CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-629

PHARMACOLOGY REVIEW

MEMORANDUM
April 16, 2004

TO: File
FROM: Kenneth L. Hastings, Dr.P.H.

SUBJECT: NDA 21-629

I have reviewed the pharmacology/toxicology information provided for Apidra® (insulin glulisine) and concur with the recommendation by the primary reviewer, Dr. Herman Rhee and the pharmacology/toxicology supervisor, Dr. Jeri El-Hage that the NDA should be approved. The final product label is acceptable.

/S/

Kenneth L. Hastings, Dr.P.H.
Associate Director for Pharmacology and Toxicology
Office of Drug Evaluation II

NDA #:21-629

Product Name : Apidra (insulin glulisine, HMR1964)

Sponsor: Aventis Pharmaceuticals, Inc.

Indication: Diabetes

Division: Metabolic and Endocrine Drug Products

Reviewer: Herman Rhee, Ph.D.

Date: January 23, 2004

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EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability: Approval
Preclinical pharmacology and toxicology recommends approval of NDA21-629, based on preclinical findings on Apidra (insulin glulisine, HMR1964) as reviewed in this document.

1.2 Recommendation for nonclinical studies: None.

Recommendations on labeling:

1. The following preclinical findings should be included in labeling instructions.

a. Carcinogenicity, Mutagenicity, Impairment of Fertility

Standard 2-year carcinogenicity studies in animals have not been performed. In Sprague Dawley rats, a 12- month repeat dose toxicity study was conducted with insulin glulisine at subcutaneous doses of 2.5, 5, 20 or 50 IU/kg twice daily (dose resulting in an exposure 1, 2, 8, and – times the average human dose, based on body surface area comparison). There was a non-dose dependent higher incidence of mammary gland tumors in female rats administered insulin glulisine compared to untreated controls. The incidence of mammary tumors for insulin glulisine were similar to human insulin. The relevance of these findings to humans is not known.

Insulin glulisine was not mutagenic in the following tests: Ames test, in vitro mammalian chromosome aberration test in V79 Chinese hamster cells and in erythrocyte micronucleus test.

In fertility studies in male and female rats, subcutaneous doses up to 10 IU/kg once daily (dose resulting in an exposure 2 times the average human dose, based on body surface area comparison) had no adverse effects on male and female fertility, or general reproductive performance of animals were observed.

b. Pregnancy - Teratogenic Effects - Pregnancy Category C

Reproduction and teratology studies have been performed with insulin glulisine in rats and rabbits using regular human insulin as a comparator. The drug was given to female rats throughout pregnancy at subcutaneous doses up to 10 IU/ kg once daily (dose resulting in an exposure equivalent to approximately 2 times the average human dose, based on body surface area comparison). Insulin glulisine did not have remarkable toxic effects on the embryo-fetal development in rats.

HMR1964 was given to female rabbits throughout pregnancy at subcutaneous doses up to 1.5 IU/ kg/day (0.5 times the average human dose, based on body surface area

comparison). Adverse effects on embryo-fetal development were only seen at maternal toxic dose levels inducing hypoglycemia. Increased incidence of post- implantation losses and skeletal defects were observed at a dose level of 1.5 IU/ kg once daily (dose resulting in an exposure 0.5 times the average human dose, based on body surface area comparison) that also caused mortality in dams. A slight increased incidence of post-implantation losses was seen at the next lower dose level of 0.5 IU/ kg once daily (dose resulting in an exposure 0.2 times the average human dose, based on body surface area comparison), which was also associated with severe hypoglycemia but there were no defects at that dose. No effects were observed in rabbits at a dose of 0.25 IU/ kg once daily (dose resulting in an exposure 0.1 times the average human dose, based on body surface area comparison). The effects of insulin glulisine did not differ from those observed with subcutaneous regular human insulin at the same doses and were attributed to secondary effects of maternal hypoglycemia. There are no well-controlled clinical studies of the use of insulin glulisine in pregnant women.

c. Nursing Mothers-It is unknown whether insulin glulisine is excreted in human milk, ——— human insulin · ———— excreted in human milk. For this reason, caution should be exercised when APIDRA is administered to a nursing woman.

d. Pediatric Use-Safety and effectiveness of APIDRA in pediatric patients have not been established.

3.1 Introduction:

Insulin glulisine (HMR1964, 3βLys-29βGlu-human insulin, hereafter referred to as Apidra or glulisine) is a recombinant rapid-acting insulin analog produced using *Escherichia coli (E. coli*). Glulisine differs from human insulin by the replacement of asparagine in position β3 by lysine and lysine at position β29 by glutamic acid [3βLys-29βGlu-human insulin]. The target indication for glulisine is the treatment of diabetes mellitus.

Preclinical and clinical studies have shown that glulisine displays a time-concentration and time-action profile with a more rapid onset, earlier peak effect in lowering blood glucose levels, and a shorter duration of action than regular human insulin. The time-concentration and time-action profiles of glulisine define it as a member of the rapid-acting insulin subfamily of short-acting insulin preparations. Other approved rapid-acting insulin analogues include insulin lispro and insulin aspart. The time-action profile of glulisine suggests that subcutaneous (s.c.) injection 0 to 15 minutes before a meal or immediately following a meal may provide convenient prandial glycemic control in patients with diabetes mellitus.

The adult clinical program for glulisine consisted of 13 clinical pharmacology studies and 4 international, well-controlled Phase III studies (3 active-controlled studies that evaluated the efficacy and safety of s.c. administered glulisine, plus 1 active-controlled safety study to support the use of glulisine administered by continuous s.c. insulin infusion). The Phase III efficacy studies were specifically designed to evaluate the safety

and efficacy of glulisine in subjects with type 1 and type 2 diabetes, and also to evaluate the immediate post meal dosing of glulisine. The total number of subjects exposed to glulisine was more than 1500.

Summary of nonclinical findings:

The nonclinical pharmacology studies were designed to demonstrate that the mode of action of insulin glulisine *in vitro* and *in vivo* is similar to that of human insulin. *In vivo* studies were also performed to demonstrate the rapid action of insulin glulisine, compared to regular human insulin. The *in vitro* nonclinical pharmacology studies compared the activity of insulin glulisine with that of human insulin and several insulin analogs, with the aim of demonstrating similar *in vitro* activities for insulin receptor binding and metabolic responses. These studies were carried out in several appropriate cell types. *In vitro* studies also investigated other pharmacological activities of insulin glulisine, compared to human insulin and several insulin analogs, including insulin-like growth factor-1 (IGF-1) receptor binding and mitogenic activity. The potential to cause proliferative activity in mammary glands was also studied *in a* 12-month toxicity study in rats.

General safety pharmacology parameters, particularly cardiovascular safety, of subcutaneous insulin glulisine were assessed in telemetered Beagle dogs. Pharmacodynamic drug interaction studies were carried out in dogs using mixtures of insulin glulisine and NPH insulin to determine whether they are suitable for mixing immediately before injection; this is a standard procedure with human insulin or insulin lispro.

Single- and repeated-dose toxicity, genotoxicity, reproductive and developmental toxicity, local tolerance and immunogenicity studies were conducted in order to predict the safety profile for the therapeutic use of insulin glulisine in humans. In all species tested and all studies conducted, insulin glulisine was injected subcutaneously because this injection route is intended for the therapeutic use of insulin glulisine in humans. Exposures at the NOAEL in all of the toxicity studies were used to predict safety margins for use of insulin glulisine in humans based on estimated average dose.

Subchronic and chronic toxicity after repeated daily doses of insulin glulisine were studied for up to 6 months in Beagle dogs and 12 months in Sprague Dawley rats. Study duration was appropriate to support the intended long-term use of insulin glulisine in diabetic patients. A 12-month repeated-dose toxicity study was performed in rats to evaluate the carcinogenic potential of the compound and the mitogenic potential was evaluated by measuring cell proliferation in mammary glands using Ki-67 immunohistochemistry in the 6- and 12-month study. Reproductive toxicology study was conducted to evaluate the effects of insulin glulisine on the reproductive performance of rats (fertility, embryo-fetal and postnatal development) and rabbits (embryo-fetal development).

In summary, the present data show that HMR 1964 behaves like human insulin with respect to receptor binding and activation of the initial insulin signaling cascade. HMR 1964 relative to human insulin shows no enhanced promotion of thymidine incorporation into newly synthesized DNA. These results are in contrast to Asp(B10) insulin which exhibits an increased insulin receptor binding and induces a prolonged phosphorylation state of the insulin receptor and receptor substrates. Insulin glulisine bound to the insulin receptor and stimulated metabolic activity with a slightly lower affinity than human insulin. The observation that the structural modifications in insulin glulisine do not substantially impair insulin receptor binding and *in vitro* activity is in accordance with published findings that both positions β3 and β29 are not directly involved in insulin receptor interactions.

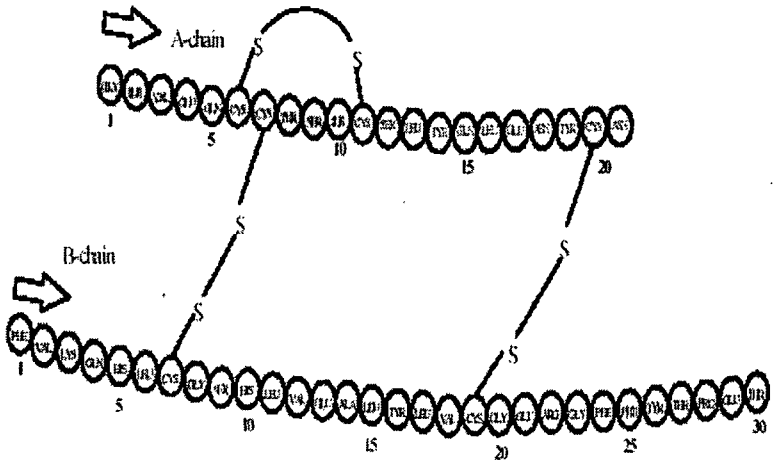
PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION AND DRUG HISTORY

NDA number: 21-629
Review number: 001
Sequence number/date/type of submission: June 18, 2003/Commercial
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Aventis Pharmaceuticals Inc., Bridgewater, NH 08807
Tel(908)304-7000

Manufacturer for drug substance: Aventis Pharmaceuticals Inc., Bridgewater, NH
Reviewer name: **Herman Rhee, Ph.D.**
Division name: Metabolic and Endocrine Drug Products
HFD #: **510**
Review completion date: Jan. 30, 2004
Drug:

Trade name: Apidra
Generic name: Insulin glulisine (rDNA origin) injection
Code name: HMR1964
Chemical name: 3βLys-29βGlu-human insulin
CAS registry number: 207748-29-6
Molecular formula/molecular weight: $C_{258}H_{384}O_{78}N_{64}S_6$/5823
Structure:



Relevant INDs/NDAs/DMFs: IND# [redacted] #61,956(HMR 1964)/NDA#19-938(Novolin R), #20-986(NovoLog), and #21-081(Lantus)

Drug class: Short acting, soluble human insulin analogue
Indication: Diabetes

Clinical formulation:
Insulin glulisine is available as a solution for injection containing 3.49 mg/mL insulin glulisine [equimolar to 100 I.U. (International Units) of insulin] and 3.15 mg/mL m-cresol. Concentrations of each component are summarized in Table 1 below.

Table 1 - Components and composition

COMPONENTS(a)	COMPOSITION		FUNCTION	REFERENCE TO STANDARDS(b)
	Percentage	Per unit (1 mL)		
Insulin glulisine equimolar to I U (International Units) of insulin	0.349	3.49 mg [100]	active substance	in-house
Metacresol [m-Cresol] (c)	0.315	3.15 mg	[redacted]	USP
Trometamol [Tromethamine]	0.600	6.00 mg	[redacted]	Ph. Eur. USP
Sodium chloride	0.500	5.00 mg		Ph. Eur.; USP
Polysorbate 20	0.001	0.01 mg		Ph. Eur.; NF
Sodium hydroxide	q.s.	q. s. ad pH 7.3	[redacted]	Ph. Eur.; NF
Hydrochloric acid, concentrated [Hydrochloric acid]	q.s.	q. s. ad pH 7.3		Ph. Eur. NF
Water for injection	ad 100	ad 1.00 mL		Ph. Eur.; USP

(a) Components are listed according to their pharmacopoeial names. If more than one monograph exists, other names are given in brackets, along with the compendial origin.

(b) The current edition of the Pharmacopoeia is always referred to.

(c) For Metacresol, the common chemical name "m-Cresol" is preferably used in other documents.

