vitro* study of drug metabolism of NK-104 Report No. 5: A study of drug-drug interaction mediated by CYP2C9; Report No. [R99035]

4.2.2.6.5 Interaction with cyclosporine A in the uptake of NK-104 using LST-1 expressing *Xenopus* oocytes; Report No. [ATR-149-035]

4.2.2.6.6 Inhibition studies of concomitant drugs on hOATP1B1 uptake of [14C]-NK-104; Report No. [FBM 06-T350]

4.2.2.6.7 Studies on plasma concentration of gemfibrozil and cerivastatin or NK-104 after single oral administration to rats; Report No. [RI102020]

4.2.2.7 Other Pharmacokinetic Studies

4.2.2.7.1 Synthesis of [¹⁴C]-NK-104; Report No. [F-01]

4.2.2.7.2 Examination of NK-104 absorption site in rats; Report No. [R95033]

4.2.2.7.3 Study on pharmacokinetics of NK-104 in rat models of liver dysfunction induced by carbon tetrachloride; Report No. [RT2001/2503]

4.2.2.7.4 Plasma concentration of NK-104 (b) (4) and NK-104 (b) (4) after single oral administration to mice; Report No. [RF9935]

4.2.3 Toxicology

4.2.3.1 Single-Dose Toxicity

4.2.3.1.1 Single dose oral toxicity study of NK-104 in mice; Report No. [RFG2510]

4.2.3.1.2 Single dose toxicity study of NK-104 by oral administration in rats; Report No. [RG25001]

4.2.3.1.3 Single oral dose toxicity study of NK-104 in Crj:CD(SD) rats (UDS dose-finding study); Report No. [RF9808]

4.2.3.1.4 NK-104 - Single dose toxicity study by the intravenous route in the rat; Report No. [387/002]

4.2.3.1.5 Single dose toxicity study of NK-104 by oral administration in dogs; Report No. [RFG2501]

4.2.3.1.6a Toxicity study of NK-104 on single oral administration in cynomolgus monkeys; Report No. [SBL17-33]

4.2.3.1.6b Plasma concentration during a single oral dose toxicological study of NK-104 in cynomolgus monkeys; Report No. [RFG2513]

4.2.3.2 Repeat-Dose Toxicity

4.2.3.2.1 One-month oral repeated dose toxicity study of NK-104 in mice; Report No. [RF9514]

4.2.3.2.2 A four-week oral dose range-finding toxicity and toxicokinetics study of NK-104 in CB6F1-Tg rasH2 mice; Report No. (b) (4) [38.01]

4.2.3.2.3 NK-104 sub-acute toxicity to mice by repeated oral administration for 13 weeks; Report No. [KOW 14/952398]

4.2.3.2.4 A 1-month oral toxicity preliminary study of NK-104 in rats; Report No. [R92034]

4.2.3.2.5 One-month repeated dose toxicity study of NK-104 by oral administration in rats; Report No. [RG25002]

- 4.2.3.2.6 NK-104 13-week preliminary study to a rat carcinogenicity study by repeated oral administration; Report No. [KOW 12/942992]
- 4.2.3.2.7 Six-month consecutive oral toxicity study of NK-104 in rats; Report No. [RFG2506]
- 4.2.3.2.8 NK-104 - 2 week intravenous toxicity study in the rat; Report No. [387/003]
- 4.2.3.2.9a A 14-day oral toxicity study of NK-104 in dogs (preliminary study); Report No. [RF2502]
- 4.2.3.2.9b Measuring plasma NK-104 levels (plasma samples from a 14-day toxicological study in dogs); Report No. [A92005]
- 4.2.3.2.10a Three-month repeated dose toxicity study of NK-104 by oral administration in dogs; Report No. [RFG2503]
- 4.2.3.2.10b Three-month repeated oral dose toxicokinetic study of NK-104 in dogs; Report No. [AG25001]
- 4.2.3.2.11a Twelve-month consecutive oral toxicity study of NK-104 in dogs; Report No. [RFG2504]
- 4.2.3.2.11b Measurement of plasma and tissue concentrations of NK-104 in a 12-month repeated oral dose toxicity study of NK-104 in dogs; Report No. [AG25004]
- 4.2.3.2.12 NK-104 – 2 week intravenous toxicity study in the beagle dog; Report No. [387/001]
- 4.2.3.2.13a Four week consecutive oral toxicity study of NK-104 in cynomolgus monkeys; Report No. [SBL17-34]
- 4.2.3.2.13b Plasma drug levels in cynomolgus monkeys in a four-week repeated dose toxicity study of NK-104; Report No. [RFG2514]
- 4.2.3.2.14a A 26-week consecutive oral toxicity study of NK-104 in cynomolgus monkeys, followed by an 8-week recovery period; Report No. [SBL17-35]
- 4.2.3.2.14b Measurement of the plasma concentrations of NK-104 in a 26-week repeated oral dose toxicity study of NK-104 in cynomolgus monkeys, followed by an 8-week recovery period; Report No. [RFG2515]
- 4.2.3.2.14c Peer review on the renal histopathological examination in the “26-week repeated oral administration toxicity study and 8-week recovery study of NK-104 on cynomolgus monkeys” (Study Number SBL17-35) [SBL17-35 Peer Review]

4.2.3.3 Genotoxicity

4.2.3.3.1 *In Vitro*

- 4.2.3.3.1.1 Mutagenicity testing of NK-104 in bacterial reverse mutation assays; Report No. [3140]
- 4.2.3.3.1.2 Chromosomal aberration test in cultured mammalian cells on NK-104; Report No. [3141]
- 4.2.3.3.1.3 *In vivo/in vitro* unscheduled DNA synthesis (UDS) test of NK-104 with rat hepatocyte; Report No. [3638 (144-014)]

4.2.3.3.2 *In Vivo*

- 4.2.3.3.2.1 Micronucleus test of NK-104 by single oral administration in mice; Report No. [RG25003]
- 4.2.3.3.2.2 Micronucleus test of NK-104 repeated oral administration in rats; Report No. [KW92117]
- 4.2.3.3.2.3 Single cell gel (SCG) assay of NK-104 with mice (Comet Assay); Report No. [A819 (144-121)]

4.2.3.4 Carcinogenicity**4.2.3.4.1 Long-Term Studies**

- 4.2.3.4.1.1 NK-104 potential tumorigenic effects in repeated oral gavage administration to mice; Report No. [KOW 16/982522]
- 4.2.3.4.1.2 Statistical analysis of tumors and non-neoplastic findings in mice in NK-104 potential tumorigenic effects in repeated oral gavage administration to mice; Report No. [KOW/016]
- 4.2.3.4.1.3 NK-104: Potential tumorigenic effects in repeated oral gavage administration to rats; Report No. [KOW 13/971903]
- 4.2.3.4.1.4 Pathology Working Group Peer Review; Report No. [EPL 668-001/002]
- 4.2.3.4.1.5 Carcinogenicity study of NK-104 in mice and rats - Analysis of tumor incidence - ; Report No. [5483 (144-048)]
- 4.2.3.4.1.6 Potential tumorigenic effects of repeated oral gavage administration of NK-104 to the rat; statistical analysis of forestomach tumors; Report No. [RF2001/37]

4.2.3.4.2 Short- or Medium-Term Studies

- 4.2.3.4.2.1 Study on the promoting effect of NK-104 on development of thyroid tumors in rats pre-treated with N-bis (2-hydroxypropyl) nitrosamine; Report No. [G2523]
- 4.2.3.4.2.2 A 26-week oral dose carcinogenicity and toxicokinetic study of NK-104 in CB6F1-Tg rasH2 mice; Report No. (b) (4) 138.03]
- 4.2.3.4.2.3 Two-stage lung carcinogenicity study of NK-104 in Tg rasH2 mice; Report No. [G2536]

4.2.3.5 Reproductive and Developmental Toxicity**4.2.3.5.1 Fertility and Early Embryonic Development**

- 4.2.3.5.1.1 Reproductive and developmental toxicity study of NK-104 in rats administered orally prior to and in the early stages of pregnancy (Seg. I); Report No. [KW92083]
- 4.2.3.5.1.2 Histopathological examination of the effect of NK-104 on the testis and epididymis of rats; Report No. [2000KW100]
- 4.2.3.5.1.3 Preliminary study of the effect of NK-104 on fertility and early embryonic development up to implantation in oral administration to rabbits; Report No. [100320P]

4.2.3.5.1.4 Study on effects of oral administration of NK-104 on fertility and early embryonic development to implantation in rabbits; Report No. [100320]

4.2.3.5.2 Embryo-fetal Development

4.2.3.5.2.1 Teratogenicity study of NK-104 in rats by oral administration (preliminary study); Report No. [R92080]

4.2.3.5.2.2 Study by oral administration of NK-104 during period of fetal organogenesis in rats (Segment II); Report No. [RFG2508]

4.2.3.5.2.3 Teratogenicity study of NK-104 in rabbits by oral administration (preliminary study); Report No. [RF2505]

4.2.3.5.2.4 Study by oral administration of NK-104 during fetal organogenesis in rabbits (Segment II); Report No. [RFG2507]

4.2.3.5.3 Prenatal and Postnatal Development, Including Maternal Function

4.2.3.5.3.1 Study by oral administration of NK-104 in rats during the perinatal and lactation periods (Segment III); Report No. [RFG2511]

4.2.3.5.3.2 Study by oral administration of NK-104 in rats during the perinatal and lactation periods (Additional study); Report No. [RFG2512]

4.2.3.7 Other Toxicity Studies

4.2.3.7.1 Antigenicity

4.2.3.7.1.1 Antigenicity study of NK-104 in guinea pigs; Report No. [SR-9241]

4.2.3.7.3 Mechanistic Studies

4.2.3.7.3.1 Study on the effect of NK-104 on the thyroid gland in rats; Report No. [RF9811]

4.2.3.7.3.2 Study on the effect of NK-104 on the thyroid gland in rats: 2. Evaluation of non-effect dose with regard to thyroid hormones; Report No. [RF9812]

4.2.3.7.3.3 Study on the effect of NK-104 to thyroxine clearance in rats by multiple oral administrations; Report No. [RF9731]

4.2.3.7.3.4 Immunohistochemical examination of cell-proliferating activity in the thyroid gland from "Six-month repeated oral administration toxicity study of NK-104 in rats"; Report No. [G2533]

4.2.3.7.3.5 Immunohistochemical examination of cell-proliferating activity in specimens from "Two-stage carcinogenicity study of NK-104 in rat thyroid"; Report No. [G2528]

4.2.3.7.3.6 Immunohistochemical examination of cell-proliferating activity in specimens from "Two-stage carcinogenicity study of NK-104 in rat thyroid" (additional examination); Report No. [G2532]

4.2.3.7.3.7 Immunohistochemical study of cell proliferative activity in specimens used for evaluation of the effects of NK-104 on rat thyroid; Report No. [RF2001/06]

4.2.3.7.3.8 Effects of a single oral dose of NK-104 on dog eyes; Report No. [RF9225]

- 4.2.3.7.3.9 Concentration of NK-104 in eye tissues after an oral administration of NK-104 to dogs; Report No. [A92012]
- 4.2.3.7.3.10 Concentration of NK-104 in lens after repeated oral administration of NK-104 for three months to dogs; Report No. [AG25002]
- 4.2.3.7.3.11 Distribution of NK-104 in eye tissues after single and repeated oral administration of NK-104 to various animals; Report No. [R93055]
- 4.2.3.7.3.12 Binding of NK-104 with lens protein of various animals; Report No. [R96010]
- 4.2.3.7.3.13 Binding of NK-104 with plasma protein of various animals by equilibrium dialysis method; Report No. [R93017]
- 4.2.3.7.3.14 Investigation of NK-104-induced cataract: Concentration of NK-104 in plasma and lens in rats by single oral administration; Report No. [RF9615]
- 4.2.3.7.3.15 Binding of NK-104 with human lens protein by equilibrium dialysis method; Report No. [R98056]
- 4.2.3.7.3.16 Investigation of NK-104-induced cataract: 4. Accumulation of NK-104 in lens by a 3-month oral administration to rats; Report No. [RF9719]
- 4.2.3.7.3.17 Accumulation of NK-104 in lens by multiple oral administration to monkeys; Report No. [RF9725]
- 4.2.3.7.3.18 Investigation of NK-104-induced cataract: 5. A 3-month oral toxicity study of NK-104 and mevalonic acid in combination in dogs; Report No. [RF9727]
- 4.2.3.7.3.19 Examination of hepatocyte-injuring and drug-metabolizing enzyme inducing activities of NK-104 in mice (non GLP study); Report No. [3842 (144-015)]
- 4.2.3.7.3.20 Effect of NK-104 by concomitant administration of mevalonic acid on rat forestomach (morphological examination); Report No. [RF2001/28]
- 4.2.3.7.3.21 Evaluation of the effects of NK-104, used in combination with mevalonic acid, on rat fore-stomach (a preliminary study); Report No. [RF2001/35]
- 4.2.3.7.3.22 Evaluation of the effects of NK-104, used in combination with mevalonic acid, on rat fore-stomach; Report No. [G2534]
- 4.2.3.7.3.23 Prevention of NK-104-induced toxicity in pregnant rats by mevalonic acid supplementation; Report No. [RF9834]

4.2.3.7.5 Metabolites

- 4.2.3.7.5.1 Single dose toxicity study of NK-104 lactone in dogs by oral administration; Report No. [RFG2517]
- 4.2.3.7.5.2 One-month repeated dose toxicity study of NK-104 lactone by oral administration in dogs; Report No. [RFG2518]
- 4.2.3.7.5.3 Mutagenicity testing of NK-104 lactone in bacterial reverse mutation assays; Report No. [3846]
- 4.2.3.7.5.4 Chromosomal aberration test in cultured mammalian cells on NK-104 lactone; Report No. [3847]
- 4.2.3.7.5.5 Two-week repeated dose oral toxicity study with 8-OH NK-104 Ca salt in rats; Report No. [RF9964]

4.2.3.7.6 Impurities

4.2.3.7.6.1 Single dose toxicity study of UK-4 (relative substance of NK-104) in mice by oral administration; Report No. [RF9501]

4.2.3.7.6.2 Single dose toxicity study of NK-104^{(b) (4)} in rats by oral administration; Report No. [RFG2519]

4.2.3.7.6.3 One-month repeated dose toxicity study of NK-104^{(b) (4)} by oral administration in rats; Report No. [G2520]

4.2.3.7.6.4 Mutagenicity testing of NK-104^{(b) (4)} in bacterial reverse mutation assays; Report No. [3844]

4.2.3.7.6.5 Chromosomal aberration test in cultured mammalian cells on NK-104^{(b) (4)}; Report No. [3845]

4.2.3.7.6.6 Micronucleus test of NK-104^{(b) (4)} by single oral administration in mice; Report No. [G2524]

4.2.3.7.6.7 Single dose toxicity study of NK-104^{(b) (4)} in rats by oral administration; Report No. [G2521]

4.2.3.7.6.8 One-month repeated dose toxicity study of NK-104^{(b) (4)} by oral administration in rats; Report No. [G2522]

4.2.3.7.6.9 Mutagenicity testing of NK-104^{(b) (4)} in bacterial reverse mutation assays; Report No. [4278]

4.2.3.7.6.10 Chromosome aberration test in cultured mammalian cells on NK-104^{(b) (4)}; Report No. [4315]

4.2.3.7.6.11 Micronucleus test of NK-104^{(b) (4)} by single oral administration in mice; Report No. [G2525]

4.2.3.7.7 Other

4.2.3.7.7.1. Review of the methods used for statistical analysis of toxicological data on NK-104; Report No. [RT2002/2502]

Studies not reviewed within this submission:

N/A

2.6.2 PHARMACOLOGY**2.6.2.1 Brief summary****Pitavastatin**

Pitavastatin is a new molecular entity of the statin class of drugs for cholesterol lowering therapy. Like other statins, pitavastatin is a selective HMG-CoA reductase inhibitor. Pitavastatin is the 3*R*, 5*S*(+)-enantiomer and has two chiral carbon atoms (positions 3 and 5 in the 3,5-dihydroxy-6-heptenoic acid side chain).

Pitavastatin is a potent statin, with an IC₅₀ for HMG-CoA of 6.8 nM. While pitavastatin is not known to lower serum cholesterol in any strain of rats (similar to other statins), it does lower serum cholesterol in mice, guinea pigs, rabbits, dogs, and monkeys (and humans). Pitavastatin has pharmacodynamic activity against HMG-CoA reductase in

rats, mice, guinea pigs, rabbits, monkeys and dogs (and humans). Pitavastatin was also active *in vitro* in human HepG2 cell cultures.

Metabolites

The primary metabolite of pitavastatin in humans is pitavastatin lactone, which is also the primary metabolite in dogs and monkeys, but is a minor metabolite in rats, and undetectable in mice and rabbits. The Sponsor presented evidence is consistent with their claim that pitavastatin lactone is not pharmacologically active, but the Sponsor did not rule this possibility out definitively.

Impurities

Process impurities for pitavastatin include two structural pitavastatin (b) (4). In addition to the lactone, another primary human metabolite includes the hydroxylated form (at the C8 position) pitavastatin. Studies have revealed that both (b) (4) are weak inhibitors of HMG-CoA reductase, but 8-hydroxypitavastatin form was more potent than parent pitavastatin.

METABOLITES AND IMPURITIES

Drugs	Inhibitory effect on HMG-CoA reductase IC ₅₀ value (nM)
NK-104 8-hydroxy form	3.5 [1.6]
(b) (4)	(b) (4) (b) (4)
(b) (4)	(b) (4) (b) (4)
NK-104	5.5 [1]

(Sponsor, M4, R99032, p9)

Inhibition of cholesterol synthesis *in vitro*

Pitavastatin inhibited cholesterol synthesis in normal human hepatocytes with an IC₅₀ of 24.5 nmol/L. In human skeletal muscle cells pitavastatin had an IC₅₀ 3.4 nmol/L for cholesterol synthesis inhibition. The sterol inhibitory activity of pitavastatin in liver was about 18 times higher than that of pravastatin and around 3 times higher than that of simvastatin. The inhibitory activity of pitavastatin in skeletal muscle was about 58 times higher than that of pravastatin and 3-fold that of simvastatin. Pitavastatin has a longer duration of action compared to other statins with significant inhibition of sterol synthesis in the liver up to 6 hours post dose when the inhibitory effect of simvastatin and pravastatin had. In guinea pigs pitavastatin significantly inhibited hepatic sterol synthesis to a similar extent 3 hours after dosing to other tested statin, however pitavastatin inhibition continued over a 12 hour duration (P<0.01 at 6 and 9 hours post dose). The pitavastatin elimination was ~10 hours in plasma and ~9 hours in the liver. The lactone was below the limit of quantification (<2.5 ng/ml) in any plasma samples.

Plasma lipid lowering in normal and hyperlipemic animals

Pitavastatin lowered TG by >20% in guinea pigs given daily oral pitavastatin for two weeks. Following 14-day oral pitavastatin (0.1 mg/kg) in dogs total plasma cholesterol was reduced significantly.

Statins lower plasma TG and not plasma cholesterol in normal, fed rats by inhibiting hepatic TG secretion. This is unlike other species (e.g. rabbit, guinea pig) in which **plasma TG doesn't change and cholesterol** lowering represents the pharmacodynamic effect. Rabbits are often used because similar to humans they transport cholesterol as LDL.