Route of administration: Subcutaneous
Proposed use: To control diabetes with insulin analogue with fast onset of action.
Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.
Studies reviewed within this submission: Please see the reviews below.
Studies not reviewed within this submission: NA

3.2 PHARMACOLOGY

3.2.1 Brief summary

Insulin glulisine given to rats s.c. in doses of 0.5 IU/kg was as effective as insulin lispro and showed a slightly higher total blood glucose-lowering activity than human insulin. The blood glucose-lowering activity of insulin glulisine after s.c. administration was further assessed in euglycemic clamp studies in dogs. Overall, insulin glulisine demonstrated the pharmacodynamic properties of a rapid-acting insulin. The time-action profile of insulin glulisine displayed a significantly faster onset and shorter duration of action than regular human insulin and was not statistically different from the profile of insulin lispro. This pharmacodynamic profile of insulin glulisine was unchanged by the presence of 0.01 mg/mL polysorbate 20 (Tween 20) in the final pharmaceutical formulation. [redacted] to the insulin glulisine formulation progressively attenuated the rapid-acting time action profile of insulin glulisine. Based on hypoglycemic activity of various mixtures of human regular insulin or rapid-acting insulin analogs with human NPH insulin it was concluded that insulin glulisine is suitable to be mixed with human NPH insulin immediately before injection, as is done with human regular insulin and insulin lispro.

The clamp studies in conscious dogs did not reveal any clinically relevant effect on vital functions. Subcutaneous injection of up to 1.0 IU/kg insulin glulisine in telemetered dogs caused a decrease in systolic blood pressure and an increase in heart rate and corrected Q-T interval as shown below. The positive chronotropic effect is known to be caused by insulin-induced hypoglycemia. The observed moderate, but statistically significant increase in corrected QT interval duration (QTc-Bazett's) is known to occur with insulin-induced hypoglycemia in man.

In summary, insulin glulisine when tested in dogs has the pharmacodynamic properties of a rapid acting human insulin. The *in vitro* and *in vivo* pharmacology data do not indicate a specific risk for clinical use of glulisine. The *in vitro* data in several cell lines indicate no clear enhanced mitogenic potential.

3.2.2 Primary pharmacodynamics

Mechanism of action:

In an effort to understand mechanistic activity HMR1964 binding to human insulin receptor was compared with that of human insulin and HMR1153 (3βLys-28βLeu-human insulin). The table below summarizes the study, which indicates that the relative binding affinity to the isolated human insulin receptor was comparable to human insulin. Amino acid substitutions might affect intracellular signaling pathways, which affected the mitogenic activity of some insulin analogues. HMR1964 had similar activities to

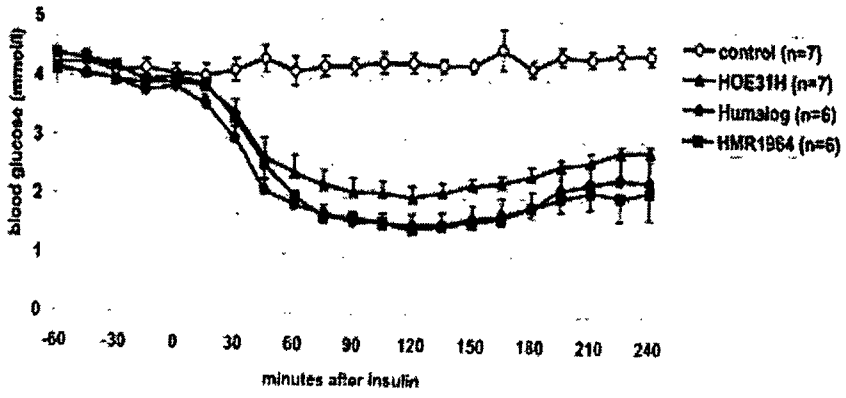
human insulin in thymidine incorporation into newly synthesized DNA and prolonged phosphorylation state of the insulin receptor and its substrate.

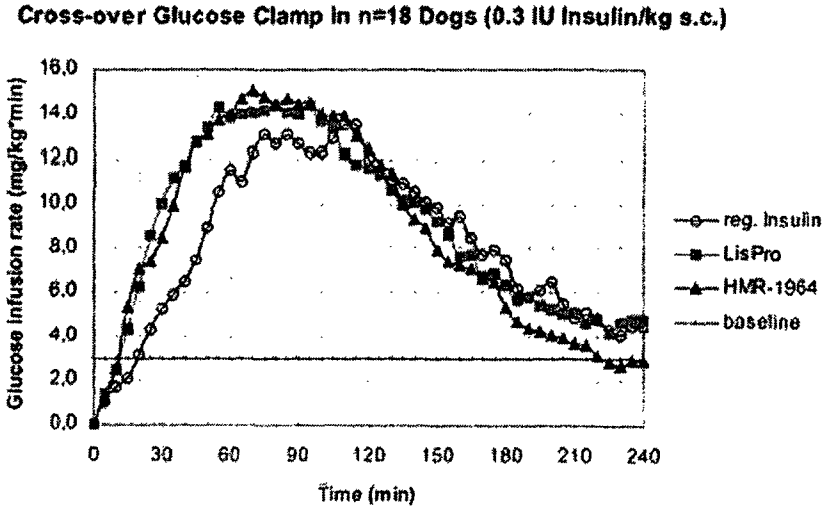
Compound	Experiment	IC_{50} values [pM]	IC_{50} Ratio (Human Insulin/ HMR xxxx)
HMR 1964	1	14.41	0.68
	2	8.668	0.71
HMR 1153	1	13.13	0.75
	2	7.942	0.78
Human Insulin	1	9.823	1.00
	2	6.165	1.00

Drug activity related to proposed indication:

The maximal response in stimulating lipogenesis and glucose transport in isolated rat adipocytes was compared between HMR1964 and human insulin. The $EC_{50\ values}$ for lipogenesis and glucose transport for HMR1964 were slight higher than the values with human insulin as shown below. Aventis comparator HMR1153 was also slightly less potent, compared to human insulin. In anesthetized starved male Wistar rats, blood glucose lowering effect of HMR1964 was also compared with several other drugs. HMR1964, Humalog, or HOE31H (human insulin) was administered at an equal dose of 0.5 IU/kg s.c. to 6 to 7 rats. All the drugs reduced blood glucose 30 minutes after drug administration, an effect which lasted for several hours as illustrated below. The magnitude of the peak effects was comparable for in the three agents. Similar results were observed also for glucose infusion rate in cross-over glucose clamp study in dog. Human insulin, LisPro, or HMR1964 were administered at a dose of 0.3 IU/kg s.c. to dogs, which required comparable amounts of exogenous glucose infusion after the three drugs (Please see the second figure below).

	EC50 values [nM]		
	Human Insulin	HMR 1964	HMR 1153
Lipogenesis	0.055 ± 0.009	0.126 ± 0.011	0.126 ± 0.013
Glucose Transport	0.049 ± 0.011	0.110 ± 0.023	0.107 ± 0.039
mean	0.052	0.118	0.116
potency ratio	1	0.44	0.45





3.2. 3 Secondary pharmacodynamics:

Although insulin has been shown to exert growth-promoting effects through its own receptor, interaction of insulin with IGF-1 receptors may significantly contribute to the mitogenic potency of this hormone, specifically in muscle tissue. The human osteosarcoma derived cell line B10 that expresses at least 30-times more IGF-I than insulin receptors was used for this study. The IGF-I receptor binding capabilities of the hormones were determined in a competition assay using ^{131}I-labeled IGF-I as tracer. Human IGF-I binds to its receptor at picomolar concentrations (IC_{50} in the range of 50 pM). In contrast, all of the investigated insulin derivatives as well as human insulin required at least 1500-times higher concentrated solutions to displace 50 % of the IGF-I

Insulin analogues HOE 901 and Asp(B10) showed significantly increased IGF-I receptor affinities (about 8-, and 4-fold, respectively) compared to human insulin. The affinity of the insulin derivative LisPro was slightly but not significantly increased compared to human insulin. The analogues HMR 1153 and Asp(B28) showed a slightly weaker affinity to the IGF-I receptor than human insulin. HMR 1964 and another analogue, HMR1747, bound with 4- to 5-fold reduced affinity against the IGF-I receptor compared to human insulin.

TITLE: HMR1964: IRS(Insulin Receptor Substrate)-1/IRS-2 Signaling in Human and Rat Myoblasts and Rat Cardiomyocytes in comparison to Human Insulin and Other Insulin analogues

Study no.: F2003PHM0032

Volume # and page #: Module 4 (Aventis 4.2.1.1.3StudyF2003phm0032)
Conducting laboratory and location:

Date of study initiation: May 1998
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Batch#1/98

Materials:
1. Apidra(HMR1964, Batch# 1/98)
2. Human insulin (Batch# 103)
3. Comparative compound 1, HMR 1153 (Batch# T4152/52)
4. Comparative compound 2, Asp(B10) Human insulin (Batch# 060493)
5. K6 myoblasts from rat heart muscle cell lines (dominantly express IGF-1)
6. Human skeletal muscle cells from M. rectus abdominis of 28-year old male Caucasian that express insulin receptors.
7. Isolated adult rat cardiomyocytes for glucose uptake study.

Methods:
IRS activation:

Observation and Results:

Tyrosine phosphorylation of IRS-1/2 in rat cardiomyoblasts:

Human insulin produced the strongest tyrosine phosphorylation of both IRS molecules after stimulation of rat cardiomyoblasts as indicated by (Table 9.1). However, Asp(B10) insulin and HMR 1153 induced a less pronounced stimulation of both IRS proteins. HMR 1964 induced only a marginal phosphorylation of IRS-1, but a strong phosphorylation of IRS-2 similar to that seen after

stimulation with human insulin (Fig. 9.1). It appears that the differential phosphorylation after human insulin (HI) and HMR1964 was the outcome of quantitative evaluation of the — because similar amounts of anti-phosphotyrosine antibodies were loaded to each gel.

Quantitative evaluation of Western Blots (Fig. 9.2) demonstrated an approximately 30-fold and a nearly 20-fold response after treatment with human insulin for IRS-1 and IRS-2, respectively. HMR 1964 exerted a marginal 2-fold effect on the activation of IRS-1, but induced a 20-fold increase of the IRS-2 phosphorylation being as effective as human insulin (Fig. 9.2). Increase of tyrosine phosphorylation was significantly lower after stimulation with Asp(B10) insulin and HMR 1153 for both IRS-1/2 (Please see Table 9.1 and Figure 9.2 below).

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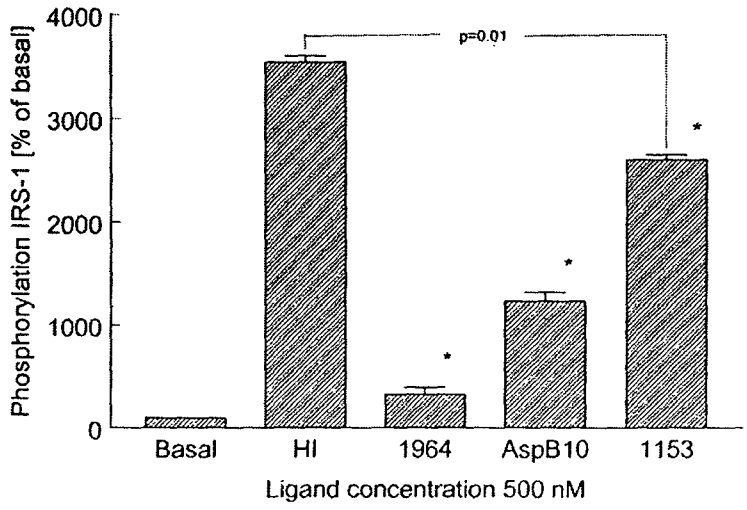
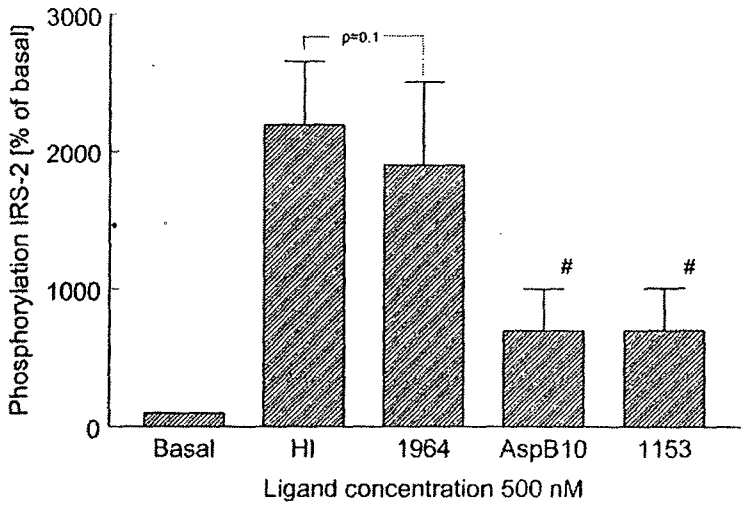
9.2 Densitometric evaluation of tyrosine phosphorylation of IRS proteins in rat K6 myoblasts





Signals as shown in Fig. 9.1 were quantified using ——— software. The data shown are mean values ± SEM of 3-4 separate experiments.
* Significantly different from basal and all other stimulated values ($p<0.05$);
# significantly different from HI and 1964 ($p<0.05$).

Tyrosine phosphorylation of IRS-1 and IRS-2 in human myoblasts

To compare the extent of insulin receptor phosphorylation in other tissues, the experiments were repeated in proliferating primary human skeletal muscle cells using exactly the same protocol as for the K6 myoblasts. Analysis of tyrosine phosphorylation of IRS-1 and IRS-2 by immunoblotting again revealed a strong phosphorylation of both IRS-1 and IRS-2 after stimulation with human insulin.

The effects of Asp(B10) insulin were almost equipotent to human insulin (Fig. 9.3). HMR 1964 induced a marginal phosphorylation of IRS-1 but a strong tyrosine phosphorylation of IRS-2 that was even higher than that seen after stimulation with human insulin (Fig. 9.4). Stimulation of tyrosine phosphorylation by AspB10 insulin and HMR 1153 was less than with human insulin for both IRS proteins.

Tyrosine phosphorylation of IRS-1 and IRS-2 was also compared in primary adult rat cardiomyocytes expressing a high level of insulin receptors but much less IGF-1 receptors than myoblasts. The same conditions were used as for K6 cells and human myoblasts. Human insulin produced a strong phosphorylation of both IRS-1 and IRS-2, whereas Asp(B10) insulin and HMR 1153 were less effective. Again, tyrosine phosphorylation of IRS-1 was only marginally activated by HMR 1964 (2-fold) which on the other hand produced an 18-fold increase of the tyrosine phosphorylation of IRS-2 reaching the same level as that seen with human insulin. This suggests that human skeletal muscle cells and adult rat cardiomyocytes were qualitatively similarly phosphorylated by HMR1964. Relative to human insulin, Asp(B10) insulin and HMR 1153 were about half as effective as human insulin at both IRS proteins.

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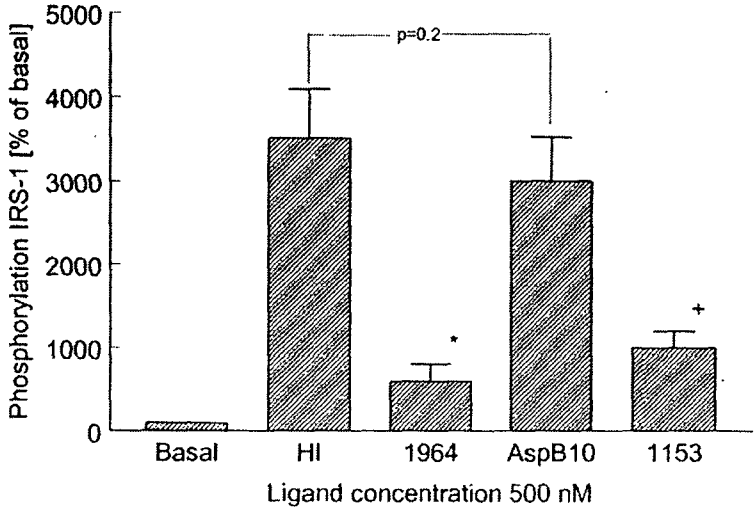
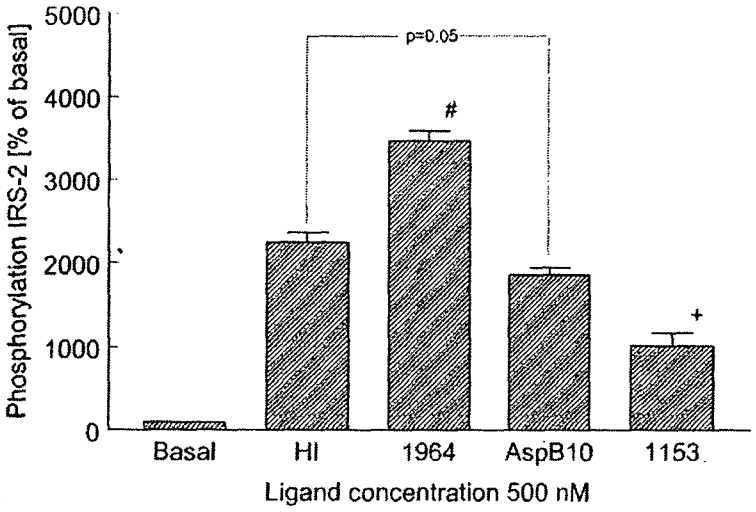
9.4 Densitometric evaluation of tyrosine phosphorylation of IRS proteins in proliferating human skeletal muscle cells



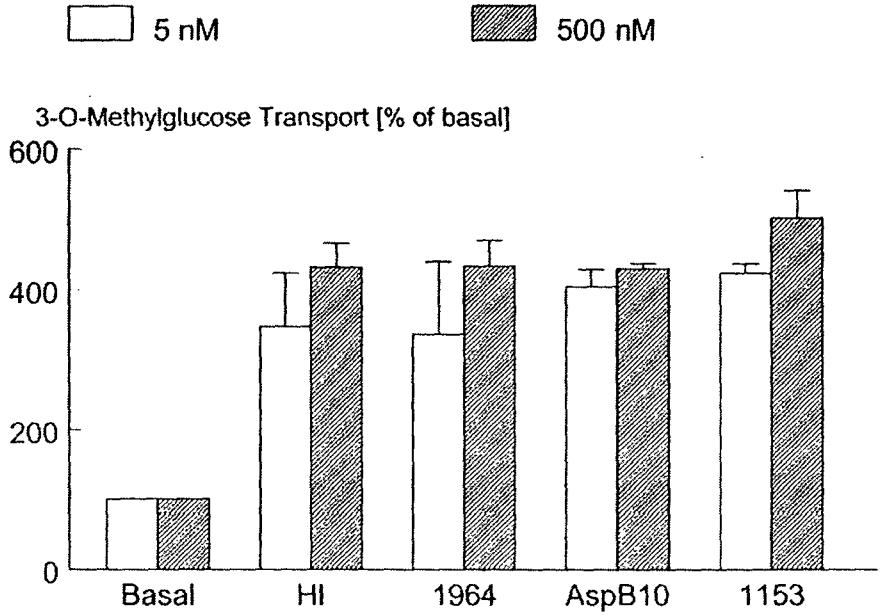


Signals as shown in Fig. 9.3 were quantified using ——— software. Data are mean values ± SEM of 4-5 separate eperiments.
* Significantly different from basal and all other stimulated values ($p<0.05$);
# significantly different from human insulin ($p<0.05$);
+ significantly different from all other values ($p<0.05$).

Glucose transport in adult rat cardiomyocytes

Stimulation of 3-0-methylglucose transport has been measured using the adult cardiomyocyte system. These cells have been extensively used for studies on insulin signaling and insulin action [Kessler et al., Am J. Physiol. Endocrinol. Metabolism 280, 2001]. The initial rate of glucose transport was increased 3.5- and 4.3-fold in response to 5 and 500 nM human insulin (HI), respectively (Fig. 9.7). Almost identical values were achieved after treatment of the cells (4×10^5 cells/ml) with HMR1964. Similar or slight increased responses were observed after treatment of cells with Asp(B10) insulin and HMR 1153 as shown in fig. 9.7 below.

9.7 Transport of 3-O-methylglucose in adult rat cardiomyocytes



4×10^5 cells/ml were incubated for 10 min in the absence (basal) or presence of the indicated concentrations of human insulin or insulin analogs. Initial rates of 3-O-methylglucose were then determined over a 10-s assay period as outlined in the Methods section. Data are mean values ± SEM of 3-4 separate experiments.

Summary and Conclusion:

Human insulin exerts a prominent and equal activation of IRS-1 and IRS-2 in both rat and human myoblasts and adult cardiomyocytes. The analog HMR 1964 produces only a marginal activation of IRS-1 [redacted] but a prominent activation of IRS-2 which is at least equal to that induced by human insulin (rat myoblasts and cardiomyocytes) or even higher (human myoblasts). Both comparators, HMR 1153 and Asp(B10) insulin do not exhibit this differential activation of IRS-1 and IRS-2 as shown for HMR 1964.

The fact that Apidra has relatively small IRS-1 stimulatory effects [redacted] which suggests its limited stimulatory action of IGF-1 like action since the IRS-1 has been implicated for mitogenic actions of IGF-1 receptors in [redacted] (Rother et al., J Biol. Chem. 273, 1998). Interestingly, it still has a strong stimulatory action on IRS-2, which is known to be related to metabolic effects of insulin.

In conclusion, the results of the present investigation demonstrate that Apidra induced a preferential activation of the IRS-2 relative to the IRS-1 signaling pathway in several animal and human muscle cells. This prominent IRS-2 but only marginal IRS-1 activation might suggest that Apidra has relatively small tissue proliferative risk without loosing its metabolic activity.

TITLE: Insulin receptor Mediated Signaling of HMR1964 in comparison to Human Insulin

Study no.: F2000pharm0627

Volume # and page # : Module 4 (Aventis 4.2. 1.1.2 StudyF2000pharm0627)
Conducting laboratory and location: [redacted]
Date of study initiation: May, 1998
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Batch#UB5/26, T4132

Objective:

In order to compare the mitogenic potentials of Apidra with those of human insulin and insulin analogues, the sponsor determined insulin receptor occupancy and activation of the insulin signaling system in the presence of Apidra and other several insulin analogues.

Methods:

[redacted]

medium with only 0.5% FCS and mixed with ^{125}I-labelled human insulin, Apidra or other insulin analogues with sufficient radioactivity (360 mCi/mg), which was counted in a gamma counter for insulin receptor binding assay. As for immunoblotting of phosphorylated proteins of signaling pathways, the proteins were separated by SDS gel

Results:

In most cases, similar insulin receptor association kinetics were observed, which was achieved within 5 minutes after binding incubation. The maximal binding of HMR1964 and HMR1423 was similar to human insulin, although Asp(B10) insulin revealed a markedly increased insulin receptor affinity compared to human insulin (10.96 ± 2.61% vs. 5.28 ± 1.78%) as illustrated below.

At a concentration of 1 nM, all tested compounds induced rapid autophosphorylation of the insulin receptor reaching a maximum after 10 minutes of stimulation. Asp(B10) insulin induced a prolonged phosphorylation state of the insulin receptor β-subunit which could still be observed after 120 minutes whereas insulin receptor autophosphorylation induced by all other compounds had already declined at this time point (Figure 9.2 a). At that time insulin β-receptor concentrations in the rat fibroblasts cells did not change significantly as demonstrated in Figure 9.2b.

Thus, a delayed dephosphorylation of the insulin receptor after a 3 minutes incubation was observed after Asp(B10) insulin induced phosphorylation of the insulin receptor but not in case of human insulin, HMR 1964 and HMR 1423 (Figure 9.4a). Similar results were obtained with insulin receptor substrate (IRS) which also showed a markedly decreased dephosphorylation rate after Asp(B10) insulin induced phosphorylation whereas HMR 1964 and HMR 1423 were very close to human insulin. A slight tendency for delayed dephosphorylation after incubation with HMR 1964 did not reach statistical significance.

To test the effect of HMR1964 on protein synthesis, its effects on thymidine incorporation was investigated. Stimulation of DNA synthesis was low initially and similar for all tested insulins at 0.01 or 0.1 nM because thymidine incorporation was significantly different (Fig. 9.8). At 1 and 10 nM, however, HMR 1964 exhibited a markedly decreased stimulation of thymidine incorporation than all other tested compounds, including human insulin.

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Summary and Conclusions:

To compare the mitogenic potency of human insulin, HMR1964 and other insulin analogues, rat-1 fibroblasts over-expressing insulin receptors have been used to estimate specific binding of HMR 1964 to the human insulin receptor. The investigation also included the kinetics of intracellular signaling (insulin receptor and insulin receptor substrate phosphorylation) and the stimulation of insulin receptor mediated DNA synthesis, i.e., thymidine incorporation.

All tested compounds showed similar insulin receptor association kinetics reaching a maximum after approximately 5 minutes. HMR 1964 and HMR 1423 appear to have similar receptor affinity to human insulin whereas Asp(B10) insulin revealed a markedly increased insulin receptor affinity. Compared to human insulin, HMR 1964 and HMR 1423 were similar regarding receptor autophosphorylation whereas different kinetics were observed with Asp (B10) insulin which induced a prolonged phosphorylation state of the insulin receptor β-subunit.

In summary, the present data demonstrate that HMR 1964 behaves like human insulin with respect to receptor binding and activation of the initial insulin signaling cascade. HMR 1964 relative to human insulin shows no enhanced promotion of thymidine incorporation into newly synthesized DNA. These results are in contrast to Asp(B10) insulin which exhibits an increased insulin receptor binding and induces a prolonged phosphorylation state of the insulin receptor and receptor substrates.