Zucker rat models of congenital hypertriglyceridemia given 1 mg/kg/day pitavastatin for 14 days resulted in significantly lowered plasma TG without an effect on plasma total cholesterol or phospholipid. VLDL-TG secretion was significantly reduced by 21% but lipoprotein lipase activity was not significantly affected.

In a postprandial rat model of hyperlipidemia, a single dose (1 mg/kg) pitavastatin significantly suppressed plasma triglycerides at 6 hours after fat loading.

Guinea pigs loaded with high lauric acid diets; serving as a hyperlipidemic model, had elevated plasma cholesterol and triglyceride, hepatic lipid content and delayed LDL clearance compared to guinea pigs fed basal diets. LDL clearance was found in the 1.0 mg/kg pitavastatin treated groups suggesting that like atorvastatin, the decrease in plasma cholesterol may occur by enhanced expression of liver LDL receptors.

In the WHHL rabbit, an established model of hyperlipidemia and atherosclerosis resulting from LDL-R deficiency, pitavastatin 0.5 mg/kg/day in drinking water for 26 weeks lowered plasma cholesterol and TG and significantly lowered VLDL-cholesterol and IDL-cholesterol but did not affect cholesterol in LDL. The plasma steady state level of pitavastatin was 40.3 ng/ml, and lactone levels were below the limit of quantitation. The pitavastatin liver concentration was 618.6 ng/g and liver concentrations of lactone were 78.8 ng/g.

Mechanism of Action

Pitavastatin significantly increased expression of LDL-R mRNA at ≥ 1000 nmol/l and significantly increased the level of binding (≥ 100 nmol/l), incorporation (≥ 10 nmol/l) and degradation (≥ 1 nmol/l) of LDL compared to controls. The maximal amount of LDL binding by Scatchard plot analysis was 1.6-fold higher with pitavastatin which was attributed to an increased amount of receptors, although affinity was unchanged and LDL dissociation to its receptor was not affected by pitavastatin. The Sponsor suggests that the increased LDL receptors are induced by the pitavastatin induced suppression of hepatic cholesterol biosynthesis. They allege that pitavastatin enhances LDL internalization (data not provided) in the liver as well as LDL clearance, which subsequently lead to reductions in LDL and total cholesterol in the plasma. Pitavastatin caused a dose-dependent decrease in VLDL-TG and ApoB (as well as VLDL-ApoB) using an isolated guinea pig liver perfusion method following daily dosing for 2 weeks

with pitavastatin (0.03, 0.3, 3 mg/kg). The suppression of VLDL secretion is thought to contribute to the reduced plasma triglyceride effects of pitavastatin.

Pitavastatin dose dependently reduced intimal thickening and fibronectin accumulation following repeat dosing (0.1-1 mg/kg) following endothelial injury with a balloon catheter. In WHHL rabbits, pitavastatin lowered lipids, suppressed progression of atherosclerosis and stabilized atherosclerotic plaques.

2.6.2.2 Primary pharmacodynamics

Pitavastatin is a selective and potent HMG-CoA reductase inhibitor. HMG-CoA reductase is the rate-limiting step in the *de novo* synthesis of cholesterol from acetyl CoA, which occurs primarily in the mammalian liver. Inhibition of HMG-CoA reductase leads to decreased cholesterol production in humans. HMG-CoA reductase is thus an effective target for the treatment of hypercholesterolemia when diet and exercise alone are unable to control plasma cholesterol levels.

Pitavastatin is the 3*R*, 5*S*(+)-enantiomer and has two chiral carbon atoms (positions 3 and 5 in the 3,5-dihydroxy-6-heptenoic acid side chain). NK-104 inhibited hepatic sterol synthesis in a dose-dependent manner with an ED₅₀ of 0.13 mg/kg in rats and 0.33 mg/kg in guinea pigs. NK-104 decreased plasma cholesterol levels in a dose-dependent manner in male beagle dogs (at ≥ 0.1 mg/kg for 14 days), rabbits (at ≥ 0.3 mg/kg for 28 days), and guinea pigs (at ≥ 0.3 mg/kg for 14 days). NK-104 reduced postprandial plasma triglyceride levels in rats.

R92068 – Pharmacological effects of NK-104 chiral compound (1): Inhibitory effect on rat liver microsomal HMG-CoA reductase

Pitavastatin is the 3*R*, 5*S*(+)-enantiomer and has two chiral carbon atoms (positions 3 and 5 in the 3,5-dihydroxy-6-heptenoic acid side chain). In a rat liver microsome assay with 30 minutes incubation, the IC₅₀ for inhibition of HMG-CoA with NK-104 was 6.8 nM, which is 6.8- and 2.4-fold greater than that observed with pravastatin and simvastatin. Pitavastatin has 2-fold higher inhibitory effect than the lactone form. T_{1/2} is 15 minutes for lactone hydrolysis to form the acid.

R93007 – Pharmacological effects of NK-104 chiral compound (3): Kinetic study of the HMG-CoA reductase inhibition by NK-104 chiral calcium salt

Pitavastatin is the 3*R*, 5*S*(+)-enantiomer and has two chiral carbon atoms (positions 3 and 5 in the 3,5-dihydroxy-6-heptenoic acid side chain). Kinetic analyses in rat liver microsomes indicated that NK-104 is a competitive inhibitor of HMG-CoA reductase with a K_i of 1.7 nM, 1.6- to 3.5-fold higher affinity than that of simvastatin (K_i 2.8 nM) or pravastatin (K_i 6.0 nM). Pitavastatin (b) (4) had a K_i of 341 nM and was a weak inhibitor in this assay.

R98048 Inhibitory effect of NK-104 on cholesterol synthesis in HepG2 cells**-Comparison among statins -**

Pitavastatin is the 3*R*, 5*S*(+)-enantiomer and has two chiral carbon atoms (positions 3 and 5 in the 3,5-dihydroxy-6-heptenoic acid side chain). NK-104 had an IC_{50} of 5.8 nM in inhibition on sterol synthesis in hepatocytes (HepG2 cells), compared to 17.0 nM with simvastatin and 32.9 nM with pravastatin.

R99038 – Inhibition of cholesterol by NK-104 in HepG2 cells - Examination of specificity toward enzyme

This assay showed that NK-104 strongly inhibits the synthesis of cholesterol from radiolabeled acetic acid ($IC_{50} = 2.0$ nM), while NK-104 only slightly inhibited synthesis of cholesterol from mevalonic acid (the product of HMG-CoA reductase, $IC_{50} = 10$ μ M). This report confirms that pitavastatin does not inhibit any enzyme downstream of HMG-CoA reductase.

R101130 - Comparison of sterol synthesis inhibiting activity in human skeletal muscle cells among pitavastatin and other pharmaceuticals with similar pharmacological effects and/or indications**Key Study Findings:**

- Study provides corollary evidence that the effects of pitavastatin in a skeletal muscle cell line are similar to currently marketed statins, and are dissimilar from that of cerivastatin

Inhibition of cholesterol biosynthesis in human skeletal muscle cell line was evaluated. Note that cerivastatin is the outlier. The cause of rhabdomyolysis is not known, however these data provide corollary evidence that the pharmacodynamic effects of pitavastatin are similar to currently marketed statins, and are dissimilar from that of cerivastatin (no longer marketed due to increased risk of rhabdomyolysis).

Table 1 Cholesterol synthesis inhibiting activities of statins in human skeletal muscle cells						
Drug	Drug concentration (μM)					
	0.0001	0.001	0.01	0.1	1	10
Pitavastatin	7.72 ± 2.78	30.02 ± 5.09	68.03 ± 6.42	76.24 ± 12.66	85.46 ± 9.37	3.4
Simvastatin	17.58 ± 2.89	25.04 ± 1.86	61.76 ± 4.17	84.81 ± 3.38	90.65 ± 4.55	4.8
Atorvastatin	16.17 ± 6.33	24.65 ± 4.08	62.17 ± 6.87	86.73 ± 3.91	92.86 ± 5.05	4.7
Fluvastatin	22.36 ± 7.01	27.55 ± 2.23	64.38 ± 2.12	84.52 ± 3.87	89.17 ± 2.88	4.1
Cerivastatin	35.12 ± 7.80	66.09 ± 3.12	90.30 ± 2.10	88.80 ± 5.36	88.56 ± 6.20	0.3
Pravastatin		3.06 ± 8.18	15.86 ± 4.27	44.31 ± 6.40	70.65 ± 2.99	76.56 ± 9.76
Numbers in the table represent the mean ± SE (n=4) of cholesterol synthesis inhibition rate (%)						
Table 2 Cholesterol synthesis inhibiting activities of statins in human hepatocytes						
Drug	Drug concentration (μM)					
	0.0001	0.001	0.01	0.1	1	10
Pitavastatin	7.50 ± 1.96	12.27 ± 2.06	36.23 ± 1.53	71.61 ± 0.77	91.63 ± 0.32	24.5
Simvastatin	8.37 ± 1.60	7.81 ± 1.92	18.76 ± 1.80	49.20 ± 1.55	80.67 ± 0.48	106.0
Atorvastatin	-0.33 ± 2.18	3.38 ± 2.33		38.32 ± 1.77	74.80 ± 1.27	209.0
Fluvastatin	6.17 ± 3.36	10.54 ± 2.67	37.21 ± 2.01	70.99 ± 0.63	92.23 ± 0.50	23.9
Cerivastatin	12.12 ± 1.78	27.32 ± 1.56	66.60 ± 1.21	89.28 ± 0.30	98.35 ± 0.12	3.8
Pravastatin		6.50 ± 5.43	-6.86 ± 9.12	-12.49 ± 5.39	1.26 ± 4.41	38.18 ± 2.24
Numbers in the table represent the mean ± SE (n=4) of cholesterol synthesis inhibition rate (%)						

(Sponsor, M4, R101130, p12)

R92069 – Pharmacological effects of NK-104 chiral compound (2): Inhibition of sterol synthesis in various tissues in rats - A potent and preferential effect in the liver -

Key Study Findings:

- The inhibitory effect of NK-104 on sterol synthesis was most potent in liver (ED₅₀ ~0.17 mg/kg), followed by the ileum (~0.6 mg/kg).
- NK-104 chiral and racemic lactones and NK-104 chiral calcium salt showed roughly equivalent inhibition of sterol synthesis at 1.5 hours post-administration to rats.
- Interconversion between lactone and hydrolyzed NK-104 are expected to have occurred in this time, such that this study does not necessarily address the potential pharmacodynamic activity of NK-104 lactone.

R95078 – Inhibitory effect of NK-104 on sterol synthesis in rats

Key Study Findings:

- Pitavastatin was approximately 2-fold more potent in rat liver than in rat skeletal muscle.

A study of inhibition of cholesterol biosynthesis by pitavastatin, pravastatin and simvastatin showed that pitavastatin is approximately 3 to 10-fold more potent than simvastatin and approximately 10 to 20-fold more potent than pravastatin.

Table 9. Summary of ED₅₀ values of inhibition of cholesterol synthesis in various organs

	ED ₅₀ mg/kg (relative to the activity in liver)		
	NK-104	pravastatin	simvastatin
Liver	0.13 (1.0)	2.4 (1.0)	0.36 (1.0)
Ileum	0.19 (1.5)	4.5 (1.9)	2.3 (6.4)
Brain	>3 (>23.1)	>30 (>12.5)	>10 (>27.8)
Kidney	0.64 (4.9)	>30 (>12.5)	2.5 (6.9)
Spleen	0.65 (5.0)	>30 (>12.5)	2.3 (6.4)
Adrenal	0.84 (6.5)	>30 (>12.5)	2.4 (6.7)
Testis	>3 (>23.1)	>30 (>12.5)	>10 (>27.8)
Skeletal muscle	0.28 (2.2)	16.2 (6.6)	0.95 (2.6)

n=4~6

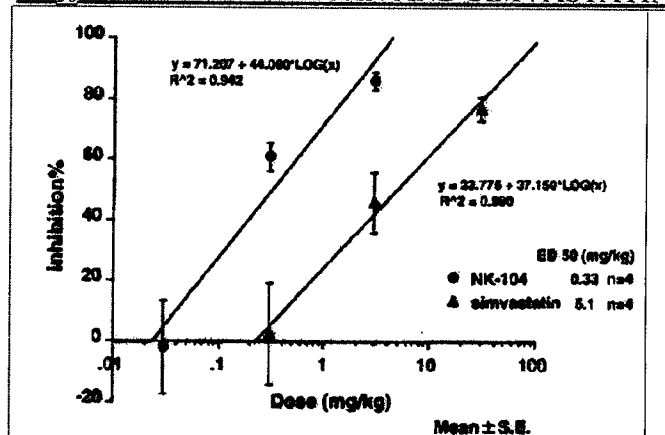
(Sponsor, M4, R95078, p21)

R98008 – Effects of NK-104 on hepatic sterol synthesis in guinea pigs**Key Study Note:**

- Pitavastatin does not lower serum cholesterol in rats

The Sponsor investigated the effect of NK-104 on sterol synthesis in an alternative species in comparison to simvastatin, in the guinea pig.

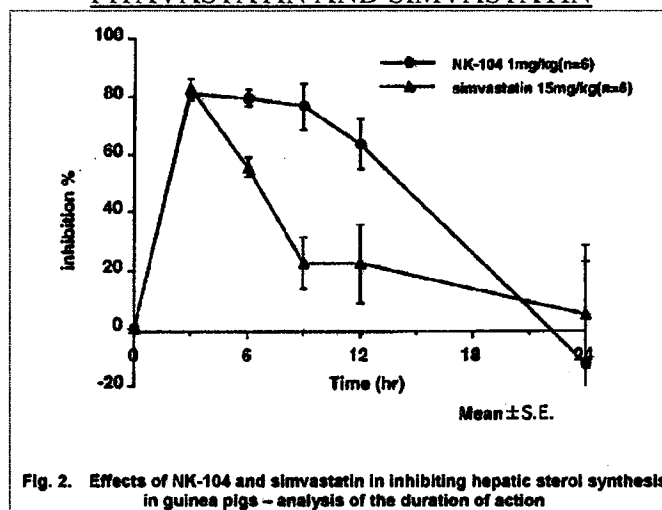
For the ED₅₀ calculation, doses for both statins were 0 (0.05% carboxymethylcellulose), 0.03, 0.3, or 3 mg/kg (4 guinea pigs/group). [¹⁴C]-Radiolabeled acetate was used to track sterol production. The ED₅₀ for pitavastatin was 0.33 mg/kg and for simvastatin was 5.1 mg/kg.

ED₅₀S FOR PITAVASTATIN AND SIMVASTATIN**Fig. 1. Effects of NK-104 and simvastatin in inhibiting hepatic sterol synthesis in guinea pigs – analysis of dose-response relationship**

(Sponsor, M4, R98008, p10)

For the duration of action calculations, doses were 1 mg/kg for pitavastatin and 15 mg/kg for simvastatin. Livers were excised at 3, 6, 9, 12 and 24 hours. At the doses utilized, the exposure (AUC) to NK-104 was greater than for simvastatin.

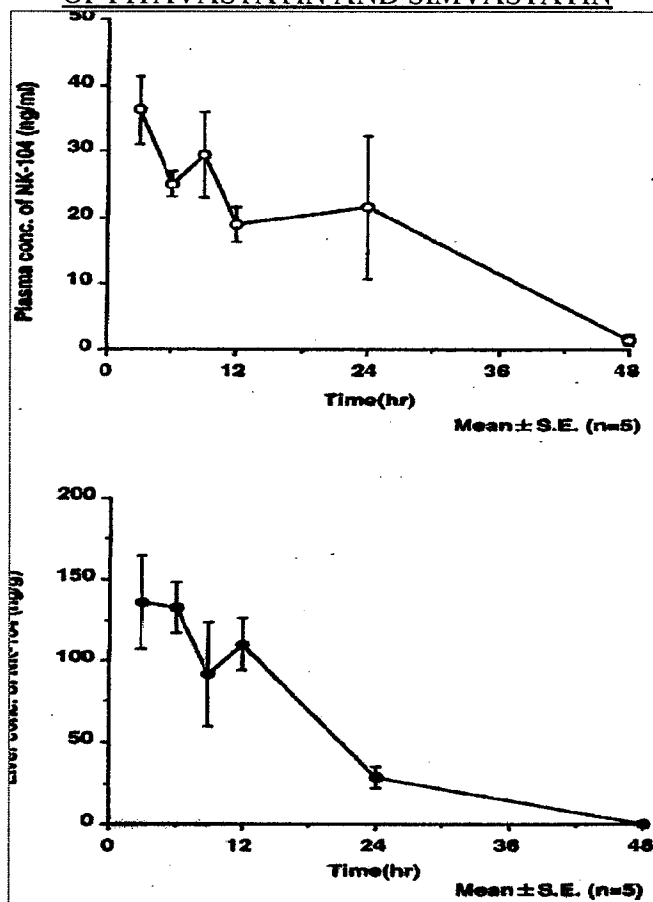
DURATION OF ACTION FOR
PITAVASTATIN AND SIMVASTATIN



(Sponsor, M4, R98008, p11)

Time-course data were collected with 1 mg/kg pitavastatin, at 3, 6, 9, 12, 24, and 48 hours for liver and plasma concentrations. The concentration of pitavastatin in liver was on the order of 2 to 4 times greater than that in plasma, and this relationship was basically constant in guinea pigs over a 24 hour time period.

**TIME-COURSE FOR LIVER AND PLASMA LEVELS
OF PITAVASTATIN AND SIMVASTATIN**



(Sponsor, M4, R98008, p13)

99058 – Measurement of NK-104 concentration in plasma after single oral administration to guinea pigs

Key Study Findings:

- The liver extraction rate (ratio of liver to plasma concentration) was ~4.1 in guinea pigs, compared to ~75 in rats and ~17.5 in rabbits.

This study examined the pharmacokinetics of NK-104 in guinea pigs. Concentrations of parent and lactone were measured for liver and plasma. Plasma T_{max} was 3 hours. $T_{1/2}$ was ~10.2 hrs for parent, while NK-104 was below the LLQ for all time points in all samples. T_{max} in liver was 3 hours and $T_{1/2}$ was ~9.1 hours. The lactone was observed in a subset of animals and thus T_{max} and $T_{1/2}$ were not able to be calculated.

R98022 – Plasma lipid lowering effect of NK-104 in guinea pigs

Plasma lipids (total cholesterol, triglycerides, and phospholipids) and ApoB concentrations, and liver lipids (triglycerides, total cholesterol, free cholesterol and phospholipids) were quantified for guinea pigs administered NK-104 at 0.03, 0.1, 0.3,

and 3 mg/kg or simvastatin at 0.3, 1, 3, 10 and 30 mg/kg for after two weeks of daily administration by oral gavage. The Sponsor utilized doses based on 1/3 the LOAEL from unspecified experiments.

In plasma, 3 mg/kg pitavastatin decreased total cholesterol by ~45%, triglycerides by ~32%, and phospholipids by ~35%. The cholesterol contents of VLDL, LDL, and HDL were decreased ~48, 49 and 46%, respectively, at the 3 mg/kg dose. The triglyceride content of VLDL was decreased ~44% at 3 mg/kg. Phospholipid content was decreased in VLDL, LDL, and HDL by ~44, 41, and 26%, respectively. VLDL-ApoB and LDL-ApoB were decreased ~58 and 46% respectively.

In liver, total cholesterol was decreased ~8% and cholesterol ester was decreased ~37% at the 3 mg/kg pitavastatin dose in guinea pigs. Free cholesterol, triglycerides and phospholipids were not decreased in liver.

99041 – Plasma lipid lowering effect of NK-104 in guinea pigs - Effect of pravastatin

-

This study did not utilize pitavastatin. A potential competitor compound was investigated in guinea pigs.

99042 – Plasma lipid lowering effect of NK-104 in guinea pigs - Effect of fluvastatin

-

This study did not utilize pitavastatin. A potential competitor compound was investigated in guinea pigs.

99043 – Plasma lipid lowering effect of NK-104 in guinea pigs - Effect of cerivastatin

-

This study did not utilize pitavastatin. A potential competitor compound was investigated in guinea pigs.

99044 - Plasma lipid lowering effect of NK-104 in guinea pigs - Effect of atorvastatin

-

This study did not utilize pitavastatin. A potential competitor compound was investigated in guinea pigs.

R94012 – Plasma lipid lowering effect of NK-104 in dogs

NK-104 was administered to dogs for two weeks by once-daily oral, along with pravastatin and simvastatin to examine the potency of pitavastatin versus potential competitor compounds. NK-104 showed pharmacodynamic activity at 1 mg/kg/day in dogs.

94053 - Hypolipidemic effect of NK-104 in Zucker rat plasma**Key Study Notes/Findings:**

- Pitavastatin does not lower serum cholesterol in rats (including Zucker rats).

Zucker homozygous rats contain two copies of a recessive (spontaneous mutant) allele specifying the leptin receptor (used as a model for obesity and hypertriglyceridemia).

Pitavastatin was administered (0.1, 0.3, and 1 mg/kg/day) for one week by oral gavage (n=5/group) and the effects on total cholesterol, triglyceride, phospholipid, free fatty acid and glucose in homozygous animals were measured. There were no statistically significant changes in any plasma lipid, though triglycerides were decreased ~28% in the 1 mg/kg/day group.

A dose was chosen (1 mg/kg/day) from the previous experiment for daily administration by oral gavage for two weeks (n=10). Total cholesterol, triglyceride and phospholipid in plasma were measured. Plasma triglycerides were decreased ~18% (p<0.05), but cholesterol and phospholipids were not different.

Two lower doses were chosen (0.03 and 0.1 mg/kg/day) for daily administration by oral gavage for two weeks (n=6/group). Total cholesterol, triglyceride and phospholipid in plasma were measured. There were no changes in any value at the lower two doses.

VLDL-TG secretion rate was measured (n=9/group) for a control and 1 mg/kg/day NK-104 groups, after once daily administration for two weeks. The VLDL-TG secretion rate was ~21% lower at 1 mg/kg/day than in vehicle-only controls.

LPL activity was measured in plasma, adipose tissue, and skeletal muscle tissues after two weeks once-daily administration (n=4/group) at 1 mg/kg/day NK-104. LPL activity at 1 mg/kg/day was not affected in plasma or tissues tested.

Zucker rats do not show a serum cholesterol lowering effect from NK-104, which is similar to other rat strains. Only triglycerides were affected in Zucker rats.

98052 - Effect of NK-104 on postprandial hyperlipidemia in rats - Comparison with atorvastatin -

This study utilized pitavastatin in comparison to atorvastatin to study the effects of postprandial hyperlipidemia in rats after a single oral dose. The doses were determined based on ED₅₀ for each compound. The ED₅₀ (0.24 mg/kg) for atorvastatin was determined in this study (the fit to the dose response curve appeared very poor), while the ED₅₀ for pitavastatin was determined in study R95078. The ED₅₀ for pitavastatin was determined to be 0.13 mg/kg, and the fit for this curve appeared quite good. No comparison of atorvastatin and pitavastatin based could be made due to a poorly fitting ED₅₀ curve for atorvastatin.

99031 - Hypolipidemic effect of NK-104 in hyperlipidemic guinea pigs fed on laurate-rich diet

Atorvastatin (3, 10 or 30 mg/kg), pitavastatin (0.3 or 1 mg/kg), or vehicle (0.5 carboxymethylcellulose) were administered to hyperlipidemic guinea pigs (n=5/group) fed high lipid content (15% palm-kernel oil and 0.04% cholesterol) for two weeks. A normal group was also utilized (n=6), which was fed a standard diet. LDL was labeled with DiI (1,1-dioctadecyl-3,3,3',3'-tetramethyl indodicarbocyanine perchlorate). After feeding of the experimental diet for 4 weeks, test substances were administered for 2 weeks. At the end of the administration period, a baseline blood draw was obtained. DiI was injected (1 mg LDL protein/kg) and blood was drawn at 2 min, and 1, 3, 6, 9 and 24 hours post DiI-administration. The 30 mg/kg/day atorvastatin group was halted due to decreased weight gain beginning ~4 days after initiation of test substance regimen.

DiI fluorescence was measured in all samples. Increasing doses of pitavastatin and atorvastatin tended to lead to decreased LDL cholesterol and ApoB pools and flux, increased the functional catabolic rate and lowered the DiI $T_{1/2}$.

Total cholesterol, triglyceride and phospholipids were measured. Atorvastatin and pitavastatin tended to lower total cholesterol, triglycerides and phospholipids compared to control guinea pigs.

Cholesterol, triglyceride, and phospholipid content of VLDL, LDL, and HDL were measured. The cholesterol component of LDL was decreased for both drugs, as was the phospholipid component of LDL; however, the triglyceride components were not different from controls.

The ApoB component of plasma, LDL, and VLDL, and liver lipids (total cholesterol, cholesterol esters, triglycerides, and phospholipids) were measured. ApoB was decreased in plasma and LDL, but not in VLDL for both drugs. There were no statistically significant effects of NK-104 or atorvastatin on total cholesterol, cholesterol esters, triglycerides, and phospholipids in liver lipids.

R98011 - Hypolipidemic effect of NK-104 in WHHL rabbits (2): No. 2. Effects on plasma lipids and atherosclerosis**Key Study Notes:**

- In the WHHL rabbit, an established model of hyperlipidemia and atherosclerosis resulting from LDL-R deficiency, pitavastatin 0.5 mg/kg/day in drinking water for 26 weeks lowered plasma cholesterol and TG and significantly lowered VLDL-cholesterol and IDL-cholesterol but did not affect cholesterol in LDL.
- Total plasma cholesterol was reduced by up to 15.6 % ($P<0.05$) compared to control WHHL rabbits.
- Plasma triglycerides were reduced by up to 35.2% ($p<0.05$) compared to controls.
- Plasma phospholipids were decreased by 26.9% ($P<0.01$) compared to controls.
- Cholesterol ester content of aortic regions was not different from controls.

- The relative lipid-deposition areas, intimal thicknesses, and intimal/medial area ratios in the aortic arch, thoracic aorta and abdominal aorta were decreased (n.s.s.) compared to controls.
- There appeared to be benefits for left coronary arterial stenosis (lower rates, n.s.s.)

In this study, suppression of progress of atherosclerosis was observed in a WHHL rabbit model. WHHL rabbits were administered pitavastatin at 0.5 mg/kg/day in drinking water for 26 weeks, between ages 3 and 10 months. The effects on lipids were examined and atherosclerotic lesions were evaluated histopathologically.

R97034 – Hypolipidemic effect of NK-104 in WHHL rabbits (2): 1. Concentration of NK-104 in plasma, liver and lens after repeated oral administration of NK-104 with drinking water for six months to WHHL rabbits

Key study findings:

- The plasma steady state level of pitavastatin was 40.3 ng/ml, and lactone levels were below the limit of quantitation.
- The pitavastatin liver concentration was 618.6 ng/g and liver concentrations of lactone were 78.8 ng/g.

Hypolipemic effects of NK-104 in WHHL rabbits (model of hyperlipemic atherosclerosis) given in drinking water for 6 months concentration in plasma, liver and lens. These rabbits lack LDL receptors.

Table 1. NK-104 and NK-104 lactone concentration of plasma, liver and eye lens after repeated oral administration of NK-104 with drinking water for 26 weeks at a dose of 0.5 mg/kg day to WHHL rabbits											
Rabbit No.	Drug	Plasma conc. (ng/ml)						Liver conc (ng/g) Week-26	Lens conc (ng/g) Week-26	Liver/Cas	Lens/Cas
		9:00am	1:00pm	3:00pm	9:00am	1:00pm	3:00pm				
7	NK-104	58.2	25.3	79.6	37.1	40.1	63.2	47.2	473.3	2.6	10.0
	Lactone	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	-	69.9	n.d. ^a	-
8	NK-104	43.1	49.4	48.4	38.7	31.9	50.2	43.3	876.0	2.3	20.1
	Lactone	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	-	109.7	n.d. ^a	-
9	NK-104	28.3	23.1	25.3	34.1	22.6	41.7	28.4	393.4	n.d. ^a	13.9
	Lactone	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	-	65.9	n.d. ^a	-
10	NK-104	81.2	48.6	50.6	50.2	39.7	48.3	53.1	720.0	2.6	13.6
	Lactone	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	-	85.1	n.d. ^a	-
11	NK-104	28.4	34.9	43.7	28.8	31.9	42.8	35.4	883.3	n.d. ^a	24.9
	Lactone	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	-	84.2	n.d. ^a	-
12	NK-104	31.3	23.2	37.8	24.8	27.6	33.2	34.0	343.2	n.d. ^a	10.7
	Lactone	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a	-	67.0	n.d. ^a	-
Mean	NK-104	41.8	33.1	47.3	34.6	32.3	50.2	40.3	618.6	1.3	13.3
S.E.		8.2	4.8	7.3	3.8	2.8	3.3	3.8	97.1	0.6	2.4
Mean	Lactone	-	-	-	-	-	-	-	78.8	-	-
S.E.		-	-	-	-	-	-	-	5.6	-	-

a), Below limit of quantification (<Ca. 5ng/ml plasma), b), Below limit of quantification (<Ca. 2.5ng/g tissue).

Table 2. Intra-day precision and accuracy measurements of NK-104 and NK-104 lactone in liver homogenate of WHHL rabbit								
Drug	Conc. spiked (ng/0.5 g)	Conc. found (ng/0.5 g, n = 5)					C.V. (%)	MRE (%)
		5.0	50.0	500.0	5000.0	50000.0		
NK-104	5	5.2	5.0	4.7	5.0	5.0	3.8	-0.4
	50	50.1	50.9	50.2	51.7	49.6	50.5	1.0
	500	496.2	494.6	499.7	508.2	503.6	498.9	0.7
	5000	49.9	49.4	48.9	49.5	49.9	49.3	-0.2
NK-104L	5	4.9	4.9	4.6	4.8	4.9	4.8	-4.4
	50	49.9	49.4	48.9	49.5	49.9	49.3	0.8
	500	492.8	493.7	493.6	492.9	493.1	493.6	0.6
	5000	-	-	-	-	-	-	-1.3

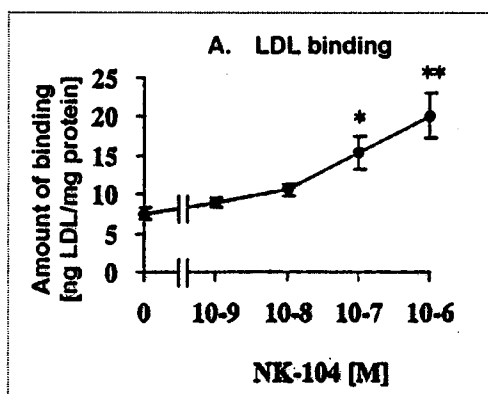
MRE, Mean relative error.

(Sponsor, M4, R97034, p7)

R99045 – Effect of NK-104 on lipid metabolism in HepG2 cells: Inhibition of cholesterol synthesis and enhancement of LDL receptor activity

Key study findings:

- NK-104 enhanced LDL-receptor binding capacity in a concentration-dependent manner when incubated with HepG2 cells by ~2.7-fold.



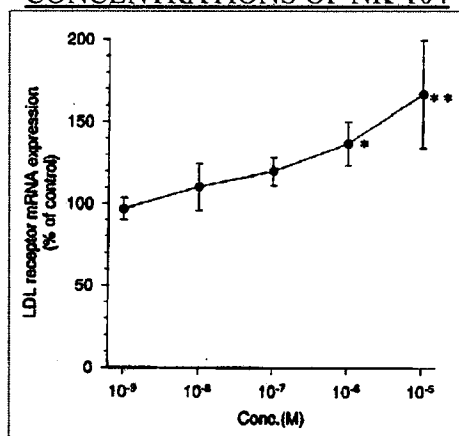
(Sponsor, M4, R99045, p13)

99037 - LDL-receptor inducing effect of NK-104 in HepG2 cells at the mRNA levels**Key Study Findings:**

- There was a statistically significant increase in LDL receptor mRNA at 1 to 10 μ M.
- Pitavastatin is similar to the other statins, several of which have been demonstrated to increase the level of LDL-receptors in the livers of a variety of mammalian species.

This study was carried out to determine if NK-104 was producing any pharmacodynamic effects on mRNA specifying genes relevant to cholesterol metabolism. NK-104 was applied to HepG2 cells in culture. Northern blotting was carried out for the LDL-receptor mRNA.

LDL RECEPTOR mRNA
EXPRESSION WITH INCREASING
CONCENTRATIONS OF NK-104



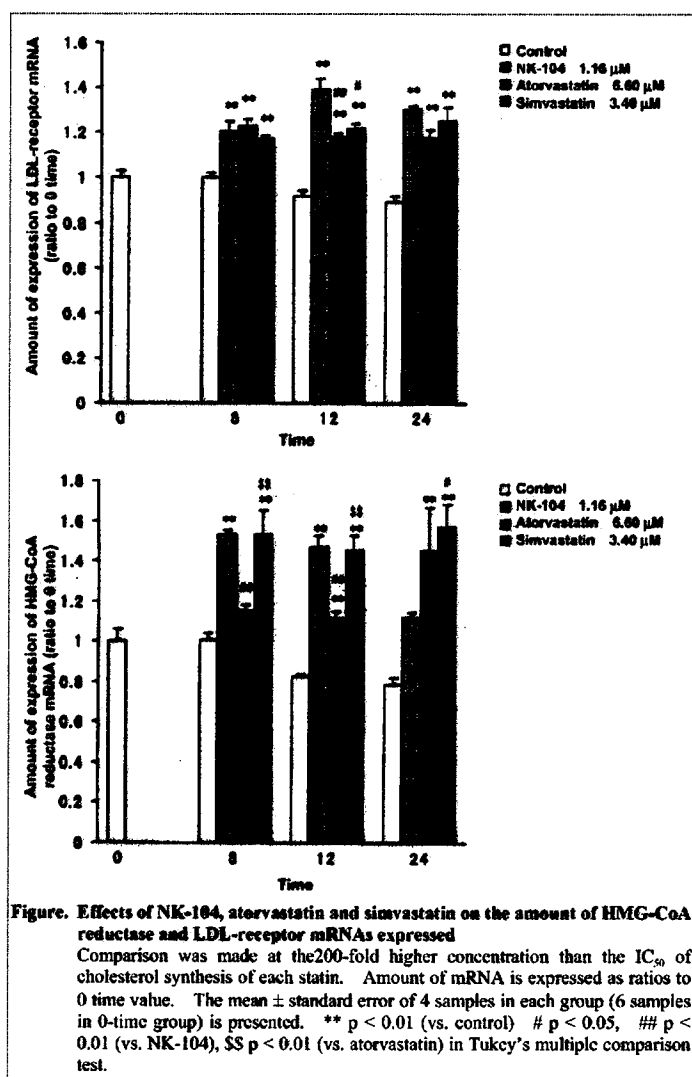
(Sponsor, M4, R99037, p10)

E-18 - Effects of NK-104 on HMG-CoA reductase and LDL-receptor mRNA expression in HepG2 cells

Key Study Findings:

- Pitavastatin, atorvastatin, and simvastatin caused increased LDL-receptor and HMG-CoA reductase mRNA expression

NK-104, atorvastatin, and simvastatin were applied to HepG2 cells. The time-course of mRNA expression for HMG-CoA reductase and LDL-receptor were monitored using the ribonuclease protection assay. All three statins caused increased LDL-receptor and HMG-CoA reductase mRNA expression for up to 24 hours, except that NK-104 did not induce HMG-CoA reductase mRNA at the 24 hour time-point.



(Sponsor, M4, E-18, p10)

R98021 – Effects of NK-104 on expression of hepatic LDL-receptor in guinea pigs**Key Study Findings:**

- NK-104 enhanced LDL-receptor binding capacity in the liver plasma membrane fraction obtained from guinea pigs treated with NK-104 at 3 mg/kg for 14 days.

Guinea pigs (n=6/group) were utilized to determine the effect of simvastatin (30 mg/kg/day) and NK-104 (3 mg/kg/day) on hepatic LDL receptor activity versus vehicle-only (0.5% carboxymethylcellulose) controls upon daily repeat-dose administration by oral gavage for 14 days. The dose-response relationship with NK-104 was calculated using data from guinea pigs (n=8/group) administered 0.03, 0.3, and 3 mg/kg/day once daily for 14 days. NK-104 produced dose-dependent decreases (see figure, below) in cholesterol (total, LDL, and VLDL), triglycerides (plasma and VLDL), and ApoB (plasma and LDL). Low-density lipoprotein was fractionated from whole blood; total cholesterol and triglycerides were measured. Apolipoprotein B was quantified in the LDL fraction by ELISA.

**PHARMACODYNAMIC EFFECT OF PITAVASTATIN
ADMINISTERED TO GUINEA PIGS**

		NK-104 (mg/kg)			
		control	0.03	0.3	3
	n	8	8	8	8
TC	mg/dl	32.7±2.8	28.5±1.4 (12.8)	21.9±3.0 (33.0) **	13.8±1.7 (57.8) **
LDL-C	mg/dl	27.1±2.6	23.3±1.4 (14.0)	17.6±2.6 (35.1) **	10.7±1.6 (60.5) **
VLDL-C	mg/dl	1.3±0.1	1.3±0.2 (0.0)	1.2±0.2 (7.7)	0.3±0.1 (78.9) **
TG	mg/dl	44.9±1.8	45.8±3.2 (-2.0)	42.1±5.2 (8.2)	32.1±2.8 (28.5) *
VLDL-TG	mg/dl	28.2±2.1	30.5±3.7 (-8.2)	27.3±5.0 (3.2)	16.8±2.6 (40.4)
Plasma-apoB	mg/dl	59.8±6.7	56.8±4.1 (4.7)	48.2±7.2 (17.4)	31.7±3.8 (46.8) **
LDL-apoB	mg/dl	51.1±3.9	45.1±3.3 (11.7)	34.3±4.8 (32.9) *	21.6±3.4 (57.7) **

mean ± SE, *p < 0.05, **p < 0.01 Dunnett, vs control. () = percent reduction from the control group

(Sponsor, M4, R98021, p13)

LDL was collected from fasted guinea pigs by fractionation; LDL was labeled with ¹²⁵I. Cell membranes were obtained from the livers of treated animals by homogenization and fractionation.