3.2.4. Safety Pharmacology:

(Please see Pharmacology Review#000 dated May 3, 2001 for detailed information)

TITLE: Effect of a subcutaneous administration of HMR 1964 on arterial blood pressure, heart rate, body temperature and electrocardiogram in conscious dogs

Study no.: F200fPHM0023/Report and Document#: 99/11004/PH/

Volume # and page # : Module 4 (Aventis 4.2.1.3.2StudyF2001phm0023)/Page2-51
Conducting laboratory and location:

Date of study initiation: June 1, 1999 / Date of Report: Nov. 18, 1999
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: HMR1964 Batch#1081

Methods:

Three Beagle dogs/sex/groups (28-33 month old, 11-14 kg body weight) were implanted with a telemetric device to monitor blood pressure, heart rate and body temperature at

least 3 weeks before the study. The three instrumented conscious Beagle dogs/sex/group received a single injection of 0(control), 0.3, or 1 I.U./kg HMR1964 subcutaneously four hours before the electrocardiographic evaluation. Using Excel software, the values of the hemodynamic parameters were selected, extracted and calculated from the recorded measurements as an average of 2 minutes of recording. Control values were taken as the mean of these three values before the drug injection, then new values were taken at 5, 15, 30, 60, 90 minutes, 2, 3 and 4 hours after injection.

Results:

After injection of HMR 1964 at the doses of 0.3 or 1 l.U./kg, systolic arterial blood pressure tended to be slightly decreased (figure 1). When compared to the initial values, the maximal decreases of systolic blood pressure were 11 % in the treated groups and 3% in the vehicle group. The slight increase in blood pressure observed in the three groups at time 5 or 15 minutes after compound administration probably reflected the stress due to injection because heart rate was also elevated in the three groups including the control as presented subsequently. Diastolic blood pressure was also raised initially as the case of systolic pressure without remarkable drug-dose dependent changes (figure 2).

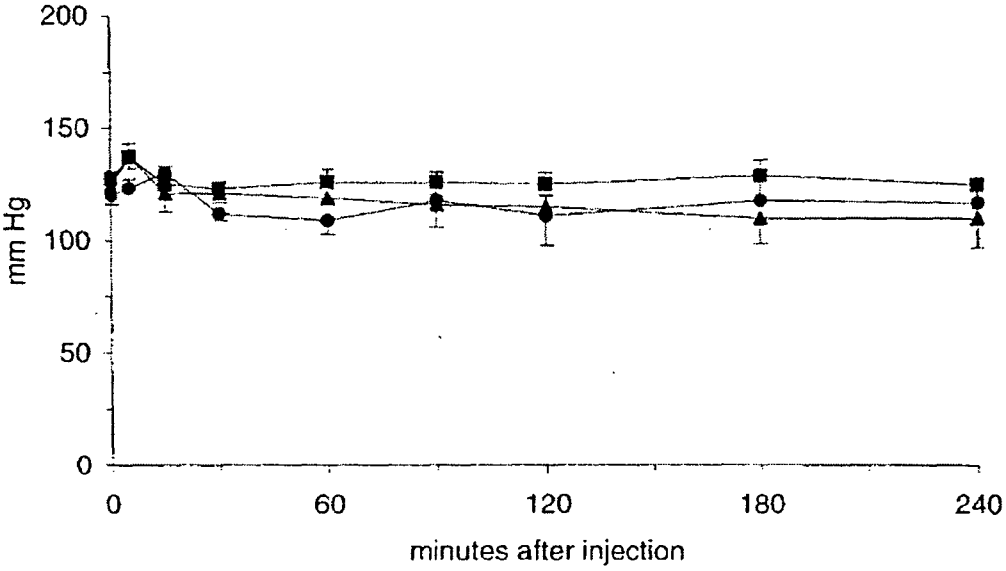

Figure 1 - Effect of a subcutaneous injection of **HMR 1964** on **systolic blood pressure** in the conscious telemetered dog

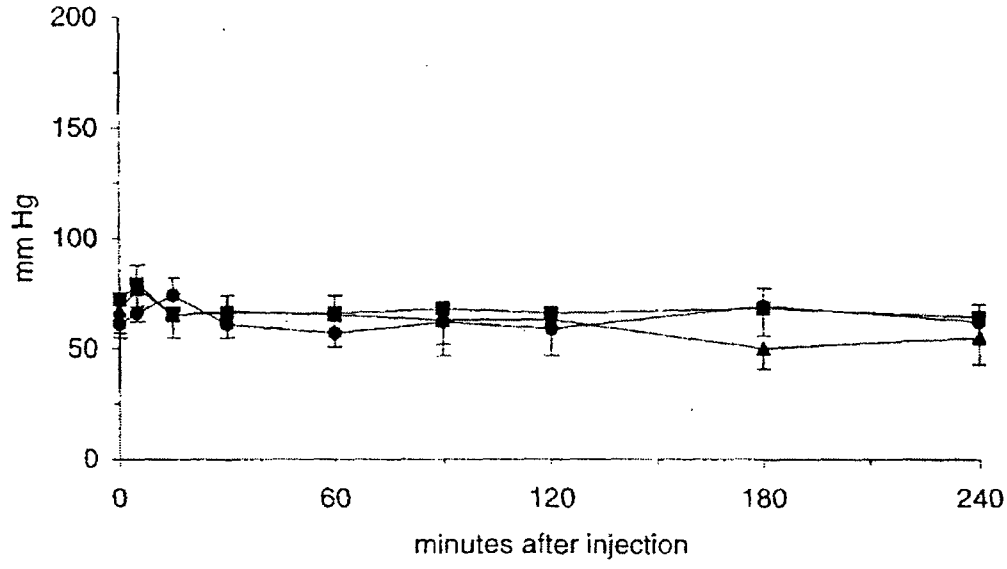

Figure 2 - Effect of a subcutaneous injection of **HMR 1964** on **diastolic blood pressure** in the conscious telemetered dog

—■— vehicle --▲— 0.3 I.U./kg —●— 1 I.U./kg (3 dogs per group)

Effect on heart rate:

Heart rate was increased in both the vehicle and 0.3 I.U./kg groups shortly after injection. After administration of the vehicle, heart rate was increased by 17 % at time 5 min after injection, then it decreased and remained below the initial value from 15 min post-injection. One of the three control dogs (1036) showed a significant effect with the change point test. When HMR 1964 was administered at the dose of 0.3 I.U./kg, heart rate was increased by 23 % five minutes after injection. Then, after a transient return to baseline and in contrast with the control group, heart rate progressively increased from 30 min after injection.

The dose of 1 I.U./kg was followed by a long lasting increase of heart rate starting 30 min after injection. The tachycardia peaked at time 2 hours after injection (115+ 1 beats/min vs. 72+ 3 beats/min initially, + 60 %) and was still present at time 4 hours (103+ 8 beats/min). In the 1 I.U./kg group, the three dogs showed a significant effect with the change point test as shown below.

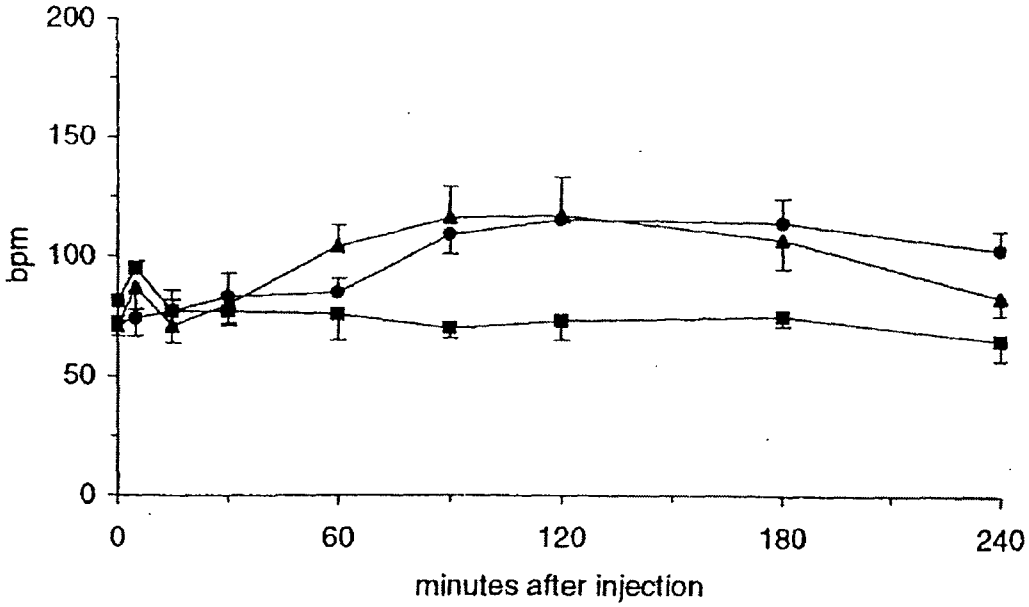

Figure 4 - Effect of a subcutaneous injection of **HMR 1964** on **heart rate** in the conscious telemetered dog

—■— vehicle —▲— 0.3 I.U./kg —●— 1 I.U./kg (3 dogs per group)

Effect on corrected-QT interval duration:

HMR1964 at both of 0.3 and 1 I.U./kg had no significant effects on body temperature, P-R interval, the duration of QRS complex, and QT interval. However, when the QT interval data were corrected according to Bazett's formula, ANOVA showed a significant effect among the groups. In the vehicle group, the QT interval duration corrected by the Bazett's formula remained close to the initial value from 15 min after injection to the end of experiment. Neither of the three control dogs showed a significant effect with the change point test. HMR1964 increased heart rate at doses of 0.3 and 1 IU/kg one hour after its administration (see Table 4). At the doses there were no significant changes in uncorrected QT interval (see Table 7).

In the 0.3 I.U./kg group, the QT interval duration corrected by Bazett's formula was increased from 30 min to 3 hours after injection. The effect peaked at time 2 hours (+ 42 ms, + 18 %, the initial value being 233 + 15 ms). Dog 1036 displayed a significant effect with the change point test. In the 1 I.U./kg group, the three dogs displayed a significant effect with the change point test. Globally, the dose of 1 I.U./kg increased the QT interval duration corrected by the Bazett's formula, starting 30 minutes post dose and reaching a maximum increase of 52 ms 2 hours after administration (+ 23 %, initial value being 224+ 2 ms). The effect was still present at time 4 hours (271 + 8 ms that is + 47 ms in comparison with initial value) as shown in Table 8.

According to statistical analysis (ANOVA) for QT interval, the p values between groups, within times and interaction group/time were 0.029, 0.003, and 0.035, respectively, as shown below. According to Fridericia's formula, the dose of 0.3 I.U./kg provoked a moderate increase of QT interval duration, starting 30 minutes post dose and reaching a maximum increase of 19 ms 2 and 3 hours after administration (+ 8 %, initial value being 227+ 15 ms). The dose of 1 I.U./kg moderately increased the QT interval duration corrected by Fridericia's formula (Table 9).

Analysis of variance (p values) for QT interval duration corrected by the Bazett's formula		
between groups	within times	interaction group/time
0.029	0.003	0.035

Table 4 - Effect of a subcutaneous injection of **HMR 1964** on **heart rate** in the conscious telemetered dog

Time minutes	**Vehicle**	**HMR 1964 0.3 I.U./kg**	**HMR 1964 1 I.U./kg**
	Beats/min	*Beats/min*	*Beats/min*
20 min bef. in.	87 ± 8	69 ± 7	68 ± 9
15 min bef. in.	79 ± 12	68 ± 5	75 ± 2
10 min bef. in.	77 ± 8	77 ± 4	73 ± 1
Initial value	*81 ± 9*	*71 ± 4*	*72 ± 3*
5	95 ± 3	87 ± 9	74 ± 7
15	77 ± 9	71 ± 7	77 ± 5
30	77 ± 6	80 ± 8	83 ± 10
60	76 ± 11	104 ± 9	85 ± 6
90	70 ± 4	116 ± 13	109 ± 8
120	73 ± 8	117 ± 16	115 ± 1
180	75 ± 4	107 ± 12	114 ± 10
240	65 ± 8	83 ± 7	103 ± 8

mean ± sem of 3 dogs/group

Mean initial values are the mean of individual initial values which were taken as the mean of the three individual values before injection (bef. in.)