LDL receptor-binding was measured by combining liver cell membranes with ¹²⁵I-labeled LDL. After incubation of one hour at 0 °C, the membrane fraction was re-isolated and washed. Radioactivity in the sediment was measured. Differential binding (+ or – EDTA) was used to calculate the non-calcium-dependent binding potential (non-LDL receptor bound radiolabeled LDL). NK-104 produced dose-dependent increases in the LDL receptor binding capacity in livers of guinea pigs administered 0.03 to 3 mg/kg/day (p<0.05 at 3 mg/kg NK-104).

**LDL-RECEPTOR-BINDING CAPACITIES IN GUINEA
PIGS ADMINISTERED PITAVASTATIN**

		¹²⁵ I-LDL binding amount	
		ng LDL protein/mg membrane protein	
	n	Ca ²⁺ -dependent	Non-Ca ²⁺ -dependent
control	8	172.3±31.4	152.9±21.6
NK-104 0.03mg/kg	8	205.1±27.2	174.9±22.5
0.3mg/kg	8	227.9±40.6	210.2±29.4
3mg/kg	8	290.2±31.0 *	158.0±22.9
mean±S.E., *p<0.05 Dunnett, vs. control			

(Sponsor, M4, R98021, p14)

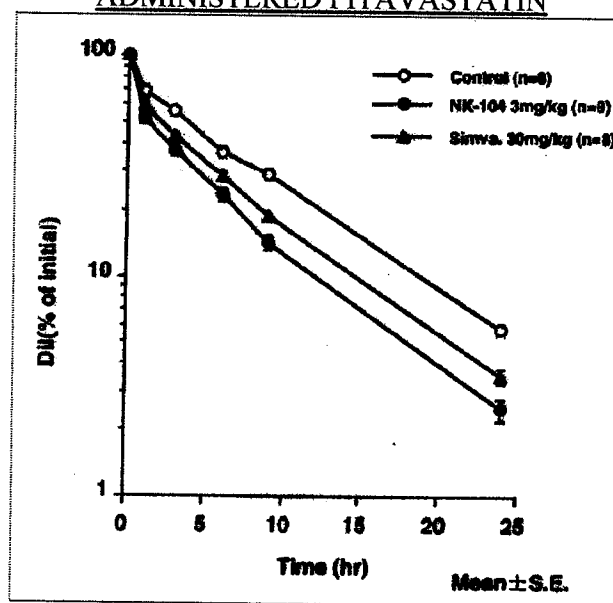
97023 – Effects of NK-104 on LDL clearance in guinea pigs

Key Study Findings:

- LDL clearance was enhanced by daily administration of NK-104 (3 mg/kg) or simvastatin (30 mg/kg) for 14 days by oral gavage.

LDL was prepared from animals administered NK-104 (3 mg/kg/day, n=16), simvastatin (30 mg/kg/day, n=16) or 0.5% carboxymethylcellulose (n=18) for 14 days. The LDL fractions for each group were pooled after collection. Total cholesterol and ApoB levels were quantified. LDL samples were labeled with two distinct fluorophore.

**LDL CLEARANCE IN GUINEA PIGS
ADMINISTERED PITAVASTATIN**



(Sponsor, M4, R97023, p13)

Guinea pigs were administered NK-104 (3 mg/kg/day, n=8), simvastatin (30 mg/kg/day, n=8) or 0.5% carboxymethylcellulose (n=8) for 14 days. On Day 14 animals were fasted

for 18 hours and plasma samples were analyzed for lipids and ApoB concentrations. The previously labeled LDL corresponding to each group was administered intravenously. Blood samples were obtained at 2 minutes, 1, 3, 6, 9, and 24 hours. Fluorescence intensity was determined in plasma. Total cholesterol and ApoB levels were measured and the rate of clearance of LDL was calculated.

**PHARMACODYNAMIC EFFECT OF ADMINISTRATION
OF PITAVASTATIN IN GUINEA PIGS**

	Control	NK-104 3mg/kg	Simvastatin 30mg/kg
n	8	8	8
Total Cho. (mg/dl)	37.1 ± 2.7 (100)	20.3 ± 2.3 (55) **	24.7 ± 1.8 (67) **
LDL-Cho. (mg/dl)	25.5 ± 1.7 (100)	14.4 ± 1.5 (56) **	18.9 ± 1.5 (74) *
LDL apoB (mg/dl)	37.6 ± 2.6 (100)	21.9 ± 2.9 (58) **	29.6 ± 2.5 (79)
LDL-C pool (mg)	5.38 ± 0.37 (100)	2.97 ± 0.31 (55) **	3.82 ± 0.30 (71) **
LDL apoB pool (mg)	7.94 ± 0.58 (100)	4.49 ± 0.59 (57) **	5.98 ± 0.54 (75) *
FCR (pools/hr)	0.137 ± 0.009 (100)	0.244 ± 0.018 (178) **	0.194 ± 0.006 (142) *
1/FCR (hr)	7.54 ± 0.57 (100)	4.23 ± 0.27 (56) **	5.19 ± 0.14 (69) *
LDL-C flux (mg/hr)	0.74 ± 0.08 (100)	0.70 ± 0.05 (95)	0.73 ± 0.05 (99)
LDL apoB flux (mg/hr)	1.09 ± 0.12 (100)	1.06 ± 0.11 (97)	1.15 ± 0.09 (106)
t _{1/2} (hr)	6.52 ± 0.12 (100)	5.46 ± 0.19 (84) **	5.89 ± 0.18 (90) *

(): % of control Mean ± S.E. *p<0.05, **p<0.01 Dunnett

(Sponsor, M4, R97023, p15)

Yanagita T, Hara E, Yotsumoto H, Rahman SM, Han S-Y, Cha J-Y *et al.* NK-104, a potent new 3-hydroxy-3-methylglutaryl Coenzyme A reductase inhibitor, enhances posttranslational catabolism of apolipoprotein B-100 and inhibits secretion of apolipoprotein B-100 and triacylglycerols from HepG2 cells; Current Therapeutic Research 1999; 60: 423-434

Key Study Findings:

- NK-104 acted to decrease cholesterol production in HepG2 cells and cholesterol secretion into the medium after 3 and 24 hours.
- Secretion of apolipoprotein B-100 into the medium was decreased with 10 µM NK-104 after 3 and 24 hours.

Cholesterol esters were decreased in cells and medium after 24 hours, but not after 3 hours. Triacylglycerol incorporation into HepG2 cells from radiolabeled glycerol was unchanged, but secretion of radiolabeled triacylglycerol into the medium was decreased ~35%. Secretion of apolipoprotein B-100 into the medium was decreased with 10 µM NK-104 after 3 and 24 hours. This was shown by pulse-labeling of apolipoprotein B-100 with radiolabeled methionine that indicated greater degradation of ApoB-100 in NK-104-treated cells compared to controls. This increase in degradation of ApoB-100 led to a significant decrease in secretion into the medium.

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(Sponsor, M4, Yanagita, T., et al, p8)

97057 - Effect of NK-104 on VLDL secretion in guinea pig liver

Livers from guinea pigs administered NK-104 (0.03, 0.3, or 3 mg/kg) or simvastatin (30 mg/kg) for 14 days (n=14/group) were perfused for 4 hours. Lipid and ApoB secretion was measured in liver perfusate. Triglyceride secretion in VLDL was reduced by ~20% in NK-104 treated animals at 3 mg/kg, but not in simvastatin treated animals at 30 mg/kg.

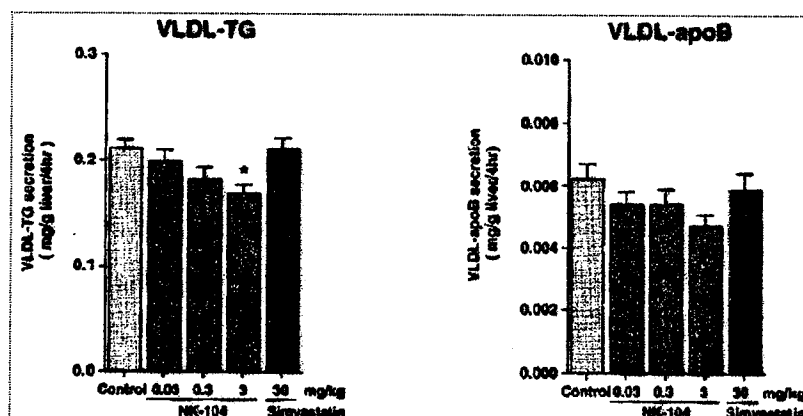


Fig. 7. Effects of NK-104 and simvastatin on levels of triglyceride and apolipoprotein B in perfusate VLDL after 4 hour liver perfusion.
Each value represents the mean $\pm$ S.E. of 14 guinea pigs.
* $p < 0.05$, significantly different from control (Dunnnett test).

(Sponsor, M4, 97057, p20)

E-20 - NK-104 suppresses oxidized LDL-induced foam cell formation in RAW264.7 macrophages by inhibiting HMG-CoA reductase

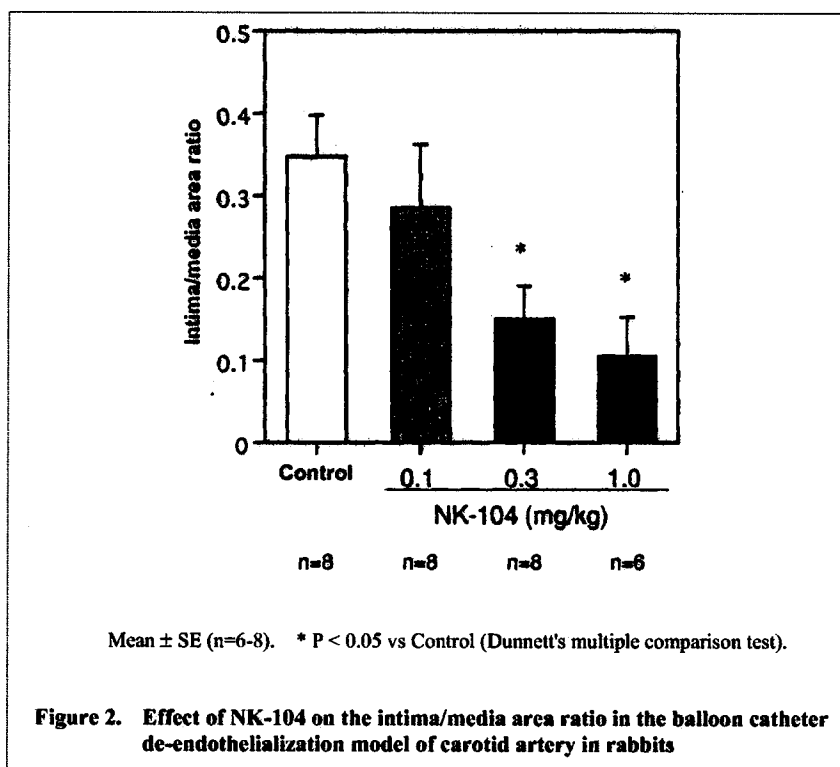
NK-104, atorvastatin, and simvastatin decrease the production of total cholesterol, free cholesterol and cholesterol esters in RAW264.7 cells induced by oxidized LDL. Addition of mevalonolactone and to a lesser extent, farnesol, led to loss of inhibition of

cholesterol synthesis. Geranylgeraniol addition did not lead to loss of inhibition by NK-104.

E-21 – NK-104 suppresses neointimal thickening and its fibronectin accumulation in balloon-injured rabbit carotid artery

Key study findings:

- In a rabbit model of carotid artery injury, pitavastatin decreased post-trauma thickening of the vascular smooth muscle tissue by 56.8-69.5% at doses of 0.3 and 1.0 mg/kg in rabbits.



(Sponsor, M4, E-21, p14)

Suzuki H, Kobayashi H, Sato F, Yonemitsu Y, Nakashima Y, Sueishi K. Plaque-stabilizing effect of pitavastatin in Watanabe Heritable Hyperlipidaemic (WHHL) rabbits; J Atheroscler Thromb 2003; 10: 109-116

This publication contains the results WHHL rabbit studies with pitavastatin that show similar to findings to those studies already reviewed.

R99032 – Inhibitory effect of NK-104-related compounds on HMG-CoA reductase

This report examined the inhibitory effect of the four optical isomers of NK-104: NK-104, two (b) (4) and an (b) (4) form on HMG-CoA reductase activity in rat liver microsomes. IC₅₀ values were reported. These results show that both (b) (4) and the (b) (4) were weak inhibitors of HMG-CoA reductase, while the 8-OH-NK-104 form is more potent than parent NK-104.

Drugs	Inhibitory effect on HMG-CoA reductase IC ₅₀ value (nM)
NK-104 8-hydroxy form	3.5 [1.6]
(b) (4)	(b) (4)
(b) (4)	(b) (4)
NK-104	5.5 [1]

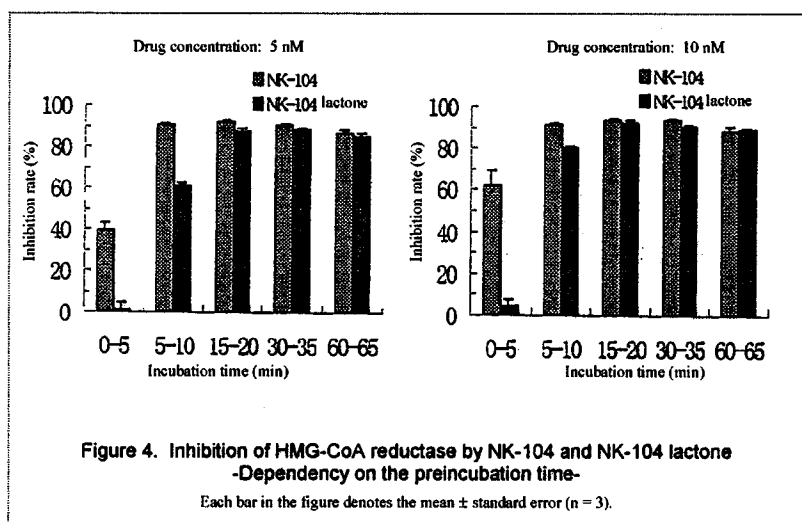
(Sponsor, M4, R99032, p9)

R101030 – Effect of NK-104 lactone on HMG-CoA reductase activity**Key Study Finding:**

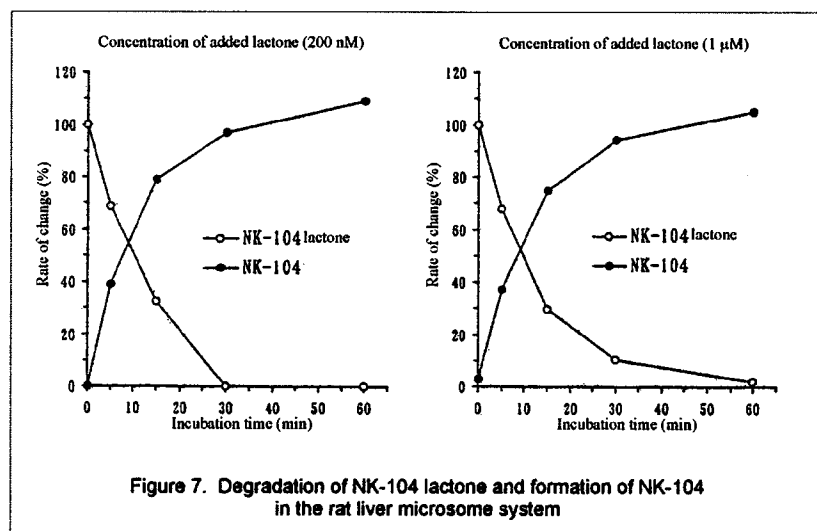
- Study indicates, albeit qualitatively, that the lactone lacks significant inhibitory activity against HMG-CoA reductase.

This issue was addressed in studies using rat liver microsomes, which presumably contain all the necessary components for production of NK-104-glucuronide and subsequent loss of the glucuronide with formation of the NK-104 lactone, and production of NK-104 from the lactone.

Sponsor shows here that inhibition of HMG-CoA reductase is not present when only the lactone is present. As parent NK-104 is formed during the incubation, inhibition of HMG Co-A reductase increases. Since the amount of lactone is only greater than NK-104 at times 0 and 5 minutes and there is no (or very little) apparent inhibition of HMG-Co-A reductase at this time point, the Sponsor has provided a reasonable argument that the lactone is not active, though not definitively.



(Sponsor, M4, R101030, p29)



(Sponsor, M4, R101030, p32)

RF9118 – Pharmacological study on NK-104: Hypolipidemic effect of NK-104 orally administered to dogs for 14 days

Dogs were administered pitavastatin lactone, pitavastatin and a racemic mixture of pitavastatin lactone. Since the lactone and parent pitavastatin are ultimately interconvertible, it is unclear as to the significance of these findings.

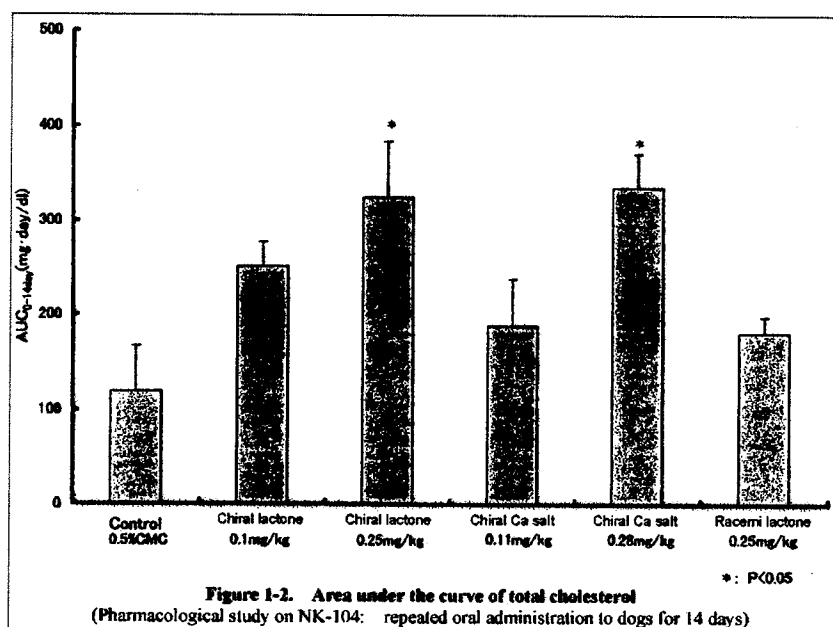
DOSE GROUP ASSIGNMENTS

Group No.	Drug name	Dose mg/kg		Concentration mg/ml	Dose volume ml/kg	Capsule		Number of animals
		Per day	Per dosing			No.	Size	
1	0.5% CMC	0	0	0	0.05	1	00	3 ^{b)}
2	Chiral lactone	0.1	0.05	2.5	0.02	1	00	4
3	Chiral lactone	0.25	0.125	2.5	0.05	1	00	4
4	Chiral Ca salt	0.11	0.056 ^{a)}	2.5	0.02	1	00	4
5	Chiral Ca salt	0.28	0.141 ^{a)}	2.5	0.05	1	00	4
6	Racemi lactone	0.25	0.125	2.5	0.05	1	00	4

a) Doses of chiral Ca salt were set to 0.141 and 0.056 mg/kg twice daily because of correction for molecular weight and water content.

b) In the examination on 2 days before the start of administration and the day of administration, 1 animal in Group 1 (control group) showed an abnormal rise in total cholesterol, and was excluded from the main study. Therefore, the number of animals in Group 1 became 3.

(Sponsor, M4, RF9118, p5)



(Sponsor, M4, RF9118, p22)

2.6.2.3 Secondary pharmacodynamics

R99028 – Effects of NK-104 on biliary lipids in hamsters

Hamsters were administered 0.3 and 1.0 mg/kg NK-104 orally, along with a vehicle-only control group. Hamster total cholesterol levels were not responsive to NK-104, but did show lower triglyceride levels, similar to rats. In the liver, cholesterol concentration was not different between NK-104 groups and vehicle controls, but bile acids were decreased in concentration by 12% and 23% at 0.3 and 1.0 mg/kg respectively.

R98051 – Effects of NK-104 on biliary lipids in guinea pigs

Guinea pigs were administered a vehicle-only control or 3 mg/kg NK-104 orally for 15 days repeat daily-dose and biliary lipids were observed. Total cholesterol in plasma was decreased by 50%, phospholipids were decreased 33%, and triglycerides were decreased 26%, while biliary total cholesterol was decreased 24% and biliary phospholipids and bile acids were not different from vehicle controls.

R101018 – Effect of repeated administration of NK-104 on blood steroid hormones in rats

NK-104 administered to rats (n=8) for 7 days orally at up to 30 mg/kg/day did not alter total cholesterol, testosterone, estrone, or corticosterone levels in plasma.

R101021 – Effect of NK-104 on blood steroid hormones in guinea pigs

Guinea pigs were administered NK-104 for 7 days orally at up to 3 mg/kg. NK-104 lowered cholesterol, and tended to correlate with lower testosterone levels (29% at 3 mg/kg, trend n.s.s.) Estrone levels were not different from controls.

2.6.2.4 Safety pharmacology**Summary:**

Pitavastatin crosses the blood-brain barrier. In mice given ≥ 3 mg/kg (≥ 1.3 -fold MRHD of 4 mg based on $C_{\max}$) there was a transient decrease in spontaneous motor activity in an Irwin test. The effect at 30 mg/kg (13-fold MRHD based on $C_{\max}$) persisted for 60 minutes post-administration. Mice given 10 mg/kg (4.5-fold MRHD based on $C_{\max}$) had a transient decrease in grip strength.

In dogs given 10 mg/kg which provided an exposure ≥ 154 -fold based on $C_{\max}$ comparisons to a human 4 mg dose, a significant treatment related increase in heart rate was seen at 3 and 6 hours post-dose. The ambulatory ECG was unremarkable. The lactone was evaluated in hERG channel and guinea pig papillary muscle assays. The NOAEL for the lactone in the hERG assay was 0.3 μM (~ 9 -fold MRHD $C_{\max}$ estimate). The NOAEL for pitavastatin lactone in the guinea pig papillary muscle was 0.1 μM (3-fold MRHD $C_{\max}$ estimate).

Urinary excretion of sodium and chloride (but potassium was unaffected) was decreased in rats given ≥ 10 mg/kg (50-fold MRHD based on $C_{\max}$).

The maximum human dose is 4 mg/day (2.5 mg/M²) which after 14-days dosing in male subjects corresponds to a $C_{\max} = 55$ ng/ml and $\text{AUC}_{0-24} = 153$ ng·h/ml with a $T_{\max}$ of 9 hours. The lactone form of pitavastatin is the major human metabolite. Pitavastatin and its subsequent lactone exist in dynamic equilibrium i.e. they interconvert *in vivo*. The lactone is also formed in dog and monkey. Based on AUC comparison approximately 0.5% (percent of pitavastatin parent concentration) exists as 8-hydroxypitavastatin. The 8-hydroxypitavastatin is formed in humans and monkeys.

Pitavastatin is pharmacologically active (inhibits HMG-CoA reductase) in mouse, rat, guinea pig, rabbit, dog and cynomolgus monkey. Therefore these test species were used in a variety of pharmacokinetic evaluations. Validated methods for the determination of pitavastatin (3R, 5S) and well as its^{(b) (4)} and pitavastatin metabolites i.e. lactone (inactive glucuronide), 5-ketone^{(b) (4)} 8-hydroxypitavastatin (M-13, active at target) and others (M-4 to M-11) have been studied. The^{(b) (4)} have shown HMG-CoA reductase inhibitory effects *in vitro* but at concentrations more than 100-fold higher than pitavastatin suggesting weak inhibitory activity. 8-hydroxypitavastatin shows an inhibitory effect at 1.5-fold lower concentration than the parent suggesting greater pharmacologic activity than the parent. The activity of the 5-ketone has not been specified.

SR-9213 – NK-104 general pharmacology study (1)

Key Study Findings:

- Pitavastatin was administered at 3, 10, and 30 mg/kg p.o. in 0.5% CMC-Na (6-10 animals/group) in mice
- General signs and behavioral effects within 3 hours post-administration
 - Decreased motor activity at 30 mg/kg NK-104 (decreased motor activity at 3, 10 mg/kg after 4 hours, n.s.s.), which is 36x MRHD by body surface area
 - Decreased alertness, grooming at 30 mg/kg NK-104
- Central nervous system effects within 3 hours post-administration
 - Decreased grip strength at ≥ 10 mg/kg/day NK-104, at 12x MRHD by body surface area
 - Decreased pinna reflex at 30 mg/kg NK-104 at 36x MRHD by body surface area
 - Sleep prolongation with hexobarbital was observed at 30 mg/kg/day, n.s.s.
 - No dose-dependent anticonvulsive or convulsive-potentiating findings
 - Decreased writhing response times at ≥ 10 mg/kg NK-104, but no effect in Haffner method (assessment of analgesia)
 - Slightly decreased body temperature at ≥ 10 mg/kg (n.s.s.)
- Autonomic nervous system
 - No effects
- Effects on isolated smooth muscle
 - No effect on acetylcholine or histamine induced contraction in guinea pig ileum, but NK-104 did shift the curve to the right and also inhibited maximum contraction response at 10^{-4} M, and inhibited barium chloride contraction responses at 10^{-4} M.
- Respiratory/circulatory system effects (NK-104 at 0.3, 1, 3 mg/kg, i.v. in dogs)
 - Effects on HR, BP, ECG, femoral blood flow and respiration were unremarkable in anesthetized dogs.
- Gastrointestinal effects
 - No effects on charcoal powder expulsion rates in mice
- Water/electrolyte metabolism

- Urinary excretion of sodium and chloride were inhibited up to 6 h post dose in the 10 and 30 mg/kg groups of rats.
- Urinary volume was decreased in the 30 mg/kg group (↓37%).
- The urinary excretion of potassium was unaffected.
- Diuresis was attributed by the Sponsor to the potentiation of water reabsorption in the kidney tubules, effect seen at ≥72-fold MRHD based on body surface area.

Neurological effects: Pitavastatin crosses the blood-brain barrier. Irwin test (mice): a decrease in spontaneous motor activity was observed in 1/6 mice in groups given 3 or 10 mg/kg NK-104 and 3/6 mice given 30 mg/kg. This was a transient observation in low-dose, mid-dose at 120 minutes after dosing which occurred after the T_{max} (0.5-1 h). At high-dose the effect in 2/6 mice had a persistent decrease in motor activity. In high-dose group 1/6 had decreased grooming and pinna reflex 30-60 minutes after dosing. In the mid-dose, 1/6 had a transient decrease in grip strength. No effect on seizure potential was observed. Decreased acetic-acid induced writhing was observed in mid-dose, high-dose groups.

Cardiovascular/Pulmonary effects (anesthetized dogs): Effects of NK-104 at 0.3, 1, 3 mg/kg, i.v. on HR, BP, ECG, femoral blood flow and respiration were unremarkable in anesthetized dogs. No effect on BP was observed at 3 mg/kg i.v. on the hypotensive response to acetylcholine or vasopressor response induced with norepinephrine.

Renal effects: Urinary excretion of sodium and chloride were inhibited up to 6 h post dose in the 10 and 30 mg/kg groups of rats. Urinary volume was decreased in the 30 mg/kg group (↓37%). The urinary excretion of potassium was unaffected. Diuresis was attributed by the Sponsor to the potentiation of water reabsorption in the kidney tubules.

SR-9214 – NK-104 general pharmacology study (2)

Key Study Findings:

- Central nervous system effects within 3 hours post-administration
 - No dose-dependent anticonvulsive or convulsive-potentiating findings
- Effects on the somatic nervous system, as determined by the traction method, were unremarkable.
- Hypotensive effects with administration of acetylcholine and pressor responses induced by norepinephrine were unremarkable.
- In pylorus-ligated rats administered 3, 10, or 30 mg/kg NK-104 p.o., there were dose-related trends towards increased volume, increased acid output, and increased pepsin output versus 0.5% carboxymethylcellulose-only controls.
- No injury to rat gastric mucosa occurred at up to 30 mg/kg/day at 5 hours post-administration.
- Bile secretion was slightly increased at 30 minutes after administration of 1 and 3 mg/kg in rats under anesthesia (n.s.s.), but not at 0.3 mg/kg.
- There were no effects on PT or APTT in rabbit plasma, or ADP/collagen-induced platelet aggregation at concentrations up to 100 μM NK-104 *in vitro*.

The potential effects of NK-104 on central nervous system (CNS), somatic nervous system, the respiratory-circulatory system, the gastrointestinal system (GI), and plasma coagulation and platelet aggregation were studied. The CNS was studied by evaluating the convulsion-potential effects of NK-104 in male mice. The somatic nervous system was studied using the traction method in male mice. The respiratory-circulatory system was evaluated by measuring effects on blood pressure response in male dogs. Gastric juice secretion, effects on gastric mucosa, and bile secretion were measured to evaluate potential effects on the GI system. Prothrombin time (PT), activated partial thromboplastin time (APTT), and induced platelet aggregation were measured to study effects on coagulation and platelet properties.

CNS effects: Doses were 3, 10, and 30 mg/kg NK-104 p.o. in mice. Pitavastatin was negative for convulsive potentiation at all doses, negative for effects on traction when hanging by hind-limbs and fore-limbs from wires.

There was no effect on norepinephrine (0.5 µg/kg) or acetylcholine (3 µg/kg) induced mean blood pressure responses in anesthetized male dogs administered 3 mg/kg NK-104.

Gastrointestinal effects: In pylorus-ligated rats administered 3, 10, or 30 mg/kg NK-104 p.o., there were dose-related trends towards increased volume (19%), increased acid output (23%), and increased pepsin output (23%) in the 30 mg/kg group versus 0.5% carboxy-methylcellulose-only controls, but no changes in pH, acidity, or pepsin activity.

EFFECTS OF NK-104 ON GASTRIC SECRETION OF PYLORUS-LIGATED MALE RATS

Group	Dose (mg/kg, p.o.)	No. of animals	Body weight (g)	Volume (ml/4 hr)	pH	Acidity (µEq/ml)	Acid output (µEq/4 hr)	Pepsin activity (mg/ml)	Pepsin output (mg/4 hr)
Control (0.5% CMC-Na)	-	8	182.1 ^{a)}	3.93	1.54	58.0	229.0	15.36	59.87
			± 2.1	± 0.22	± 0.07	± 3.1	± 21.4	± 0.82	± 3.83
NK-104	3	8	185.0	3.89	1.53	56.6	220.0	16.30	62.56
			± 2.5	± 0.22	± 0.04	± 3.2	± 17.9	± 1.05	± 4.30
NK-104	10	8	183.3	4.18	1.44	64.4	277.8	15.48	64.20
			± 2.2	± 0.47	± 0.08	± 3.6	± 42.1	± 0.32	± 6.74
NK-104	30	8	183.0	4.66	1.49	59.9	282.1	15.89	73.74
			± 2.3	± 0.27	± 0.03	± 2.5	± 24.0	± 0.60	± 4.25

^{a)}: Each value represents the mean ± S.E.

(Sponsor, M4, SR-9214, p27)

There were no remarkable effects of NK-104 doses of up to 30 mg/kg on the gastric mucosa of male rats or bile secretion at doses of up to 3 mg/kg p.o. in rats.

No effect on charcoal propulsion at 3, 10, 30 mg/kg p.o. NK-104 to mice. No effect on gastric secretion in the 3 and 10 mg/kg groups in rat but an increased tendency for secretion (acid, pepsin) in the 30 mg/kg group (↑23%, n.s.s.). No effect on gastric mucosa of rat.

Pitavastatin was administered at 0.3, 1, and 3 mg/kg i.v. for evaluation of bile secretion in anesthetized male rats. Slight increase in bile secretion observed in the 1 and 3 mg/kg groups but not at 0.3 mg/kg i.v. in rats (↑12%, n.s.s.).

Clotting effects: There were no effects on PT or APTT in rabbit plasma, or ADP/collagen-induced platelet aggregation at concentrations up to 100 μ M NK-104 *in vitro*.

FBM06-1114 – Effect of NK-104 on central nervous function in mice

The effects of NK-104 on central nervous function (Irwin test) in mice was evaluated after single-dose administration to two mice/group of 0, 3, 10, or 30 mg/kg by oral gavage (4 groups of 8 males). Results of an Irwin test in CD-1 mice were unremarkable at doses up to 30 mg/kg.

FBM 06-4115 - Effects of single oral dose administration of NK-104 on cardiovascular and respiratory function in conscious dogs**Key Study Findings:**

- In conscious Beagle dogs, heart rate was slightly but significantly increased after administration of the highest dose, 10 mg/kg dose of NK-104 at 3 and 6 hours post-dose ($p < 0.05$, $p < 0.01$), respectively.
- All other parameters, including PR, QRS, and QT/QTc intervals measured pre-dose and 0.5, 1, 3, 6, 12, and 24 hours post-dose, were unremarkable.
- Increased heart rate occurred at 10 mg/kg, which represents 154-fold human exposure at the MRHD of 4 mg/day (based on AUC in 3 month TK study in dogs)

Male dogs ($n=4$) were administered escalating oral doses of 0.1, 1.0, and 10 mg/kg NK-104, and one dog was administered an empty capsule. Frequency of administration was weekly. Ten mg/kg approximated 154-fold human exposure at the MRHD. Dogs were monitored daily for clinical signs. Behavior was monitored from 1 hour pre-dose to 6 hours post-dose later by videotape.

Ambulatory electrocardiograms of conscious dogs were obtained once prior to the first administration, then until 24 hours post-administration. ECGs were examined by automated analyzer at 0.5, 1, 3, 6, 12, and 24 hours after dosing for PR, QRS, QT, and QTc. Blood pressure and heart rate were recorded by an implanted automatic telemetry system from 9-hours pre-dose to 1-day post-dose. Respiratory rate was measured by systolic blood pressure baseline variation. Gas samples were analyzed for pH, partial pressure of CO₂, O₂ and saturation fraction of O₂ measured in arterial blood.

Heart rate was slightly but significantly increased after administration of the highest dose, 10 mg/kg dose of NK-104 at 3 and 6 hours post-dose ($p < 0.05$, $p < 0.01$, respectively). All other parameters were unremarkable. Increased heart rate occurred at 154-fold human exposure to NK-104 at the MRHD of 4 mg/day.

KOW002HG – Effects of NK-104 and its main metabolite, NK-104 lactone, on the hERG current
Key Study Findings:

- The highest concentration of NK-104 in blood, as measured by $C_{\max}$ after three weeks repeat dose administration of a 4 mg oral dose of NK-104 in humans was 1.34×10^{-7} mol/L (or 134 nM).
- The plasma protein unbound-to-bound ratio is approximately 0.01 (or ~1% free), which yields an effective concentration of approximately 1.34×10^{-9} mol/L (or 1.34 nM).
- The highest concentration of NK-104 lactone that tested negative for hERG current reduction (~NOAEL) was 3×10^{-7} mol/L (300 nM).

The negative control was DMSO (0.1% by volume) and reduced hERG current by ~10.5% after 10 minutes. The positive control was E-4031, which reduced hERG current by ~88% after 10 minutes. NK-104 did not reduce hERG currents at concentrations up to 1×10^{-5} mol/L (10 μ M), the highest concentration of NK-104 tested after factoring in controls. NK-104 lactone, the primary metabolite of NK-104, inhibited hERG currents at 1×10^{-6} mol/L (1 μ M) by ~12.9% after adjustment of results based on the negative control, but did not significantly inhibit hERG currents at 3×10^{-7} mol/L (0.3 μ M) or 1×10^{-7} mol/L (0.1 μ M) after compensating for the vehicle-only control ($p < 0.05$). The NOAEL for hERG current inhibition by NK-104 lactone in this assay was 0.1 μ M.

hERG ASSAY DESIGN

Group		Concentration of test preparation	Number of cells*
Article	Concentration of test solution †		
Vehicle control (DMSO)	0.1 vol% ‡	-	5
NK-104	1×10^{-7} mol/L	1×10^{-4} mol/L	5
	1×10^{-6} mol/L	1×10^{-3} mol/L	5
	1×10^{-5} mol/L	1×10^{-2} mol/L	5
NK-104 lactone	1×10^{-7} mol/L	1.1×10^{-4} mol/L	5
	3×10^{-7} mol/L	3.3×10^{-4} mol/L	5
	1×10^{-6} mol/L	1.1×10^{-3} mol/L	5
Positive control (E-4031)	1×10^{-7} mol/L	1×10^{-4} mol/L	5

* The number of cells from which data were recorded for 10 minutes after beginning the application following the 1-minute superfusion of the test solution for achieving a steady-state concentration of the applying article in the chamber are indicated.

† Vehicle (DMSO) was diluted 1000 times with the superfusing solution.

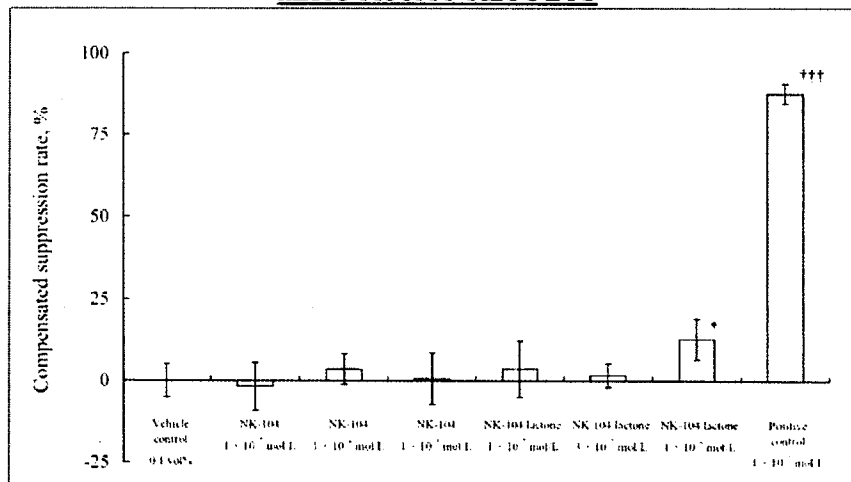
‡ The concentrations of test solutions are targeted final concentrations in the application chamber after completing the application.