Table 7 - Effect of a subcutaneous injection of **HMR 1964** on **QT interval duration** in the conscious telemetered dog

Time minutes	Vehicle	HMR 1964 0.3 I.U./kg	HMR 1964 1 I.U./kg
	ms	*ms*	*ms*
20 min bef. in.	195 ± 16	206 ± 9	212 ± 5
15 min bef. in.	199 ± 15	212 ± 11	216 ± 8
10 min bef. in.	201 ± 14	231 ± 32	213 ± 2
Initial value	*198 ± 15*	*216 ± 17*	*214 ± 5*
5	186 ± 5	197 ± 9	210 ± 12
15	199 ± 13	214 ± 14	214 ± 10
30	203 ± 14	220 ± 16	215 ± 16
60	199 ± 15	197 ± 5	216 ± 10
90	210 ± 13	193 ± 8	192 ± 21
120	202 ± 8	196 ± 13	199 ± 10
180	203 ± 11	204 ± 16	181 ± 15
240	212 ± 15	209 ± 10	202 ± 6

mean ± sem of 3 dogs/group

Mean initial values are the mean of individual initial values which were taken as the mean of the three individual values before injection (bef. in.)

Table 8 - Effect of a subcutaneous injection of **HMR 1964** on **QT interval duration corrected by the Bazett's formula** in the conscious telemetered dog

Time minutes	**Vehicle**	**HMR 1964 0.3 I.U./kg**	**HMR 1964 1 I.U./kg**
	ms	*ms*	*ms*
20 min bef. in.	229 ± 12	213 ± 15	226 ± 3
15 min bef. in.	200 ± 8	224 ± 8	218 ± 7
10 min bef. in.	210 ± 5	262 ± 39	226 ± 3
Initial value	*213 ± 7*	*233 ± 15*	*224 ± 2*
5	232 ± 19	229 ± 9	223 ± 11
15	207 ± 5	227 ± 7	235 ± 6
30	226 ± 18	251 ± 14	258 ± 6
60	218 ± 18	249 ± 13	260 ± 4
90	214 ± 9	263 ± 7	262 ± 14
120	217 ± 5	275 ± 10	276 ± 16
180	209 ± 8	271 ± 24	258 ± 13
240	216 ± 11	238 ± 8	271 ± 8

mean ± sem of 3 dogs/group

Table 9 - Effect of a subcutaneous injection of **HMR 1964** on **QT interval duration corrected by the Fridericia's formula** in the conscious telemetered dog

Time minutes	Vehicle	HMR 1964 0.3 I.U./kg	HMR 1964 1 I.U./kg
	ms	*ms*	*ms*
20 min bef. in.	217 ± 13	210 ± 13	221 ± 0
15 min bef. in.	199 ± 7	219 ± 3	217 ± 4
10 min bef. in.	206 ± 7	251 ± 36	222 ± 3
Initial value	*207 ± 9*	*227 ± 15*	*220 ± 2*
5	215 ± 14	218 ± 9	217 ± 8
15	203 ± 6	222 ± 9	227 ± 7
30	217 ± 16	239 ± 12	242 ± 9
60	211 ± 15	230 ± 9	244 ± 6
90	211 ± 9	237 ± 5	236 ± 16
120	212 ± 5	246 ± 10	247 ± 13
180	207 ± 8	246 ± 20	229 ± 14
240	214 ± 11	228 ± 7	245 ± 7

mean ± sem of 3 dogs/group

Mean initial values are the mean of individual initial values which were taken as the mean of the three individual values before injection (bef. in.)

ATOX 10.2 ARTEMIS II RUN DATE: 01-FEB-2001

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2000.0325 - HMR 1964 6 MONTHS + RECOVERY SUBCUTANEOUS

ELECTROCARDIOGRAM
Q-T interval

SUMMARY AND STATISTICS

			GROUP 1 0 IU/kg b.wt.	GROUP 2 0.5 IU/kg b.wt.	GROUP 3 1.0 IU/kg b.wt.	GROUP 4 2.0 IU/kg b.wt.
PRELIMINARY VALUE NE (Seconds)	MALES	MEAN	0.20	0.19	0.20	0.20
		S.D.	0.01	0.01	0.02	0.01
		N	4	4	4	5
	FEMALES	MEAN	0.19	0.21	0.19	0.21
		S.D.	0.02	0.02	0.02	0.01
		N	4	4	4	5
INTERMEDIATE VALUE (Seconds)	MALES	MEAN	0.19	0.20	0.20	0.21
		S.D.	0.01	0.01	0.02	0.01
		N	4	4	4	4
	FEMALES	MEAN	0.20	0.21	0.20	0.21
		S.D.	0.01	0.01	0.01	0.01
		N	4	4	4	5
PREMATURE FINAL VALUE (Seconds)	FEMALES	MEAN				0.20 M
		S.D.				
		N				1
FINAL VALUE (Seconds)	MALES	MEAN	0.18	0.18	0.18	0.19
		S.D.	0.02	0.01	0.02	0.01
		N	4	4	4	4
	FEMALES	MEAN	0.19	0.19	0.18	0.20
		S.D.	0.02	0.01	0.02	0.02
		N	4	4	4	4
RECOVERY (Seconds)	MALES	MEAN	0.18	0.18 M	0.18 M	0.20 M
		S.D.				
		N	1	1	1	1

WILCOXON-TEST. TWO-TAILED TEST FOR THE HIGHEST DOSE AND ONE-TAILED TESTS FOR LOWER DOSES,
IF ALL HIGHER DOSES ARE SIGNIFICANTLY DIFFERENT FROM CONTROL ($P < 0.05$).
COMMON CONTROL GROUP OR COMPLETELY POOLED ANALYSIS IF MORE THAN TWO DOSE*SEX GROUPS CONTAIN LESS THAN FOUR OBSERVATIONS.

+/- : SIGNIFICANTLY DIFFERENT FROM CONTROL (STATISTICAL ANALYSIS BASED ON CHANGES VERSUS PRELIMINARY VALUES).
M : EVALUATION NOT POSSIBLE NE : NO STATISTICAL EVALUATION

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Summary and Conclusion:

Three conscious Beagle dogs/sex/group were implanted with a telemetric device and were treated with HMR1964 at doses of 0, 0.3 or 1 I.U./kg. An analysis was performed on arterial blood pressure, heart rate, ECG parameters and body temperature and the change point test was used for a descriptive analysis of the results.

Both doses tended to slightly decrease systolic blood pressure. The diastolic and mean blood pressures were unchanged. HMR 3964 dramatically increased heart rate. After the doses of 0.3 and 1 I.U./kg, heart rate was increased from 30 min to the end of experiment, the effect peaking 2 hours after administration; at that time, the increases were of 65 and 60% as compared to respective initial values. Tachycardia was still present 4 hours after injection of the higher dose.

An increase of corrected QT interval duration was observed after administration of HMR 1964. P value for QT interval corrected by Bazett's formula between groups was 0.029, although it did not change the uncorrected Q-T interval. A decrease in uncorrected QT interval was observed as would be expected as a consequence of increased heart rate. QT interval corrected by Fridericia's formula in insulin treated groups was not significantly different from control (p=0.148). Therefore, it is possible that the increase in Bazett's corrected QT interval is an artifact secondary to increased heart rate (Spence et al., Toxicol.Sci 1998 Oct 45 (2):247-258). In addition, it is well established that hypoglycemia increases QT interval in humans. The increase of corrected QT interval duration will have to be taken into consideration in at risk patients.

Because of the unexpected increase in corrected QT interval in this study as presented above, the reviewer rechecked the 6-month subcutaneous toxicity study in dog (Study#:2000.0325/ Report#:2000.1131/ Document#F2000TOX0570), which was reviewed in the original IND#61,956 dated Oct. 5, 2001. In the 6-month study (4 dogs/sex/group), the doses of HMR1964 were 0, 0.5, 1, 2 I.U./kg, which were injected subcutaneously for 6 month. In this study no prolongation in QT interval was observed as shown below because the sponsor determined QTc value 24 hours after the test article administration.

Abuse liability:

No relevant study has been performed.

Other: Drug Interaction:

The activity of rapid-acting insulin analogue HMR1964 when mixed with NPH insulin has been evaluated in healthy Beagle dogs after subcutaneous administration. Constant mixing ratio of either two parts of rapid-acting insulin (0.2 IU/kg) and one part of NPH insulin was used to compare the hypoglycemic effects of HMR1964 (1964:NPHmix),

insulin lispro (Lispro:NPHmix) or regular human insulin (Ins:NPHmix) when given as mixture with NPH insulin. Additionally one group of animals was treated with HMR1964 0.2 IU/kg s.c. and NPH insulin 0.1 IU/kg s.c. administered as two separate injections at two different sites (1964+NPHsep).

The hypoglycemic activity of a mixture consisting of two parts of rapid-acting insulin HMR1964 and NPH insulin was not significantly different from a respective mixture made of lispro insulin and NPH insulin. Time-action profile of a mixture of regular human insulin and NPH insulin showed the tendency towards later onset of action, later mean time of action as well as longer duration of action although none of these effects was statistically significant as compared to the HMR1964:NPH insulin mixture. Compared to a separate injection of HMR 1964 and NPH insulin all tested mixtures showed a significantly later onset and mean time of action but no significant loss of total hypoglycemic effect.

Taken together the results of the present study in healthy, normoglycemic dogs it could be concluded that the rapid-acting insulin analog HMR 1964 is suitable to be mixed with NPH insulin immediately before injection similar to human regular insulin with insulin lispro.

3.3 PHARMACOKINETICS/TOXICOKINETICS (Please see Pharmacology Review#001 dated May 3, 2001 for detailed information)

3.3.1 Brief summary:

Single-dose pharmacokinetic studies (iv and sc) in male rats and dogs, in vitro stability investigations in human and rat plasma and toxicokinetics in rats and dogs (14- days, 1-month, 6- month, 12 month toxicology studies) were conducted. Toxicokinetic studies in rats and rabbits (reproduction toxicity studies), determination of insulin antibodies in rats and dogs (6- month, 12- month) and a distribution/excretion study in rats were also performed.

The main pharmacokinetic findings were: Insulin glulisine was well absorbed following subcutaneous injection in male rats and dogs. The toxicokinetic data indicated a systemic exposure in rats and dogs of substantial concentrations of insulin glulisine in all repeated dose toxicity studies and developmental toxicity studies. From the toxicokinetic data, AUC and Cmax generally increased with escalating dose, without gender differences or cumulative effects.

Insulin antibodies were detected in a few dogs in the 6- month toxicity study; however, there was no clear dose-effect relationship. After a single subcutaneous injection of ^{125}I-insulin glulisine in rats, the radioactivity was ubiquitously distributed within the organism, with the highest levels of radioactivity at the injection site. ^{125}I- human insulin had a similar distribution, although radioactivity remained at the injection site for a longer time. This difference indicates a more rapid absorption of insulin glulisine.

3.3.3 Absorption:

Single dose nonclinical pharmacokinetic studies were performed in rats and dogs. Pharmacokinetic results from GLP studies of single i. v. and s. c. injections of insulin glulisine in rats(F1999KIN0168) and dogs (F1999KIN0169) are summarized in the table below: After subcutaneous administration of insulin glulisine to male rats, the compound was completely absorbed. The onset of absorption was rapid. Elimination from blood and serum was relatively fast and virtually completed within 4 h. A very similar elimination profile was observed following intravenous dosing.

Pharmacokinetic parameters for insulin glulisine in rats and dogs (mean)

Variable	Rats		Dogs	
	0.5 mg/kg i.v.	2 mg/kg s.c.	0.05 mg/kg i.v.	0.05 mg/kg s.c.
C_{max} (ng/mL)	1083	2170	338.7	16.8
t_{max} (h)	0.083*	0.167	0.083*	1
$t_{1/2}$ (h)	0.25	0.35	0.92	1.11
AUC∞ (ng*h/mL)	488.5	2025.6	130.2	55.1

* first measuring point

3.3.4 Distribution:

Distribution of radioactivity was studied after single subcutaneous administration of 125I- insulin glulisine in rats at a dose of 50 IU/ kg and was investigated with whole body autoradiography. The radioactivity was distributed ubiquitously within the organism, although distributed radioactivity in the central nervous system was low. At 30 min after administration, the highest radioactive drug equivalents were found at the injection site, followed (in decreasing order) by the thyroid gland (^{125}I resulting from deiodination), urinary bladder, kidney, gastric contents, urine in bladder, skin, adrenal gland, pancreas, blood, intestinal contents, spleen, lung, and liver. The distribution pattern of radioactivity was similar 1, 2, and 4 hr after sc administration. At 8 hr after administration, the overall distribution of radioactivity had decreased in most organs and tissues including the thyroid gland.

3.3.5.Metabolism:

In vitro stability (non-GLP study) was measured in human and rat plasma. In human plasma, the molecule is very stable, with little degradation observed after 8 hours at 37 ° C. In contrast, rapid degradation is seen in rat plasma with less than 5% of insulin glulisine remaining after 8 hours at 37°C. The main products were iodo- tyrosine and a second peak that remains structurally unknown. The specific metabolite, des (1B- 3B) insulin glulisine resulting from cleavage at the [B3Lys] modification, was not found in rat plasma in vitro. This metabolite might have been expected due to peptidase activities in plasma because [B3- Lys] is the only structural change in insulin glulisine that is relevant for biodegradation in comparison to endogenous insulin. Therefore, it can be assumed that proteolytic degradation of insulin glulisine proceeds by cleavage at various positions (see figure below) as known for the human insulin molecule.

Study system	Heparinized rat and human plasma / 37°C			
Time	Insulin glulisine human plasma/0.5h	Insulin glulisine human plasma/2h	Insulin glulisine human plasma/8h	Insulin glulisine rat plasma/8h
Concentration	10 ng/mL	10 ng/mL	10 ng/mL	10 ng/mL
Parent				
P-1				
P-3 (3-Iodo-tyrosine)				
Others				

3.3.6 Excretion:

No report is available.

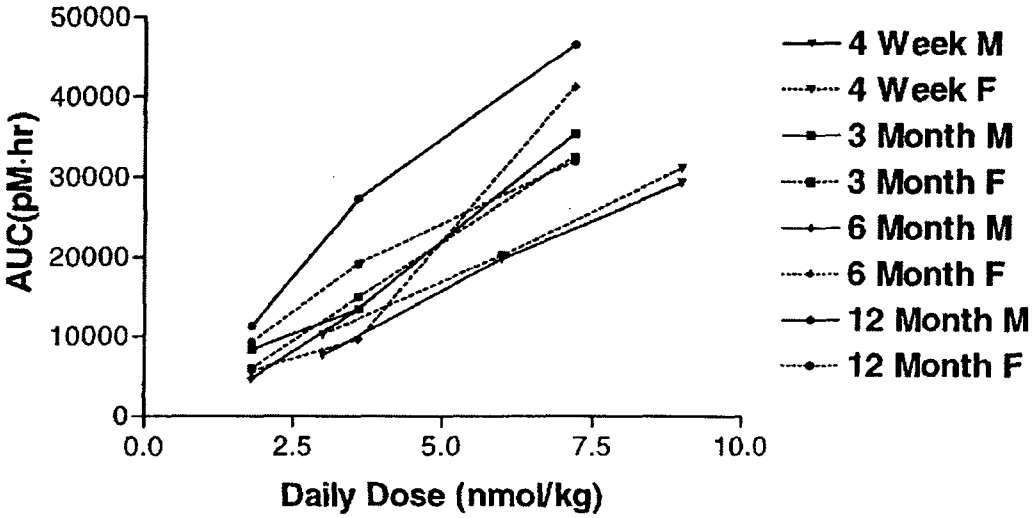
3.3.10 Pharmacokinetic Tables and Figures to Include Comparative TK Summary:

The sponsor presented the following table and Figure 5 for pharmacokinetic data of HMR1964 along with insulin lispro and regular human insulin. AUC values of the two comparators and HMR1964 (0.3 IU/kg) were comparable in dogs as shown below.

	Sample mean (N = 16)			
Variable	**HMR 1964 (0.3 IU/kg)**	**HMR 1964 (0.1 IU/kg)**	**Insulin lispro**	**Regular human insulin**
$AUC_{(0\text{-}1h)}$ [mg/kg]	310.5[$]	417.4	309.4	202.8
$AUC_{(0\text{-}1.5h)}$ [mg/kg]	620.7[$]	903.2	671.5	441.1
$AUC_{(0\text{-}2h)}$ [mg/kg]	937.8[$]	1407.6	1018.4	703.8
$AUC_{(0\text{-clamp end})}$ [mg/kg]	3255.2[$]	4205.9	2890.5	3278.6
Maximum GIR* [mg/min/kg]	12.1[#]	19.9[#]	13.1[#]	11.9[#]
Time of maximum GIR* [min]	172.6[#]	134.9[#]	102.5[#]	174.9[#]
Onset of action [min]	19.4[#]	28.4[#]	24.3[#]	28.9[#]
Duration of action [min]	351.1[#]	269.3[#]	317.5[#]	409.4[#]
Early $t_{50\%}$* [min]	27.5[#]	34.3[#]	32.8[#]	38.6[#]
Late $t_{50\%}$* [min]	300.7[#]	268.7[#]	264.6[#]	346.1[#]

* Maximum GIR, time of maximum GIR as well as time to early and late half-maximum GIR's were determined from "smoothed" GIR profiles.
[#] Median values
[$] Dose-normalization based on 0.1 IU/kg

Figure 5 Overview of toxicokinetics performed in dogs.



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3.4 TOXICOLOGY

3.4.1 Overall toxicology summary:

The sponsor performed the following in vitro or in vivo toxicological studies in mouse, rat, dog, or rabbit utilizing insulin glulisine in final formulation. Single- and repeated-dose toxicity studies were performed to assess potential acute, subchronic, chronic and reproductive effects related to the compound insulin glulisine. Generally, it appears that there were no unexpected findings because they were no different from those observed with regular insulins as reviewed subsequently. All toxicity studies have been performed in compliance with GLP. The table below summarizes all of the toxicology studies performed with insulin glulisine. Most of the studies were reviewed previously on Oct. 5, 2001 under IND#61,956 as appended (Page 88). In this NDA, new toxicology studies are reviewed subsequently.

List of Toxicology Studies That Were Performed By The Sponsor@				
Study type	Route	Species	Study#	Review Date
Single-dose	sc, iv	Rat		
Single-dose	Sc	Mouse, Dog		
Repeated dose studies				
1 Month	sc	Rat	F1999Tox0178	10/5/2001
6 Months	Sc	Rat	F2000Tox0572	10/5/2001
1 Month	Sc	Dog	F1999Tox0166	10/5/2001
6 Months	Sc	Dog	F2000Tox0570	10/5/2001
Segment 1	Sc	Rat	F2001Tox0147	10/5/2001
Segment II	Sc	Rat	F2001Tox0033	10/5/2001
Segment II	Sc	Rabbit	F2001Tox0046	10/5/2001
Segment III	Sc	Rat	F2001Tox0292	2/18/2002
Genotoxicity				10/5/2001
12 Months	Sc	Rat	F2001Tox0209	Carcinogenicity
Local tolerability	sc, iv, im	Rabbit	F2000Tox0499	
Immunogenicity	Sc	Rabbit	F2000Tox0572	
@Most of pharmacology & toxicology studies were reviewed under IND#61,956 on 10/5/2001.				

3.4.2 General toxicology:

Single- and multiple-dose toxicology studies were carried out in mice, rats, and dogs after various doses of insulin glulisine. In general, there were drug dose dependent decreases in blood glucose concentrations. There were also minor clinical signs after a large dose of insulin glulisine, which appeared to be secondary effects to the drug's primary effect on hypoglycemia. There were no histopathological changes in the studies.

3.4.3 Repeated dose toxicity:

TITLE: 12 month subcutaneous toxicity and carcinogenicity studies in rats

Study no.: DSE 2001-0534 (HMR 1964 Placebo and HMR 1964: Groups 1 - 5) and DSE 2001-0534 V1 (HR 1799: Groups 6 - 8)

Volume #/Document#/Page #:Module 4.2.3.4.2.3./ F2001Tox0209/1-1805
Conducting laboratory and location: Aventis Pharma, Drug Innovation and Approval, Lead Optimization, Drug Safety Evaluation, D-65926 Frankfurt am Main, Germany
Date of study initiation: June 18, 2001
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Batch#1352 and 1376/1

Methods
Doses: 5, 10, 40, and 100 I.U./kg/day insulin glulisine and 5, 40, and 100 I.U./kg/day human regular insulin (HR1799). See Table 1 below for study design, dose, and animal numbers in details. HMR 1964 dose levels of 50, 20 and 5 IU/kg twice per day based on toxicological and toxicokinetic considerations derived from previous 1- and 6- month-toxicity studies performed in rats. The doses were recommended by US FDA CAC committee.

Twice per day dosing was selected to permit evaluation of maximal doses, while attempting to minimize loss of animals secondary to hypoglycemia. The low dose of 2.5 IU/kg twice per day was chosen in case mortality decreased the number of available animals at the high and possibly intermediate high dose. It was still a multiple of the expected clinical dose in humans. HR 1799 (human insulin) dose levels are based on previous experience with different insulin derivatives and analogues. Dose levels of 5, 20 and 50 I. U./ kg twice per day were chosen for comparison to HMR 1964.

Table 1: Test and control groups / Dose levels / Formulation of test compound

Group	No. of animals M	F	Dose * I.U./kg	Concentration mg/ml	Volume ml/kg	Vehicle
HMR 1964						
1	30	30	2 x 0	0	1	HMR 1964 Placebo-solution
2	30	30	2 x 2.5	2.5	1	
3	30	30	2 x 5	5	1	
4	30	30	2 x 20	20	1	
5	30	30	2 x 50	50	1	
HR 1799						
6	30	30	2 x 5	5	1	HR 1799 Placebo-solution
7	30	30	2 x20	20	1	
8	30	30	2 x 50	50	1	

* Each group was treated twice daily with the respective dose level. Second treatment / day occurred approx. 8 hours after the first / day

Species/strain: Rat/SD, [redacted]
Number/sex/group or time point (main study): 30 rats/sex/group
Route, formulation, volume, and infusion rate: Subcutaneous administration(twice daily with 8 hours interval), formulation(please see below), volume(one ml/kg), stability was acceptable for max. 8 days at 2-8 ° C under light protection.

Formulation : HR 1799 [redacted]
(equimolar to I.U. insulin
m-Cresol [redacted]
$NaH_2PO_4x2\ H_2O$
Glycerol (85 %) [redacted]
NaOH
HCl, conc. [redacted]
Water for injection

Satellite groups used for toxicokinetics or recovery: 6 rats/sex/group
Age: 6-7 weeks old
Weight: Male, 235 g; female, 170 g
Unique study design or methodology (if any):

Observation times and Results:

Mortality: Twice daily except weekends and holidays when mortality was checked once daily.

Clinical signs: The behavior and general health condition of the animals were examined twice daily while the neurological condition, the refracting media of the eyes, the oral mucosa and the teeth were examined monthly. Palpation for masses was performed once monthly.
Body weights: All rats were weighted on arrival, on Day 1 and weekly thereafter.
Food consumption: Weekly
Palpable masses: Monthly for 6 months, from 6 month onwards twice monthly.
Ophthalmoscopy:
EKG: Not determined in this rat study.

Hematology: Blood samples were collected from a retro-bulbar venous plexus of non-fasted animals at various time intervals. In addition, blood samples were also obtained from the animals which are to be sacrificed in a moribund status. Red cell count parameters such as red blood cell counts, mean corpuscular hemoglobin, mean corpuscular volume, hematocrit, hemoglobin, mean corpuscular hemoglobin concentration and reticulocyte counts were measured. White cell counts parameters include differential leukocyte counts and leukocyte counts(WBC). Activated partial thromboplastin time, thrombocyte counts(platelets), and thromboplastin time were also determined on weeks 53 to 55 as shown below.

Table 2: Times and number of animals

Parameter	Times	Animals per group	Anesthesia
Hematology	Final value / weeks 53 - 55	all surviving animals	Ketamine hydrochloride / Xylazine combination (67/6.7 mg/kg i.p.)
Clinical chemistry	Final value / weeks 53 - 55	all surviving animals	Ketamine hydrochloride / Xylazine combination (67/6.7 mg/kg i.p.)

Clinical chemistry: Standard clinical chemistry parameters were determined in the blood obtained for hematologic study as indicated below in Table 6.

Table 6: Clinical Chemistry parameters

Alanine Aminotransferase (ALAT or GPT)	**Calcium**	**Potassium** (K^+)
Albumin	**Chloride** (Cl^-)	**Sodium** (Na^+)
Albumin/Globulin ratio (calculated)	**Cholesterol**	**Total Protein**
Alkaline Phosphatase	**Creatinine**	**Triglycerides**
Aspartate Aminotransferase (ASAT or GOT)	**Globulin** (calculated)	**Urea**
Bilirubin total	**Glucose**	
	Inorganic Phosphorous	

Urinalysis: Not performed.

Antibody Determinations:

On the day of regular necropsy (day after the last dose) blood samples from all surviving anesthetized (intraperitoneal injection of 67 mg Ketamine hydrochloride/6.7 mg Xylazine /kg body weights) rats per group respectively were collected from a retro-bulbar venous plexus of nonfasted animals. After centrifuging serum was taken and kept frozen. The frozen serum samples were sent to the Department of Drug Metabolism & Pharmacokinetics. Analyzes were done in accordance with a separate study protocol which specifies the methods used.

Gross pathology: Gross lesions were documented during necropsy. All tissues/organs were preserved in 4 % neutral buffered formaldehyde solution.
Organ weights (specify organs weighed if not in histopath table): See Histopath table on page xxx .