(Sponsor, M4, KOW002HG, p16)

The highest concentration of NK-104 in blood, as measured by $C_{\max}$ after three weeks repeat dose administration of a 4 mg oral dose of NK-104 in humans was 1.34×10^{-7} mol/L (or 134 nM). The plasma protein unbound-to-bound ratio is approximately 0.01 (or ~1% free), which yields an effective concentration of approximately 1.34×10^{-9} mol/L (or 1.34 nM). If plasma exposure were the same as cardiac exposure (excluding an effect of protein-binding), then NK-104 would be present at a 4-fold lower concentration

than those that may impact cardiac function via the hERG channel. The highest concentration of NK-104 lactone that tested negative for hERG current reduction (~NOAEL) was 3×10^{-7} mol/L (300 nM), which provides a safety margin for hERG current inhibition of 8.8 (assumes no protein-binding of the lactone and those plasma exposures are equal to cardiac exposures).

hERG ASSAY RESULTS



(Sponsor, M4, KOW002HG, p22)

Inhibition of hERG Currents				
Substance	Concentration (mol/L)			
	1×10^{-7}	3×10^{-7}	1×10^{-6}	1×10^{-5}
DMSO (-ctrl)	N/A	N/A	N/A	N/A
NK-104	-	N/A	-	-
NK-104 lactone	-	-	↓11%	N/A
E-4031 (+ctrl)	↓88%	N/A	N/A	N/A

N/A, not tested at indicated concentration

-, negative for reduced hERG current

↓% indicates positive response for reduced hERG current as % decrease from maximum

KOW001MG – Effects of NK-104 lactone on action potentials in isolated guinea pig papillary muscles

Key Study Findings:

- NK-104 lactone, the primary metabolite of NK-104, affected action potentials at 3×10^{-6} mol/L (3 μ M) by ~4-5% at action potential duration at 30, 60, and 90% of repolarization (APD₃₀₋₉₀).
- The theoretical NOAEL for the lactone was 1×10^{-7} mol/L (0.1 μ M), an 87-fold safety margin by estimated C_{max}.
- The highest concentration of NK-104 that tested negative for affecting the action potential in isolated guinea pig papillary muscles was 1×10^{-6} mol/L, which is approximately 29-fold higher than the estimated effective concentration of NK-104 in

blood, assuming that plasma exposure is the same as tissue (cardiac) exposure, which may not be the case.

The negative control was DMSO (0.1% by volume) and had no effect on action potential in isolated guinea pig papillary muscles. The positive control was E-4031, a selective potassium channel inhibitor, which caused mean percent changes of 12.8%, 26.2%, and 25.9% at APD₃₀, APD₆₀, and APD₉₀, respectively. NK-104 lactone, the primary metabolite of NK-104, affected action potentials at 3×10^{-6} mol/L (3 μ M) by ~4-5% at APD₃₀₋₉₀, but did not significantly affect action potentials at 1×10^{-7} mol/L (0.1 μ M), 3×10^{-7} mol/L (0.3 μ M), or 1×10^{-6} mol/L (1 μ M).

ACTION POTENTIALS IN ISOLATED PAPILLARY MUSCLE. ASSAY DESIGN

Group		Concentration of test preparation	Number of samples*
Article	Concentration of test solution †		
Vehicle control (DMSO)	0.1 vol% ‡	-	5
NK-104 lactone	1×10^{-7} mol/L	1.2×10^{-4} mol/L	5
	3×10^{-7} mol/L	3.6×10^{-4} mol/L	5
	1×10^{-6} mol/L	1.2×10^{-3} mol/L	5
	3×10^{-6} mol/L	3.6×10^{-3} mol/L	5
Positive control (E-4031)	1×10^{-7} mol/L	1×10^{-4} mol/L	5

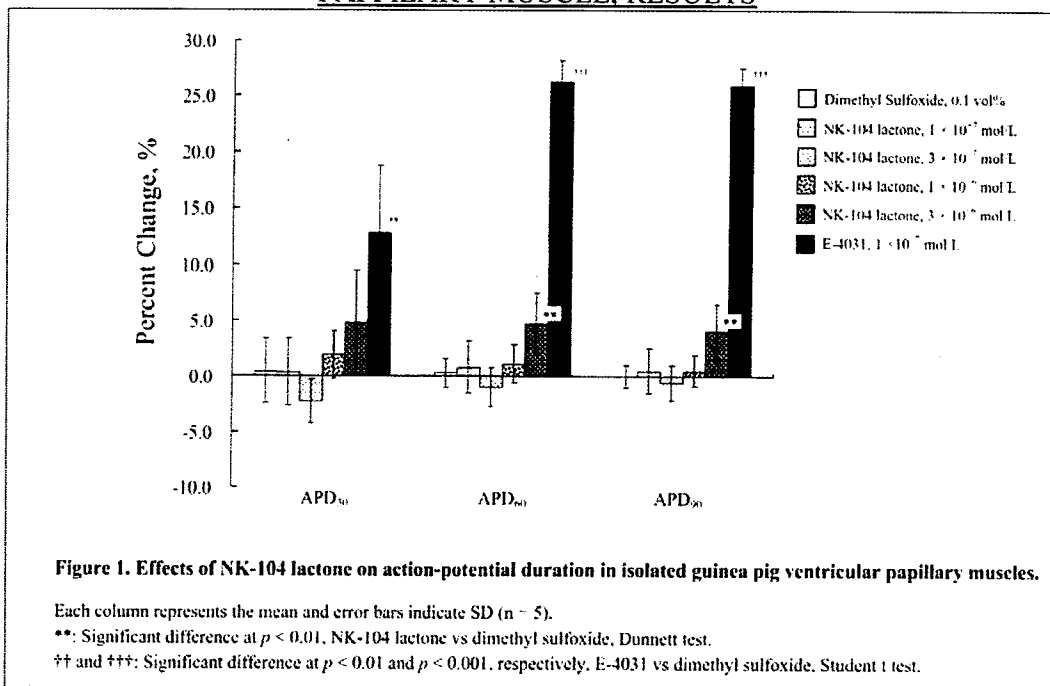
*: Data collected for 30 minutes after the concentration of the test article in the chamber reached a steady state were judged as valid. Samples from which data were not collected for 30 minutes after the concentration of the test article reaches steady state were excluded.

†: Vehicle (DMSO) was diluted 1000 times with Tyrode solution.

‡: The concentrations of test solutions are targeted final concentrations in the application chamber after completing the application.

(Sponsor, M4, KOW001MG, p14)

ACTION POTENTIALS IN ISOLATED PAPPILARY MUSCLE, RESULTS



(Sponsor, M4, KOW001MG, p20)

The highest concentration of NK-104 in blood, as measured by $C_{\max}$ after administration of 4 mg oral dose of NK-104 was 1.34×10^{-7} mol/L (or 134 nM). The plasma protein unbound to bound ratio is approximately 0.01 (or ~1% free), which yields an effective concentration of approximately 1.34×10^{-9} mol/L (or 1.34 nM). The highest concentration of NK-104 that tested negative for affecting the action potential in isolated guinea pig papillary muscles was 1×10^{-6} mol/L, which is approximately 29-fold higher than the estimated effective concentration of NK-104 in blood, assuming that plasma exposure is the same as tissue (cardiac) exposure, which may not be the case.

Pitavastatin lactone affected action potentials at 3×10^{-6} mol/L (3 μ M) by ~4-5% at APD₃₀₋₉₀. Assuming no protein-binding and that assuming that plasma exposure is the same as tissue (cardiac) exposure, which may not be the case, safety margins would be 87-fold at the 4 mg MRHD.

Recall that in conscious Beagle dogs in study FBM 06-4115, heart rate was slightly but significantly increased after administration of the highest dose, 10 mg/kg dose of NK-104 at 3 and 6 hours post-dose ($p < 0.05$, $p < 0.01$), respectively. All other parameters, including PR, QRS, and QT/QTc intervals measured pre-dose and 0.5, 1, 3, 6, 12, and 24 hours post-dose, were unremarkable. Increased heart rate occurred at 154-fold human exposure to NK-104 at the MRHD of 4 mg/day.

Increased Action Potential Duration – Isolated Papillary Muscle					
APD		Concentration (mol/L)			
		1×10^{-7}	3×10^{-7}	1×10^{-6}	3×10^{-6}
DMSO (-ctrl)	N/A	N/A	N/A	N/A	N/A
NK-104 lactone	30	-	-	-	↑4.8%
	60	-	-	-	↑4.7%
	90	-	-	-	↑4.0%
E-4031 (+ctrl)	30	↑13%	N/A	N/A	N/A
	60	↑26%			
	90	↑26%			

N/A, not tested at this concentration

-, negative for significant change (as a % change from DMSO control)

↑XX%, percent increase from DMSO control

*Theoretical safety margin calculation based on C_{max} from 4 mg human dose of 134 nM plasma concentration, and free vs. protein-bound of ~1% (or ~1.34 nM), for informational purposes in labeling decisions. Assumes plasma exposure is equivalent to cardiac exposure.

2.6.2.5 Pharmacodynamic drug interactions

99029 – Drug interaction of NK-104 - Combination with warfarin -

Key study findings:

- Pitavastatin is metabolized, at least in part, by the 2C9 isoform of the cytochromes P450 (CYP2C9).
- Pitavastatin caused slight, dose-related decreases in warfarin-related PT increases compared to warfarin-only controls

CYP2C9 is known to play an important role in metabolizing warfarin, a potent anticoagulant. Here, the Sponsor administered simvastatin NK-104 (and simvastatin, a CYP3A4 substrate) to rats concomitantly with warfarin to evaluate any interaction between these two drugs. A second experiment was carried out after administration of the P450 inducer phenobarbital (general P450 inducer, specifically CYP2B6). Prothrombin time was measured in controls, pitavastatin and simvastatin treated animals after a single-dose administration or repeat-dose administration. Warfarin was administered at 1.5 mg/kg and 18 hours later NK-104 or simvastatin were administered (3, 10, or 30 mg/kg, and 10, 30, or 100 mg/kg, respectively). Neither pitavastatin nor simvastatin showed a significant effect on the effects of warfarin in rats. However both showed a slight, dose-related decrease in warfarin-related PT increases compared to

warfarin-only controls. Since simvastatin has been anecdotally reported to potentially affect PT, and such an effect was not seen here, there may be limitations to the methodology used here; an interaction between pitavastatin and warfarin cannot be ruled out based on this study.

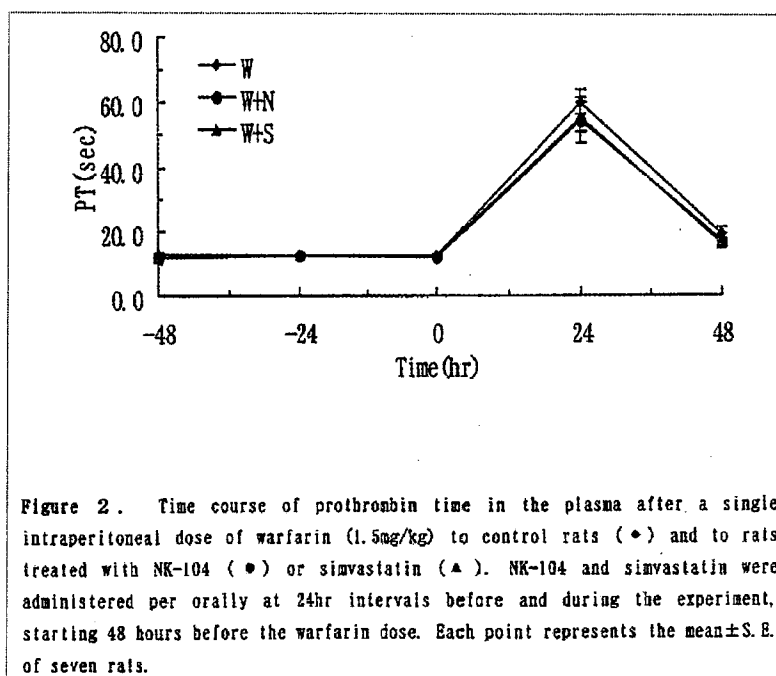


Figure 2. Time course of prothrombin time in the plasma after a single intraperitoneal dose of warfarin (1.5mg/kg) to control rats (•) and to rats treated with NK-104 (•) or simvastatin (▲). NK-104 and simvastatin were administered per orally at 24hr intervals before and during the experiment, starting 48 hours before the warfarin dose. Each point represents the mean $\pm$ S.E. of seven rats.

(Sponsor, M4, R99029, p12)

PHARMACOLOGY TABULATED SUMMARY

2.6.3.2 Primary Pharmacodynamics				Test Article: Pitavastatin	Page 1 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin and/or Other HMG-CoA Reductase Inhibitors</i>					
<i>HMG-CoA Reductase Inhibition</i>					
HMG-CoA reductase inhibition	Rat hepatic microsomes	In vitro	Pitavastatin, fluvastatin, simvastatin, pravastatin: 1, 10, 100, 1000 nmol/L	Pitavastatin, fluvastatin, simvastatin and pravastatin inhibited HMG-CoA reductase in a dose-dependent manner with IC ₅₀ values of 6.8, 1.2, 16 and 46 nmol/L, respectively.	[R92068]
HMG-CoA reductase inhibition kinetics	Rat hepatic microsomes	In vitro	Pitavastatin: 1, 3, 10 nmol/L Pravastatin: 5, 10 nmol/L Simvastatin: 1, 3 nmol/L	The Ki values for HMG-CoA reductase inhibition for pitavastatin, pravastatin and simvastatin were 1.7, 6.0 and 2.8 nmol/L, respectively. Inhibition was competitive.	[R93007]
<i>Cholesterol Synthesis Inhibition</i>					
Cholesterol synthesis inhibition	HepG2 cells	In vitro	Pitavastatin, simvastatin, pravastatin, atorvastatin, mevastatin ^a : 1, 10, 100, 1000 nmol/L	The IC ₅₀ values for cholesterol synthesis inhibition for pitavastatin, simvastatin, pravastatin, atorvastatin and mevastatin were 5.8, 17.1, > 1000, 32.9 and 8.1 nmol/L, respectively.	[R98048]
Cholesterol synthesis inhibition	HepG2 cells	In vitro	Pitavastatin: 1, 10, 100, 1000, 10000 nmol/L	[¹⁴ C]-acetic acid or [¹⁴ C]-mevalonolactone were used as substrates. Dose-dependent inhibition of cholesterol synthesis using [¹⁴ C]-acetic acid was observed (IC ₅₀ 2 nmol/L). Only minimal inhibition of cholesterol synthesis using [¹⁴ C]-mevalonolactone was observed (IC ₅₀ > 10000 nmol/L). Pitavastatin appears to selectively inhibit HMG-CoA reductase.	[R99038]
Cholesterol synthesis inhibition	Human skeletal muscle cells and human hepatocytes	In vitro	Pitavastatin, atorvastatin, cerivastatin, fluvastatin, simvastatin: 0.1, 1, 10, 100, 1000 nmol/L Pravastatin: 1, 10, 100, 1000, 10000 nmol/L	Comparison of IC ₅₀ values for cholesterol synthesis inhibition of six HMG-CoA reductase inhibitors showed that cerivastatin was the most potent in both cell lines. Pravastatin was least potent. Pitavastatin was less potent than cerivastatin in cholesterol synthesis inhibition and approximately equipotent to fluvastatin in both cell lines. In both cell lines pitavastatin was more potent than simvastatin and atorvastatin.	[R101130]
a: Described as compactin in the pharmacology reports IC ₅₀ : Median (50%) inhibitory concentration Ki: Inhibition affinity constant					
2.6.3.2 Primary Pharmacodynamics cont.				Test Article: Pitavastatin	Page 2 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin and/or Other HMG-CoA Reductase Inhibitors cont.</i>					
<i>Cholesterol Synthesis Inhibition cont.</i>					
Sterol synthesis inhibition	Male Jla:Wistar rats	Oral; single dose	Pitavastatin: 0.03, 0.1, 0.3, 1, 3 mg/kg Pravastatin: 1, 3, 10 mg/kg Simvastatin: 0.1, 0.3, 1, 3 mg/kg Fluvastatin: 0.03, 0.1, 0.3, 1, 3 mg/kg	Marked inhibition of hepatic sterol synthesis was seen 3 hours post-dosing with pitavastatin. The ED ₅₀ values were 2.1, 0.50, 0.17 and 0.072 mg/kg for pravastatin, simvastatin, pitavastatin and fluvastatin, respectively. The level of pitavastatin inhibition was around 12 times greater than that of pravastatin, three times greater than that of simvastatin and 0.4 that of fluvastatin.	[R92069]
Sterol synthesis inhibition	Male Jla:Wistar rats	Oral; single dose	Pitavastatin: 0.03, 0.1, 0.3, 1, 3 mg/kg; or 0.26 mg/kg Pravastatin: 1, 3, 10, 30 mg/kg; or 4.8 mg/kg Simvastatin: 0.1, 0.3, 1, 3, 10 mg/kg; or 0.72 mg/kg	Significant inhibition of sterol synthesis in the liver was seen 3 hours post-dosing with all three compounds. The ED ₅₀ values were 0.13, 2.4 and 0.36 mg/kg for pitavastatin, pravastatin and simvastatin, respectively. In this model, pitavastatin had a longer duration of action than simvastatin and pravastatin. The inhibitory effect of pitavastatin on sterol synthesis in the liver showed no difference between fed and fasted conditions. There was no significant difference in pitavastatin concentrations in plasma and liver in fed and fasted rats ^a .	[R95078]
a: High performance liquid chromatography using column-switching and ultraviolet detection (HPLC-UV) as described in [R92052] ED ₅₀ : Median (50%) effective dose					
2.6.3.2 Primary Pharmacodynamics cont.				Test Article: Pitavastatin	Page 3 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin and/or Other HMG-CoA Reductase Inhibitors cont.</i>					
<i>Cholesterol Synthesis Inhibition cont.</i>					
Sterol synthesis inhibition	Male Hartley guinea pigs	Oral; single dose	Pitavastatin: 0.03, 0.3 or 3 mg/kg; or 1 mg/kg Simvastatin: 0.3, 3 or 30 mg/kg; or 15 mg/kg	The ED ₅₀ values for inhibition of hepatic sterol synthesis were 0.33 mg/kg and 5.1 mg/kg for pitavastatin and simvastatin, respectively. At a dose approximately 3 times the ED ₅₀ , pitavastatin significantly inhibited hepatic sterol synthesis at 3, 6, 9 and 12 hours after dosing, whereas simvastatin significantly inhibited hepatic sterol synthesis at 3 hours only after dosing. The pitavastatin elimination half-life (t _{1/2}) was approximately 10.2 hours in plasma and approximately 9.1 hours in liver ^a . Lactone was not detected in any plasma samples (BLQ of 2.5 ng/mL) and was detected in the liver of one animal at the 3-hour time point only. The liver/plasma pitavastatin ratio was approximately 4.1.	[R98008], [R99058]
Plasma lipid lowering effect	Male Hartley guinea pigs	Oral, repeat dose for 2 weeks	Pitavastatin: 0.03, 0.1, 0.3, 1, 3 mg/kg Simvastatin: 0.3, 1, 3, 10, 30 mg/kg Atorvastatin: 3, 10, 30 mg/kg Pravastatin: 3, 10, 30, 100 mg/kg Fluvastatin: 3, 10, 30 mg/kg Cerivastatin: 0.01, 0.03, 0.1, 0.3, 1, 3 mg/kg	All HMG-CoA reductase inhibitors, except cerivastatin, significantly lowered plasma total cholesterol (TC) and phospholipid (PL). All HMG-CoA reductase inhibitors significantly reduced LDL-cholesterol (LDL-C). Only pitavastatin and atorvastatin significantly lowered triglycerides (TG), VLDL-cholesterol (VLDL-C) and liver cholesterol content. Pitavastatin, simvastatin, atorvastatin, cerivastatin, fluvastatin and pravastatin significantly lowered high-density lipoprotein-cholesterol (HDL-C).	[R98022], [R99041], [R99042], [R99043], [R99044]
a: HPLC-UV as described in [R95077] BLQ: Below limit of quantification					

2.6.3.2 Primary Pharmacodynamics cont.				Test Article: Pitavastatin	Page 4 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin and/or Other HMG-CoA Reductase Inhibitors cont.</i>					
<i>Lowering of Plasma Lipids</i>					
Plasma lipid lowering effect	Male beagle dogs	Oral, repeat dose for 14 days	Pitavastatin: 0.03, 0.1, 0.3, 1 mg/kg Pravastatin or simvastatin: 0.3, 1, 3 mg/kg	Pitavastatin significantly lowered plasma TC in a dose-dependent manner from 0.1 mg/kg after 14 days dosing. This effect was seen at 10-fold and 30-fold lower doses compared to pravastatin and simvastatin, respectively. In addition, pitavastatin had a pronounced and significant reducing effect on plasma TG, whereas pravastatin and simvastatin were less effective.	[R94012]
Plasma lipid lowering effect	Male kvl:Zucker rats	Oral; repeat dose for 1 or 2 weeks	Pitavastatin: 0.03, 0.1, 0.3, 1 mg/kg	Daily oral administration of 1 mg/kg pitavastatin for 2 weeks significantly decreased plasma TG but did not affect plasma TC or PL. The VLDL-triglycerides (VLDL-TG) secretion rate was significantly reduced but lipoprotein lipase activity and hepatic triglyceride lipase was not significantly affected.	[R94053]
Plasma lipid lowering effect	Male Wistar rats; post-prandial hyperlipidaemia	Oral, single dose	Pitavastatin: 0.5, 1, 2 mg/kg Atorvastatin: 1, 2, 4 mg/kg	Pitavastatin significantly suppressed plasma TG elevation at 6 hours after fat loading at ≥ 1 mg/kg and significantly decreased the TG- area under the concentration-time curve (AUC) up to 12 hours after fat loading in all groups. Pitavastatin had a greater effect on TG than atorvastatin.	[R98052]
2.6.3.2 Primary Pharmacodynamics cont.				Test Article: Pitavastatin	Page 5 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin and/or Other HMG-CoA Reductase Inhibitors cont.</i>					
<i>Lowering of Plasma Lipids cont.</i>					
Plasma lipid lowering effect	Male Hartley guinea pigs; lauric-rich diet-induced hyperlipidaemia	Oral*, repeat dose for 2 weeks	Pitavastatin: 0.1, 0.3, 1 mg/kg Atorvastatin: 3, 10, 30 mg/kg	In guinea pigs loaded with a high lauric acid diet both drugs decreased TC and LDL-C in a dose-dependent manner, with the potency of pitavastatin approx 10-fold higher than that of atorvastatin. Drug treatment decreased PL concentrations, with significant effects at 10 mg/kg atorvastatin. Accelerated LDL clearance was seen at 1 mg/kg pitavastatin and 10 mg/kg atorvastatin. Plasma apoB and LDL-apoB were significantly decreased in all treated groups in a dose-dependent manner. Results suggest that the drugs decrease blood cholesterol by enhancing expression of liver LDL-R.	[R99031]
a: The route of administration was not stated in the report. LDL-apoB: Apolipoprotein B-100 within the low-density lipoprotein particles					
2.6.3.2 Primary Pharmacodynamics cont.				Test Article: Pitavastatin	Page 6 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin and/or Other HMG-CoA Reductase Inhibitors cont.</i>					
<i>Lowering of Plasma Lipids cont.</i>					
Effect on plasma lipids and atherosclerosis	Male WHHL rabbits (test animals) and male Kbs-JW rabbits (normal animals)	Oral*, repeat dose for 26 weeks (continuous exposure in drinking water)	Pitavastatin: equivalent to 0.5 mg/kg/day	Pitavastatin lowered TC (10 to 20%), TG (25 to 40%), PL, VLDL-C (61.8%) and intermediate-density lipoprotein (IDL)-cholesterol (IDL-C) (48.8%) but not LDL-C which is consistent with the expression of only very low levels of LDL-R in the WHHL rabbit. Pitavastatin decreased the surface lesion area in the aortic arch and prevented medial smooth muscle cell degeneration in the thoracic aorta ($p < 0.01$). Anti-atherosclerotic effects of pitavastatin were attributed to the reduction of TG-rich lipoproteins, especially VLDL and IDL. The pitavastatin plasma concentration at steady state in Week 24 was 40.3 ng/mL; lactone was BLQ in plasma*. The pitavastatin liver concentration in Week 26 was 618.6 ng/g and the liver concentration of lactone was 78.8 ng/g.	[R98011], [R97034]
Intra-day precision and accuracy measurements of pitavastatin and lactone in liver homogenates of WHHL rabbits were performed; the measurements were within $\pm 15\%$ and were deemed acceptable. a: HPLC-MS as described in [R95077]					
2.6.3.2 Primary Pharmacodynamics cont.				Test Article: Pitavastatin	Page 7 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin and/or Other HMG-CoA Reductase Inhibitors cont.</i>					
<i>Mechanism of Action</i>					
<i>Enhancement of LDL-R Expression</i>					
LDL-R activity	HepG2 cells	In vitro	Pitavastatin: 1, 10, 100, 1000 nmol/L	Pitavastatin inhibited cholesterol synthesis in a concentration-dependent fashion with an IC_{50} value of 5.5 nmol/L. The LDL binding was significantly increased at 100 and 1000 nmol/L. LDL incorporation was significantly increased at 10, 100 and 1000 nmol/L and degradation of LDL was significantly increased at all dose levels compared to controls. The increased LDL binding was attributed to an increase in LDL-R number. The increase in LDL-R number correlated with inhibition of cholesterol synthesis.	[R99045]
LDL-R mRNA expression	HepG2 cells	In vitro	Pitavastatin: 1, 10, 100, 1000, 10000 nmol/L	Pitavastatin significantly enhanced LDL-R mRNA expression at ≥ 1000 nmol/L.	[R99037]
LDL-R and HMG-CoA reductase mRNA expression	HepG2 cells	In vitro	Pitavastatin: 1.16 μ mol/L Atorvastatin: 6.60 μ mol/L Simvastatin: 3.40 μ mol/L	Significant elevation of HMG-CoA reductase mRNA expression, relative to controls, was seen at 8 and 12 hours after pitavastatin treatment, 12 and 24 hours after atorvastatin and 8, 12 and 24 hours after simvastatin. Similarly, expression of LDL-R mRNA, relative to controls, was significantly higher with all drugs at all time points. Elevation by pitavastatin was significantly higher than that of atorvastatin and simvastatin at 12 hours.	[F-18]

2.6.3.2 Primary Pharmacodynamics cont.				Test Article: Pitavastatin	Page 8 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin and/or Other HMG-CoA Reductase Inhibitors cont.</i>					
<i>Mechanism of Action cont.</i>					
<i>Enhancement of LDL-R Expression cont.</i>					
Effects on expression of LDL-R	Male Hartley guinea pigs	Oral, repeat dose for 14 days	Pitavastatin: 3 mg/kg, or 0.03, 0.3, 3 mg/kg Simvastatin: 30 mg/kg	Pitavastatin (3 mg/kg) and simvastatin (30 mg/kg) significantly increased hepatic LDL-R binding activity to similar levels (% change compared to control group of 52.4% and 54.6%, respectively) with a significant reduction in LDL-C. When pitavastatin was orally administered for 2 weeks at 0.03, 0.3 and 3 mg/kg, LDL-C was decreased dose-dependently ($p < 0.01$ at 0.3 and 3 mg/kg), with a dose-dependent elevation in hepatic LDL-R binding activity (% change compared to control group of 71.9% at 3 mg/kg; $p < 0.05$).	[R98021]
Effects on LDL clearance	Male Hartley guinea pigs	Oral, repeat dose for 14 days	Pitavastatin: 3 mg/kg Simvastatin: 30 mg/kg	Both pitavastatin and simvastatin significantly increased LDL clearance, showing 1.8-fold and 1.4-fold greater fractional catabolic rate (pools/hour). The effect of pitavastatin in reducing LDL-C and increasing LDL clearance was greater than that with simvastatin.	[R97023]
<i>Decrease of VLDL Secretion</i>					
Effects on apoB and lipid metabolism	IlePG2 cells	<i>In vitro</i>	Pitavastatin: 1, 10 μ mol/L	Pitavastatin dose-dependently inhibited cholesterol synthesis, decreased intracellular cholesteryl ester (CE) content and inhibited secretion of cholesterol. Pitavastatin non-significantly decreased secretion of TG without affecting intracellular TG synthesis. Pitavastatin also accelerated degradation of intracellular apoB and decreased apoB secretion.	[Yanagita et al., 1999]
Effects on VLDL secretion	Male Hartley guinea pigs (<i>in situ</i> liver perfusion)	Oral, repeat dose for 14 days	Pitavastatin: 0.03, 0.3, 3 mg/kg Simvastatin: 30 mg/kg	Pitavastatin dose-dependently reduced VLDL-TG accumulation in the 4-hour liver perfusate. A significant reduction (19.9%) in liver perfusate VLDL-TG concentration was seen only at 3 mg/kg pitavastatin. Simvastatin (30 mg/kg) did not affect the accumulation of VLDL-TG in the liver perfusate.	[R97057]
2.6.3.2 Primary Pharmacodynamics cont.				Test Article: Pitavastatin	Page 9 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin and/or Other HMG-CoA Reductase Inhibitors cont.</i>					
<i>Mechanism of Action cont.</i>					
<i>Other Activities</i>					
Oxidised LDL-induced cholesterol accumulation	RAW264.7 cells	<i>In vitro</i>	Pitavastatin: 30, 100, 300, 1000, 3000 nmol/L Atorvastatin and simvastatin: 100, 300, 3000 nmol/L	Pitavastatin dose-dependently suppressed intracellular accumulation of CE and TC with IC_{50} values of 56.3 and 190 nmol/L, respectively. Pitavastatin also dose-dependently decreased cellular free cholesterol which was statistically significant at 100 nmol/L and above. Maximum inhibition (35.4%) was observed at 3000 nmol/L. The IC_{50} values for CE accumulation for atorvastatin and simvastatin were 2200 nmol/L and 168 nmol/L, respectively. The inhibitory effect of pitavastatin was abolished by co-administration of mevalonate.	[L-20]
Effect on carotid neointimal hyperplasia	Male Japanese white rabbits (balloon-injured carotid artery)	Oral, repeat dose for 24 days	Pitavastatin: 0.1, 0.3, 1.0 mg/kg Pravastatin: 9.6 mg/kg Simvastatin: 3.0 mg/kg	Pitavastatin dose-dependently suppressed intimal thickening (intima/media ratio). In addition, in comparison with pravastatin and simvastatin, pitavastatin showed greater potency in its suppression of intimal thickening and accumulation of fibronectin.	[R-21]
Effect on atherosclerotic plaque	Male WHHL rabbits	Oral, repeat dose for 16 weeks (continuous exposure in drinking water)	Pitavastatin: equivalent to 0.5 mg/kg/day	Pitavastatin lowered plasma TC, VLDL-C, LDL-C, and LDL-C and prevented the progression of aortic atherosclerosis. Furthermore, pitavastatin markedly reduced accumulation of macrophages and increased collagen and α -smooth muscle actin-positive area in the aortic plaque.	[Suzuki et al., 2003]
2.6.3.2 Primary Pharmacodynamics cont.				Test Article: Pitavastatin	Page 10 of 10
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number
<i>Studies Conducted with Pitavastatin Metabolites and/or Related Substances</i>					
<i>HMG-CoA Reductase Inhibition</i>					
HMG-CoA reductase inhibition	Rat hepatic microsomes	<i>In vitro</i>	Lactone and pitavastatin: 1, 10, 100, 1000 nmol/L	Lactone inhibited HMG-CoA reductase in a dose-dependent manner with an IC_{50} value of 12 nmol/L. The IC_{50} value of pitavastatin was 6.8 nmol/L.	[R92068]
HMG-CoA reductase inhibition	Rat hepatic microsomes	<i>In vitro</i>	8-hydroxy pitavastatin (b) (4) and pitavastatin: 1, 10, 100, 1000, 10000 nmol/L	The IC_{50} values for 8-hydroxy pitavastatin (b) (4) and pitavastatin were 3.5, 800, 1800 and 5.5 nmol/L, respectively.	[R99032]
HMG-CoA reductase inhibition	Rat hepatic microsomes	<i>In vitro</i>	Lactone and pitavastatin: 0.1, 1, 10, 100, 1000 nmol/L	The IC_{50} values for lactone and pitavastatin were 19.3 and 6.3 nmol/L, respectively. With pre-incubation for 30 minutes the corresponding IC_{50} values for lactone and pitavastatin were 2.7 and 1.7 nmol/L, respectively. The HMG-CoA reductase inhibition by lactone was attributed to pitavastatin produced in this test system from lactone and not due to lactone <i>per se</i> .	[R101030]
HMG-CoA reductase inhibition kinetics	Rat hepatic microsomes	<i>In vitro</i>	(b) (4)	The K_i values for (b) (4) and pitavastatin were 341 and 1.7 nmol/L, respectively.	[R93007]
<i>Cholesterol Synthesis Inhibition</i>					
Sterol synthesis inhibition	Male Jla:Wistar rats	Oral; single dose	Lactone and pitavastatin: 0.03, 0.1, 0.3, 1, 3 mg/kg	Marked inhibition of hepatic sterol synthesis was seen 3 hours post-dosing with lactone and pitavastatin; the LD_{50} values were 0.15 and 0.17 mg/kg, respectively. Both lactone and pitavastatin showed liver selectivity.	[R92069]
<i>Lowering of Plasma Lipids</i>					
Plasma lipid lowering effect	Male beagle dogs	Oral, repeat dose for 14 days	Lactone: 0.05, 0.125 mg/kg twice daily Pitavastatin: 0.056, 0.141 mg/kg twice daily	Plasma TC, PL, TG and HDL-C were decreased.	[R19118]
a: HPLC-MS as described in [R95077]					

2.6.3.3 Secondary Pharmacodynamics				Test Article: Pitavastatin		Page 1 of 1
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose	Noteworthy Findings	Report Number	
Studies Conducted with Pitavastatin						
Effect on biliary lipids	Male Syrian hamsters	Oral, repeat dose for 14 days	Pitavastatin: 0, 0.3, 1 mg/kg	At 1 mg/kg, pitavastatin significantly lowered plasma TG, but did not change plasma TC or plasma PL concentrations. In the bile, pitavastatin did not affect TC, but significantly raised PL at 0.3 mg/kg, and significantly lowered bile acid concentration in a dose-dependent manner. No change in lithogenic index was found.	[R99028]	
Effect on biliary lipids	Male Hartley guinea pigs	Oral, repeat dose for 15 days	Pitavastatin: 0, 3 mg/kg	Pitavastatin significantly lowered plasma TC and PL concentrations but did not significantly affect biliary lipids or the lithogenic index.	[R98051]	
Effect on steroid hormones	Male Jla:Wistar rats	Oral, repeat dose for 7 days	Pitavastatin: 0, 3, 30 mg/kg	Pitavastatin had no effect on plasma concentrations of testosterone, oestrone, corticosterone or adrenocorticotrophic hormone, however, even at 30 mg/kg, pitavastatin had no effect on plasma TC concentrations.	[R101018]	
Effect on steroid hormones	Male Hartley guinea pigs	Oral, repeat dose for 7 days	Pitavastatin: 0, 0.3, 3 mg/kg	Pitavastatin had no effect on plasma concentrations of testosterone or oestrone at either dose level. Pitavastatin significantly decreased plasma TC concentrations at 3 mg/kg.	[R101021]	

2.6.3.4 Safety Pharmacology				Test Article: Pitavastatin		Page 1 of 4
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance Report Number
Studies Conducted with Pitavastatin						
General Pharmacology	Slc:ICR mice ^a	Oral, single dose	Pitavastatin: 0, 3, 10, 30 mg/kg	6 to 10 male mice / group	3 mg/kg: No treatment-related effects. 10 mg/kg: Significant inhibition of writhing frequency. 30 mg/kg: Decreased spontaneous motor activity and prolonged hexobarbital sodium induced sleeping time were observed; both these observations were not statistically significant. Significant inhibition of writhing frequency.	No [SR-9213]
	Jcl:Wistar rats ^b	Oral, single dose	Pitavastatin: 0, 3, 10, 30 mg/kg	6 to 8 male rats / group	3 mg/kg: No treatment-related effects. 10 mg/kg: Significant decrease in urinary excretion of electrolytes. 30 mg/kg: Significant decrease in urinary excretion of electrolytes and urine volume.	No
	Beagle dogs ^c	i.v., single dose	Pitavastatin: 0, 0.3, 1, 3 mg/kg	3 males/group	0.3, 1, 3 mg/kg: No treatment-related effects.	No
	Hartley guinea pigs	In vitro (isolated ileum)	Pitavastatin: 0, 0.1, 1, 10 and 100 µmol/L	5 males/group	0.1, 1, 10 µmol/L: No treatment-related effects. 100 µmol/L: Significant inhibitory effect on acetylcholine-, histamine- and barium chloride-induced contractions in guinea pig ileum.	No

a: Effects of pitavastatin on general signs and behaviour; the central nervous system (CNS), namely, spontaneous motor activity, sleep-prolonging activity, anti-convulsant activity, convulsion potentiating activity, analgesic activity using Haflner method and acetic acid-induced writhing method; and the gastrointestinal tract (GIT), namely, small intestinal transit; nalidixic acid (1000 mg/kg; oral) used as a positive control for pentylenetetrazole induced convulsions.

b: Effects of pitavastatin on body temperature and water and electrolyte metabolism.

c: Effects of pitavastatin on heart rate, blood pressure, femoral blood flow, respiration rate and electrocardiogram (ECG).