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes (x), no ( )

Tissues or organs indicated in the histopathology table were embedded by conventional histological techniques. Light microscopic samples were stained with hematoxylin-eosin while other tissues were used for electron microscopic observations.

Toxicokinetics: NA

Results:

Antibody Determinations:

The HMR 1964 tracer binding values in the control and treatment groups do not differ significantly. They are in the range of non- specific binding as determined with the blank control at Time 0 (mean 5.16 % B/ T). The HR 1799 tracer binding values in the different treatment groups of male rats do not differ significantly and are in the range of the HMR 1964 tracer binding values (no vehicle control). However, distinctly higher HR 1799 tracer binding values (approx. 18- 28 % B/ T) were found with the female animals.

Mortality:
Overall mortality in glulisine and human insulin (HR1799) groups are summarized below in study duration-dependently. There were no deaths in weeks 1-13 in male rats with glulisine, although 9 rats died in HR1799 group in the comparable study period. There were clear dose-dependent increases in deaths in both sexes with glulisine or HR1799 in all study duration, which is evident in percent increase in all groups. Generally, males were more severely affected than females, and mortality at the comparable dose levels was always higher in the HR 1799 treated groups. Incidence of mortality in HMR 1964 high dose groups was comparable to HR 1799 mid dose groups. At the high HR 1799

dose level, excessive mortality was observed during the course of the study, and only 2 or 4 males or females, respectively, survived up to the scheduled study termination.

Histopathologically, 22 (9 males, 13 females) of the 157 compound treated rats dying early exhibited eosinophilic neuronal necrosis within the hippocampus, consistent with marked hypoglycemia resulting from the application of HMR 1964 or HR 1799. These brain lesions and/or several clinical signs in 40 of the rats dying early strongly indicate hypoglycemia as cause of death. Although in the remaining 107 prematurely dying compound- treated rats, cause of death remained undetermined, it appears likely that deaths in compound-treated rats were also due to marked hypoglycemia resulting from the known pharmacological properties and the exaggerated dose levels of both compounds.

	HMR 1964					HR 1799		
Dose (I.U./kg)*	2 x 0	2 x 2.5	2 x 5	2 x 20	2 x 50	2 x 5	2 x 20	2 x 50
	Males (30 animals / group)							
week 1 - 13	-	-	-	-	-	2	1	6
week 14 - 26	1	1	-	2	4	-	6	10
week 27 - 39	1	1	1	5	10	1	9	9
week 40 - 53	3**	2**	1	2	6	3**	5	3
total death	5	4	2	9	20	6	21	28
% total death	16.7	13.3	6.7	30.0	66.7	20.0	70.0	93.3
	Females (30 animals / group)							
week 1 - 13	1	-	-	1	-	-	1	3
week 14 - 26	-	1	-	-	2	-	3	12
week 27 - 39	-	-	-	2	5	2	4	7
week 40 - 53	-	1**	2	4	9	1	3	4
total death	1	2	2	7	16	3	11	26
% total death	3.3	6.7	6.7	23.3	53.3	10.0	36.7	86.7

twice daily; ** these figures contain one prematurely sacrificed animal each

Clinical observation:

In 25 of the 159 rats dying early, clinical signs typical for hypoglycemic episodes (lateral or prone position, stilted gait, convulsions, and/or hypoactivity) were observed one or several occasions preceding death. No other clearly compound- related clinical

observations were made. Several male animals of all study groups including the vehicle control showed an induration at the injection site in the dorsal neck region. This symptom started to occur in the different groups between study weeks 35 (group 8) and 52 (group 7). The number of affected animals ranged from 2 (groups 4, 5, 7, 8) to 13 (control group) per group.

Individual females of several groups also showed an induration at the injection site. First observations in the different groups (groups 1, 2, 3, 5, 6) were made between study weeks 46 (group 6) and 50 (group 3). Compared to males, the number of affected animals was lower and ranged from 0 (groups 4, 7, 8) to 4 (control group) per group.

In some of the animals showing induration at the injection site, additional observations like edema or wounds (oozing or scabby) were made. However, these symptoms were also seen in animals without indurations in the neck region. All other observations made in general health and behavior at all dose levels are considered to be spontaneous. Monthly inspection of teeth, eyes, visible mucous membranes and neurological condition revealed no treatment- induced macroscopically visible pathological findings. Other specific clinical findings are summarized in tables below.

Clinical Observations - Symptoms By Period (with Animal Count)

Study : 2001-0534 - HMR 1964 - 12 month subcutaneous toxicity study in rats

Week numbers relative to Start Date

Group Sex	Clinical Sign	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1m	ANIMALS ALIVE	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
	ANIMALS NORMAL	30	30	29	28	29	29	28	27	28	28	28	26	26	25	26	27	27	27	28
	Gait stilted	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Hypoactivity	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Edema	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Induration	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Scabbing	.	.	.	.	.	.	.	2	2	2	1	2	2	1	1	1	.	.	.
	Prone position	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Alopecia	.	.	.	1	.	.	1	1	.	.	.	1	2	2	2	3	3	3	2
	Scabby wound	.	.	.	1	.	1	2	1	1	1	1	1	1	1	1	.	.	.	.
	Oozing wound	.	.	1	1	1	1	.	.	.	.	1	.	1	1	1	.	.	.	.
	Bulbus blood red discolored	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Microphthalmia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Lens opacity	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	General condition poor	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Respiratory sounds	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Died	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Killed: intercurrent	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Killed: end of study	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

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Clinical Observations - Symptoms By Period (with Animal Count)

Study : 2001-0534 - H&R 1964 - 12 month subcutaneous toxicity study in rats

Week numbers relative to Start Date

Group Sex	Clinical Sign	3 9	4 0	4 1	4 2	4 3	4 4	4 5	4 6	4 7	4 8	4 9	5 0	5 1	5 2	5 3	5 4	5 5
2f	ANIMALS ALIVE	29	29	29	29	29	29	29	28	28	28	28	28	28	26	26	24	9
	ANIMALS NORMAL	19	18	17	16	16	14	15	14	14	12	12	13	13	13	12	5	0
	Gait stilted	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Head inclined	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.
	Hypoactivity	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	.
	Edema	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	.
	Induration	.	.	.	.	.	.	.	.	.	1	1	1	1	1	1	1	.
	Scabbing	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Alopecia	7	8	9	9	9	9	9	10	10	10	10	10	10	10	10	7	3
	Injury	.	1	.	.	.	1	1	1	1	.	.	.	.	.	.	.	.
	Scabby wound	.	.	.	1	1	1	.	.	.	1	1	.	.	.	.	.	1
	Oozing wound	.	.	.	.	.	.	.	.	.	1	1	1	.	.	1	1	.
	Purulent wound	.	.	.	.	.	.	.	.	.	.	.	1	1	1	1	1	.
	Tail tip missing	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	.
	Eyeball destroyed	.	.	.	.	.	.	.	.	1	1	1	1	1	1	1	1	1
	Eyelid margin(s) encrusted	.	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	Cornea dull	.	.	.	.	.	1	1	1	1	.	.	.	.	.	.	.	.
	Cornea uneven	.	.	.	1	1	1	1	1	1	1	1	1	1	1	1	1	.
	Cornea ulcerated	.	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	Corneal opacity	.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	.
	Lens opacity	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	.
	General condition poor	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.
	Snout encrusted blood colored	.	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.
	Died	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Killed: intercurrent	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.
	Killed: end of study	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	15	9

Nominal Dose: Group 1 - 0 I.U./kg Group 2 - 2.5 I.U./kg Group 3 - 5 I.U./kg Group 4 - 20 I.U./kg
Group 5 - 50 I.U./kg Group 6 - 5 I.U./kg Group 7 - 20 I.U./kg Group 8 - 50 I.U./kg

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Palpable masses:

Incidences of palpable masses were comparable in all treated male groups because there was one each in groups 1 and 8. In females, the incidences were slightly increased in the MD and HD HR1799 treated groups as summarized below.

	HMR 1964					HR 1799		
Dose (I.U./kg)*	2 x 0	2 x 2.5	2 x 5	2 x 20	2 x 50	2 x 5	2 x 20	2 x 50
	Males (30 animals / group)							
Animals with mass	1	-	-	-	-	-	-	1
No. of masses	1	-	-	-	-	-	-	1
	Females (30 animals / group)							
Animals with mass	1	1	-	2	2	2	4	4
No. of masses	1	2	-	2	2	2	4	7

* twice daily

Body weight:

Mean body weights in glulisine HD males were significantly higher (beginning on study day 50) when compared to controls. Significant differences calculated for high mid dose males on study days 267 and 288 only are considered to be incidental according to the sponsor's evaluation. Mean body weights in low mid, high mid and high dose females were dose-dependently elevated, being statistically significant from study day 8 onwards (low mid dose up to day 141, high mid dose up to day 309, high dose up to study end).

Prior study end differences of mean body weights compared to controls reached approx. 16 % or 13 % in high dose males and females respectively. Overall body weight gain in these groups was approx. 20 or 24% higher, respectively.

In the groups that were treated with HR 1799, the mean body weights in HD males were statistically significantly higher (beginning on study day 22) when compared to controls. Due to the high mortality in this group, statistical evaluation was no longer possible beginning on study day 288. Mean body weights in all HR 1799 treated female groups were dose-dependently elevated, being statistically significant from study day 8 onwards (low dose up to day 155, mid and high dose up to study end).

Differences of mean body weights compared to controls reached maximal 19 % (study day 162) or 27 % (study end) in high dose males and females, respectively. Study overall body weight gain in these groups was not calculated due to the low number of animals remaining at study end (2 and 4 males and females, respectively). The graphic presentations of body weight data on female rats are attached below, of which finding are essentially similar to the findings in male rats.

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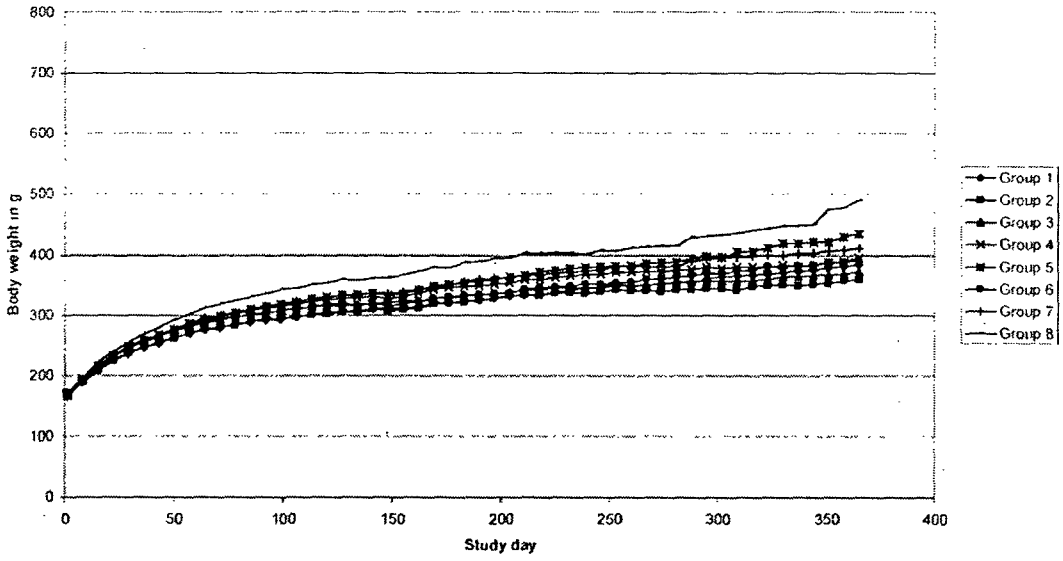
2001-0534: HMR 1964 - 12 MONTH SUBCUTANEOUS TOXICITY STUDY
Body weight development of female rats



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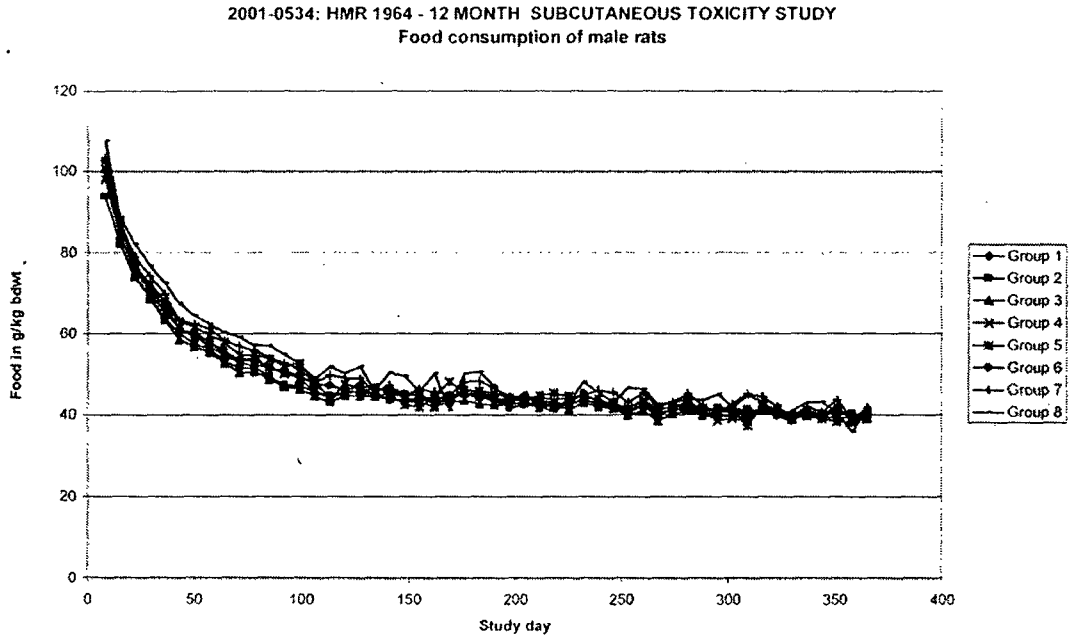
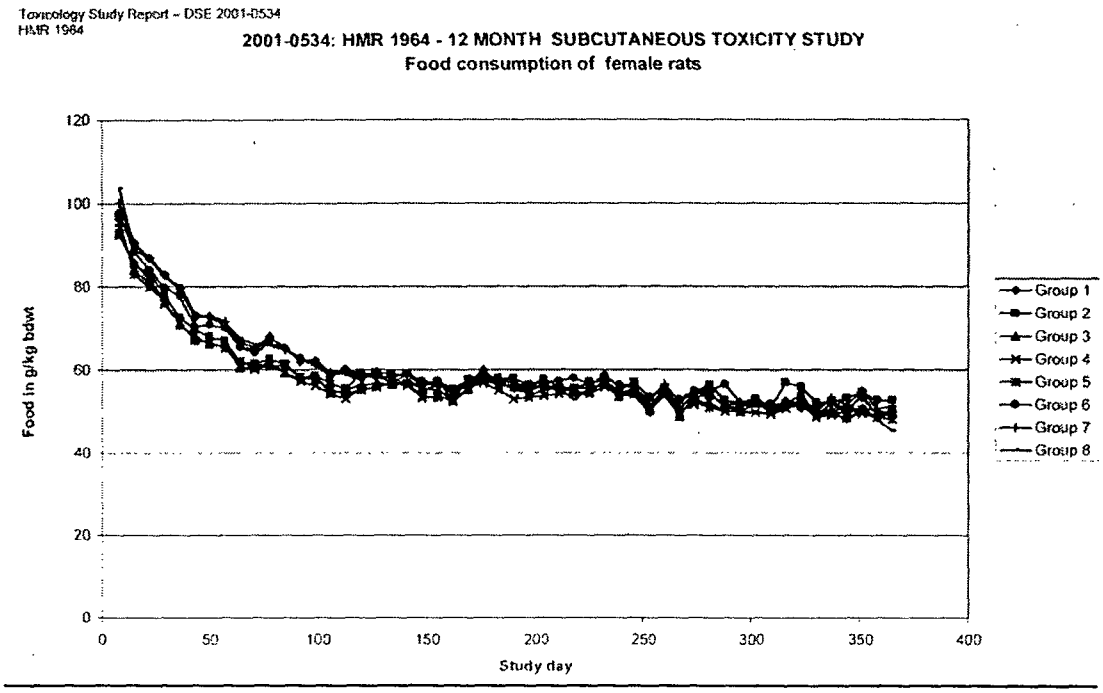
Food consumption:

In glulisine group, overall mean relative food consumption was generally comparable in all compound-treated groups except HD males, which had slightly higher relative food intake (+ 8%) as shown below. The findings in female rats were qualitative similar to those in males (see figure below). Taking in account the elevated mean body weights in different male and female groups, this indicates that the absolute food consumption was proportionally increased in the respective groups.

Similar findings were confirmed in HR 1799-treated group because overall mean relative food consumption was generally comparable in LD and MD female groups. In mid dose males (+ 14%) and high dose groups (males: + 31%; females + 13%), study overall relative food consumption was elevated when compared to controls. Taking in account the elevated mean body weights in different male and female groups, this indicates that the absolute food consumption was proportionally increased in LD groups and in the MD females, and over-proportionally higher in MD males and HD groups.





Hematological findings:

In glulisine treated groups, there were significant increases in hemoglobin, hematocrit and MCV in the MD and HD male groups. It appears that the increases were compound-related and dose-dependent. When concentrations are compared to age-matched male control rats of the same stock from the sponsor's laboratory, it becomes obvious, however, that hemoglobin concentrations in control, low, and low mid dose-groups are unusual low, with concentrations in high mid and high dose-groups being within the normal range. The effects observed in the present study are considered, therefore, not relevant toxicologically.

Hematocrit increased statistically significantly in male rats of the high mid and high dose-groups and in female rats of the high dose-group when compared to those of the control group as illustrated in tables below. Since increases were only marginal, they are considered not relevant toxicologically.

A mild, but statistically significant prolongation of prothrombin time was determined in male rats of the MD and mid high dose-groups and all treated female rats vs. concurrent controls as shown below. Since increases were only marginal and not clearly dose-dependent, they are considered not relevant toxicologically.

A significant increase of MCV was calculated in male rats of low mid, high mid, and high dose groups and in female rats of the high dose- group. Since this effect on MCV was only minor, it is considered not relevant toxicologically.

HR 1799: After twelve months of treatment, a statistically significant compound-related, dose-dependent increase of hemoglobin was seen in male rats of low and mid dose-groups compared to rats of the control group, but was not seen in the sample from the one male rat available for evaluation at termination of the study. Hemoglobin concentrations in rats of low and mid dose-groups are, however, within the normal range of our laboratory. The increases, therefore, are considered not relevant toxicologically.

Likewise, hematocrit was statistically significantly higher in male rats of low and mid dose-groups than in concurrent controls. Since differences are only marginal, they are considered to be not relevant toxicologically.

It should be mentioned that blood from only one male rat of the high dose- group was available for study. Statistical evaluations of parameters assessed were not performed therefore.

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FINAL VALUE

HEMATOLOGY - RED CELL COUNT (MALE)

			GROUP 1 0 I.U./kg	GROUP 2 2.5 I.U./kg	GROUP 3 5 I.U./kg	GROUP 4 20 I.U./kg	GROUP 5 50 I.U./kg
Red blood cell count (10E12/L)		MEAN	8.47	8.23	8.34	8.50	8.57
		S.D.	0.36	0.85	0.48	0.33	0.37
		N	24	25	27	20	10
Hemoglobin (g/L)		MEAN	148	144	148	152 +	162 +
		S.D.	8	14	8	9	8
		N	24	25	27	20	10
Hematocrit (unity)		MEAN	0.44	0.43	0.45	0.45 +	0.48 +
		S.D.	0.02	0.04	0.02	0.02	0.03
		N	24	25	27	20	10
MCV (10E-15 L)		MEAN	52	52	53 +	54 +	56 +
		S.D.	2	2	2	2	2
		N	24	25	27	20	10
MCH (10E-12 g)	NE	MEAN	18	18	18	18	19
		S.D.	1	1	1	1	1
		N	24	25	27	20	10
MCHC (g/L)	NE	MEAN	335	335	334	335	338
		S.D.	9	11	10	10	12
		N	24	25	27	20	10
Reticulocytes (unity)		MEAN	0.032	0.032	0.031	0.027	0.028
		S.D.	0.006	0.014	0.007	0.004	0.006
		N	24	25	27	20	10

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STUDY : 2001-0534 - HMR 1964 - 12 month subcutaneous toxicity study in rats

FINAL VALUE

HEMATOLOGY - RED CELL COUNT (FEMALE)

			GROUP 1 0 I.U./kg	GROUP 2 2.5 I.U./kg	GROUP 3 5 I.U./kg	GROUP 4 20 I.U./kg	GROUP 5 50 I.U./kg
Red blood cell count (10E12/L)		MEAN	7.65	7.44	7.39	7.54	7.54
		S.D.	0.38	0.76	1.06	0.32	0.29
		N	26	27	27	23	14
Hemoglobin (g/L)		MEAN	144	140	139	144	146
		S.D.	5	12	16	7	6
		N	26	27	27	23	14
Hematocrit (unity)		MEAN	0.43	0.42	0.42	0.42	0.44 *
		S.D.	0.02	0.03	0.04	0.02	0.02
		N	26	27	27	23	14
MCV (10E-15 L)		MEAN	56	56	58	56	58 *
		S.D.	2	3	8	2	2
		N	26	27	27	23	14
MCH (10E-12 g)	NE	MEAN	19	19	19	19	19
		S.D.	1	1	1	1	1
		N	26	27	27	23	14
MCHC (g/L)	NE	MEAN	339	337	334	339	334
		S.D.	7	12	18	7	3
		N	26	27	27	23	14
Reticulocytes (unity)		MEAN	0.026	0.033	0.048	0.024	0.027
		S.D.	0.004	0.038	0.106	0.007	0.010
		N	26	27	27	23	14

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ATOX 10.2 PROVANTIS RUN DATE: 19-SEP-2002

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2001-0534 - HMR 1964 - 12 month subcutaneous toxicity study in rats

FINAL VALUE

HEMATOLOGY - BLOOD COAGULATION (MALE)

		GROUP 1 0 I.U./kg	GROUP 2 2.5 I.U./kg	GROUP 3 5 I.U./kg	GROUP 4 20 I.U./kg	GROUP 5 50 I.U./kg
Platelets (10E9/L)	MEAN	1098	1050	1137	1054	1003
	S.D.	183	181	163	180	142
	N	24	25	27	20	10
Prothrombine Time (Seconds)	MEAN	11.79	12.03	12.23	11.92	12.30
	S.D.	0.47	0.71	0.46	0.44	0.43
	N	25	26	28	21	10
APTT (Seconds)	MEAN	18.2	18.1	18.6	18.2	17.6
	S.D.	2.2	2.1	1.6	1.8	1.5
	N	25	26	28	21	10

APPEARS THIS WAY
ON ORIGINAL

ATOX 10.2 PROVANTIS RUN DATE: 19-SEP-2002

SUMMARY AND STATISTICAL EVALUATION

STUDY : 2001-0534 - HMR 1964 - 12 month subcutaneous toxicity study in rats

FINAL VALUE

HEMATOLOGY - BLOOD COAGULATION (FEMALE)

		GROUP 1 0 I.U./kg	GROUP 2 2.5 I.U./kg	GROUP 3 5 I.U./kg	GROUP 4 20 I.U./kg	GROUP 5 50 I.U./kg
Platelets (10E9/L)	MEAN	949	945	1012	1021	905
	S.D.	136	229	150	141	92
	N	26	27	27	23	14
Prothrombine Time (Seconds)	MEAN	11.32	11.45 +	11.62 +	11.52 +	11.94 +
	S.D.	0.64	0.37	0.57	0.42	0.41
	N	29	28	28	23	14
APTT (Seconds)	MEAN	16.2	17.3	16.6	16.8	16.4
	S.D.	2.0	2.5	2.1	1.6	2.4
	N	29	28	28	23	14

Clinical Chemistry:

HMR 1964: Compound-related, dose-dependent statistically significant elevation of serum glucose concentration was recorded in male rats of all dose-groups as shown in a table below. The parameter in female rats was significant only in the high mid and high dose-groups when compared to concurrent controls(No table presented). Because blood was collected approximately 16 hours post administration of HMR 1964, elevated serum glucose concentrations are considered to reflect a rebound effect to compound- related, dose-dependent hypoglycemia and, therefore, not relevant toxicologically.

Blood urea nitrogen was significantly decreased dose-dependently in male rats of high mid and high dose-groups and female rats of the high dose-group. Presumably, the alterations observed might reflect the anabolic state of the rats and are considered, therefore, to be due to the pharmacologic effect of the compound.

Creatinine was statistically significantly lower in male rats of the high dose-group and increased in female rats of all dose-groups when compared to concurrent controls. Since differences were only marginal and showed no clear-cut dose-dependency, they are considered incidental.

Serum sodium concentrations were significantly decreased in male and female rats of the high dose- group. Differences observed were only marginal and, therefore, considered not relevant toxicologically. Serum albumin concentrations and, consequently, the calculated albumin/ globulin ratio were statistically significantly lowered in female rats of the high dose-group. Since the difference was only marginal, it is considered incidental.

HR 1799: Statistically significant, dose- dependent elevations of serum glucose concentrations were seen in male rats of low and mid dose- groups and in female rats of mid and high dose- groups vs. control rats. The increments are considered to reflect