2.6.3.4 Safety Pharmacology				Test Article: Pitavastatin		Page 2 of 4
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance Report Number
Studies Conducted with Pitavastatin cont.						
General Pharmacology	Slc:ICR mice ^a	Oral, single dose	Pitavastatin: 0, 3, 10, 30 mg/kg	10 male mice / group	3, 10 and 30 mg/kg: No treatment-related effects.	No [SR-9214]
	Jcl:Wistar rats ^b	Oral, single dose	Pitavastatin: 0, 3, 10, 30 mg/kg	8 male rats/group	3 mg/kg: No treatment-related effects. 10 mg/kg: A small, non-significant, increase in gastric acid output. 30 mg/kg: A small, non-significant, increase in gastric acid output, total output of pepsin and gastric acid secretion.	No
	Jcl:Wistar rats ^c	i.v., single dose	Pitavastatin: 0, 0.3, 1, 3 mg/kg	8 males/group	0.3: No treatment-related effects. 1 mg/kg and 3 mg/kg: A small, non-significant, increase in bile secretion.	No
	Beagle dogs ^d	i.v., single dose	Pitavastatin: 0, 3 mg/kg	4 males/group	3 mg/kg: No treatment-related effects effect.	No
	NZW rabbits ^e	In vitro (plasma and PRP)	Pitavastatin: 0, 0.1, 1, 10, 100 µmol/L	5 males/group	0.1, 1, 10, 100 µmol/L: No treatment-related effects.	No

a: Effects of pitavastatin on CNS (convulsion potentiating activity effects on pentylenetetrazole induced convulsions) or somatic nervous system (muscle relaxant effect on grip strength in the traction test); caffeine (200 mg/kg; oral) used as a positive control for pentylenetetrazole induced convulsions

b: Effects of pitavastatin on GIT (gastric juice secretion and gastric mucosa)

c: Effects of pitavastatin on bile secretion

d: Effects of pitavastatin on acetylcholine-induced hypotensive response and norepinephrine-induced pressor response

e: Effects of pitavastatin on platelet aggregation in plasma or prothrombin time (PT) and activated partial thromboplastin time (APTT) in rabbit PRP

2.6.3.4 Safety Pharmacology cont.				Test Article: Pitavastatin			Page 3 of 4
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report Number
<i>Studies Conducted with Pitavastatin cont.</i>							
Effect on CNS function (Irwin test) in mice	Crlj:CD1 (ICR) mice	Oral, single dose	Pitavastatin: 0, 3, 10, 30 mg/kg	8 males/group	All dose levels ^a . No treatment-related effects.	Yes	[F3M 06-1114]
Effect on cardiovascular and respiratory system in dogs	Conscious beagle dogs	Oral, single dose	Pitavastatin: 0, 0.1, 1, 10 mg/kg	4 males/group	0.1 and 1 mg/kg ^a . No treatment-related effects. 10 mg/kg: Significant treatment-related increase in heart rate at 3 and 6 hours after dosing. At this dose (10 mg/kg) exposure to pitavastatin, based on the maximum plasma drug concentration (C_{max}), was 44 to 110 times higher than that seen following the clinical dose of 4 mg.	Yes	[F3M 06-4115]
a: Supportive toxicokinetics data at 25 mg/kg on Day 1 from 13 week dose range finding study in Crl:CD-1(ICR)BR mice [KOW 14/952398]; C_{max} was 417 ng/mL and AUC_{0-24} 508 ng*μh/mL in male mice compared to a C_{max} of 47 ng/mL and an AUC_{0-24} of 102 ng*μh/mL in fed healthy male Caucasian subjects following a single dose of 4 mg [PKH/NKN98389N/NK-104.1.01]. b: Supportive toxicokinetics data at 10 mg/kg on Day 1 from 3 month toxicity study in beagle dogs [AG25001]; C_{max} was 5179 ng/mL in male beagle dogs at 10 mg/kg compared to a C_{max} of 47 ng/mL in fed healthy male Caucasian subjects following a single dose of 4 mg [PKH/NKN98389N/NK-104.1.01] and a C_{max} of 118 ng/mL in Japanese subjects following a single dose of 4 mg [NK-104-01].							
2.6.3.4 Safety Pharmacology cont.				Test Article: Pitavastatin			Page 4 of 4
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report Number
<i>Studies Conducted with Pitavastatin cont.</i>							
Effect on hERG current	hERG-transfected HEK293 cells	<i>In vitro</i>	Pitavastatin: 0.1, 1, 10 μ mol/L ^a	N/A	All concentrations. No treatment-related effects. The highest concentration, 10 μ mol/L, is equivalent to 4404.9 ng/mL which is approximately 10000-fold the estimated clinical plasma protein-free concentration of pitavastatin ^b .	Yes	[KOW002HG]
<i>Studies Conducted with Lactone</i>							
Effect on action potential in cardiac muscle	Hartley guinea pigs	<i>In vitro</i> (isolated papillary muscle)	Lactone: 0, 0.1, 0.3, 1, 3 μ mol/L Positive Control (E-4031): 0.1 μ mol/L ^a	5 males/group	0.1, 0.3 and 1 μ mol/L: No treatment-related effects. The concentration of 1 μ mol/L is equivalent to 403.45 ng/mL which is approximately 1000-fold the estimated clinical plasma protein-free concentration of lactone ^b . 3 μ mol/L: The action-potential duration (APD) at 60 and 90% of repolarisation (APD ₆₀ and APD ₉₀) was significantly prolonged without affecting APD at 30% of repolarisation (APD ₃₀), as compared with the control group ($p < 0.01$).	Yes	[KOW001MG]
Effect on hERG current	hERG-transfected HEK293 cells	<i>In vitro</i>	Lactone: 0.1, 0.3, 1 μ mol/L ^a	N/A	0.1 and 0.3 μ mol/L: No treatment-related effects. The concentration of 0.3 μ mol/L is equivalent to 121 ng/mL which is approximately 300-fold the estimated clinical plasma protein-free concentration of lactone ^b . 1 μ mol/L: The compensated suppression rate was 12.9% ($p < 0.05$).	Yes	[KOW002HG]
a: Pitavastatin and/or lactone stability and concentrations were determined in the test preparations. b: The C_{max} of pitavastatin and lactone were 55 and 35 ng/mL, respectively, in fed healthy male Caucasian subjects following repeat dosing for 14 days at 4 mg [PKH/NKN98389N/NK-104.1.01]. The protein unbound ratio in human plasma used in the calculations is 0.5% (range of 0.4 to 0.5%) [Fujino et al., 1999b] for pitavastatin and 1.1% (range of 0.67 to 1.05%) for lactone [R107017]; this is equivalent to 0.275 ng/mL unbound pitavastatin (55×0.005) and 0.385 ng/mL unbound lactone (35×0.011). For pitavastatin 1 μmol/L is equivalent to 440.49 ng/mL assuming a molecular weight of 440.49 (the molecular weight of 880.1 is used for two molecules of pitavastatin combined with one molecule of calcium). For lactone 1 μmol/L is equivalent to 403.45 ng/mL assuming a molecular weight of 403.45.							
2.6.3.5 Pharmacodynamic Drug Interactions				Test Article: Pitavastatin			Page 1 of 1
Organ / System Evaluated	Species/ Strain	Method of Administration	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Report Number
<i>Studies Conducted with Pitavastatin</i>							
Interaction with warfarin	Jla: Wistar rats	Oral, single and repeat dose in combination with warfarin (intraperitoneal route)	Single dose: Control (vehicle) Pitavastatin: 3, 10, 30 mg/kg Simvastatin: 10, 30, 100 mg/kg Repeat dose: Control (vehicle) Pitavastatin: 30 mg/kg Simvastatin: 100 mg/kg	7 males/group	Pitavastatin and simvastatin had no significant effect on the warfarin-induced prolongation of PT.		[R99029]

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Twenty analytical methods and validation reports, including lower limit of quantitation (LLOQ), upper limit, and brief description of methods, were submitted to the NDA.

2.6.4.1 Brief summary

Absorption

Pharmacokinetics in mice reveals higher C_{max} and AUC in female compared to male mice and rats. However the terminal half-life was longer in males than females. No difference in exposure was seen in Wistar compared to Sprague Dawley rats. Saturation of

absorption was seen at ≥ 250 mg/kg based on a less than dose proportional plasma increase with oral dosing in rats.

Bioavailability in was 80-97% following dosing of 1 mg/kg in rats, rabbits and dogs. In monkeys bioavailability was 19%. Rabbits showed the highest exposure per dose suggesting the highest bioavailability.

Distribution

The highest plasma protein-binding was detected in humans >rabbits >rats >monkeys >dogs >mice. Plasma protein binding (albumin, α 1-acid glycoprotein) was >96% in all species including humans.

Pitavastatin distributes to the liver where liver $C_{\max} = 3812$ ng/ml and liver $AUC_{0-\infty} = 10853$ ng·h/ml ($t_{1/2} = 3$ h in liver) in rats given a single oral dose of 1 mg/kg. Except for the liver and gastrointestinal tract, distribution was similar or lower than the plasma in other organs.

In male monkeys following a single oral dose of 3 mg/kg [^{14} C]-pitavastatin levels are highest in the bile, liver and kidney at 2308, 21.9, and 2.7 times plasma, respectively, at 4 hours post-dose. Other tissue levels were lower than plasma. The cerebrum and cerebellum were much lower than plasma radioactivity. Pitavastatin crosses the blood brain barrier.

In rats, pitavastatin was distributed (relative to plasma) to liver(27) >kidney(1.9) >plasma(1.0) >heart(0.5) >lung(0.4) >skeletal muscle(0.1) >brain(not detected).

Distribution of pitavastatin was highest into lens of dogs >mice compared to other non-clinical species (and were lowest in humans). Distribution of pitavastatin to lens/eye in pigmented rats suggests an absence of appreciable levels in the eye of pigmented rats suggesting that pitavastatin does not bind to melanin. Therefore higher distribution/binding to ocular tissue alone can not explain the lens findings.

Pitavastatin crosses the placenta and is secreted into milk. Pitavastatin (parent) was the primary form in rat milk. Pitavastatin (1 mg/kg) given to pregnant rats during organogenesis resulted in detectable fetal levels (~10% of maternal plasma levels). Pitavastatin given to lactating female rats (1 mg/kg) resulted in 7-fold higher levels in milk compared to plasma 6 hours post-administration.

The proposed mechanism of uptake of pitavastatin from the plasma by the liver is OATP1B1. Single intravenous (1 mg/kg) dosing in P-glycoprotein knockout mice results increase levels compared to normal mice at 1 hour post-dose, but a similar distribution pattern as observed in normal mice was present at 6 hours post-dose. These findings suggest that pitavastatin may interact with P-glycoprotein.

Metabolism

The major metabolic pathways of pitavastatin include glucuronidation with subsequent hydrolysis of the glucuronide and cyclization forming the lactone, beta-oxidation of the side chain (5-keto-pitavastatin), and hydroxylation of the ring (8-hydroxypitavastatin). The lactone may further hydrolyze, which returns the lactone to the parent form. Thus, pitavastatin and pitavastatin lactone are in dynamic equilibrium *in vivo*. The lactone is a circulating metabolite in monkey and dog that is undetectable in rat and mouse plasma. The lactone is considered the major human metabolite. While the Sponsor submitted data to show that the lactone metabolite lacks a pharmacodynamic effect on the HMG-CoA reductase enzyme, the currently available data do not exclude some pharmacodynamic effect by the lactone.

Metabolites M-6 and M-8 were found in heart and skeletal muscle after repeated dosing (1 mg/kg) pitavastatin at levels 2-4 times higher than parent. Other metabolites (M-2, 4-8, 10, 11; lactone, 5-ketone) were found in bile or feces at levels below that of parent. In dog, lactone was present with a C_{max} 14% of the parent following a single 1 mg/kg oral dose. In monkey following a single oral dose of 3 mg/kg, M-14 and 5-ketone were detected in plasma and lactone, M-14, 8-hydroxypitavastatin and other polar metabolites were observed in the bile/feces. M-14 was the major urinary metabolite in monkeys. The Sponsor reported that 8-hydroxypitavastatin was formed in microsomes from all species although dog had the lowest level (0.4% of total) and monkey had the highest (38% of total). *In vivo*, urinary and fecal detection of 8-hydroxypitavastatin was observed in monkeys (<2%) but plasma levels were undetectable. 8-Hydroxypitavastatin is pharmacologically active and may be more potent than parent based on *in vitro* assessments. Together this data indicates that the major as well as active metabolites of pitavastatin were observed in the test species. CYP2C8 and 2C9 are the oxidative enzymes that form 8-hydroxypitavastatin in humans. Several enzymes including UGT-glucuronidases are involved in the formation of the lactone which is in equilibrium with the parent compound across species. The potential for optical isomerization does not appear to occur in dogs as no detectable (b) (4) were detected. A similar study in rats for *in vivo* isomerization was confounded by peak interference in the analysis.

Excretion

Fecal excretion was the major route of elimination in rats, dogs, monkeys and humans. Urinary excretion predominates in rabbit. Enterohepatic recirculation occurs in rats (up to 70%) and dogs as determined through bile-cannulated models

Acute, subchronic and chronic toxicology studies were performed in a variety of animal species including mice, rat, dog and monkey with pitavastatin. The chronic 6-month rat toxicity study establishes a NOAEL of 1 mg/kg/day (1,170 ng·h/ml) which based on AUC comparisons to the 4 mg (153 ng·h/ml) maximum human dose (MRHD) of 8-fold. The chronic 12-month dog study establishes a NOEL of 0.3 mg/kg/day (630 ng·h/ml) which based on AUC comparisons to the 4 mg (153 ng·h/ml) maximum human dose (MRHD) of 4-fold. Similarly a 6-month monkey toxicity study establishes a NOAEL of 3 mg/kg/day (850 ng·h/ml) which based on AUC comparisons to the 4 mg (153 ng·h/ml)

maximum human dose (MRHD) of 6-fold. These studies would support chronic dosing at 4 mg/day in humans. Additional studies were performed to explore potential mechanisms of the identified target organ toxicities (lens, kidney, thyroid, liver, skeletal muscle, lung, forestomach) as well as qualification of various metabolites (lactone, 8-hydroxypitavastatin formed in humans) and impurities^{(b) (4)}

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

Exposure to NK-104 (AUC) as a function of dose was greatest in rabbits >>dogs ≥humans >rats >monkeys. NK-104 lactone was not detected in mouse plasma at oral doses up to 300 mg/kg. Only very small amounts of NK-104 lactone were identified in male and female rats (<1% of NK-104 concentrations). NK-104 lactone was the primary metabolite in humans and dogs.

A95001 – Plasma drug level in mice (Crj:CD-1) in single oral dose toxicology study of NK-104

Key study findings:

- NK-104 lactone was not detected in mouse plasma at oral doses up to 300 mg/kg.

Pharmacokinetics of NK-104 and NK-104 Lactone in the Mouse				
Drug	Dose (mg/kg)	T _{max} (hr)	C _{max} (µg/mL)	AUC (µg·hr/mL)
NK-104	30	0.5	1.6	1.8
	100	0.5	14.2	14.3
	300	0.5	26.7	36.9
NK-104 lactone	30	ND	ND	ND
	100	ND	ND	ND
	300	ND	ND	ND

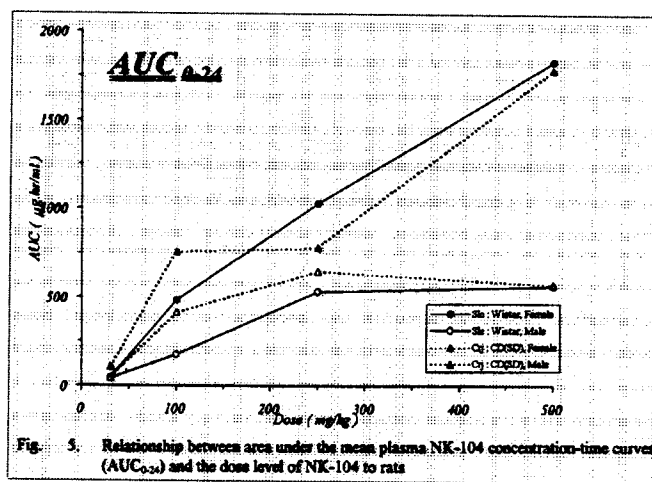
Male and female Crj:CD-1(ICR) mice (n=8/sex/group) were administered a single oral dose of 30, 100, and 300 mg/kg NK-104. Blood samples were obtained at 0.5, 1, 2, 4, 6, and 8 hours post-administration and pharmacokinetic parameters (C_{max} and AUC) were determined. C_{max} and AUC tended to increase more sharply in female than in male mice. T_{max} was 0.5 hours at all concentrations and in both sexes.

RF9933 – Plasma concentration of NK-104 after single oral administration to rats

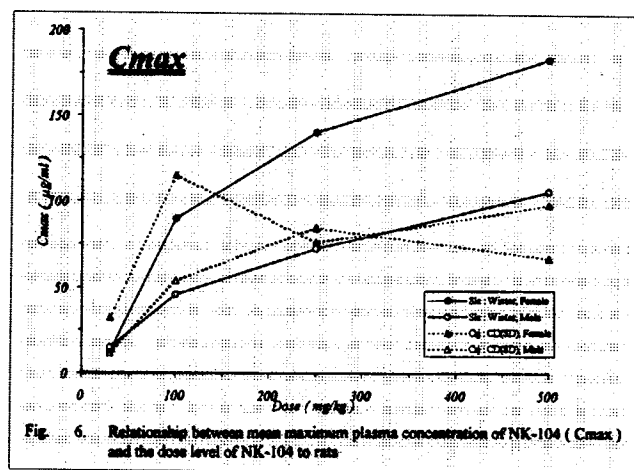
Key study findings:

- Only very small amounts of NK-104 lactone were identified in male and female rats (<1% of NK-104 concentrations).
- Saturated absorption was postulated at doses of 250 mg/kg and above, based the shape of the exposure curve.

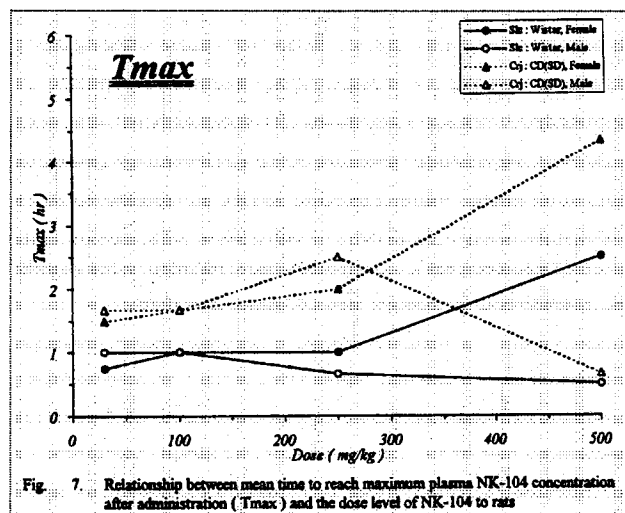
Male and female Crj:CD (SD) rats (n=3/sex/group) and male and female Slc:Wistar rats (n=3/sex/group) were administered single oral doses of 30, 100, 250, or 500 mg/kg NK-104. The maximum tolerated dose was previously determined to be approximately 500 mg/kg in rats (See RG25001). There was little difference between pharmacokinetic parameters based on strain. However, sex-specific differences emerged at doses of 100 mg/kg and higher; C_{max} and AUC were 32-68% lower in males in comparison with females. Only very small amounts of NK-104 lactone were identified in male and female rats (<1% of NK-104 concentrations). Saturated absorption was postulated at doses of 250 mg/kg and above, based the shape of the exposure curve.



(Sponsor, M4, RF9933, p17)



(Sponsor, M4, RF9933, p17)



(Sponsor, M4, RF9933, p18)