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APPLICATION NUMBER:

21-511

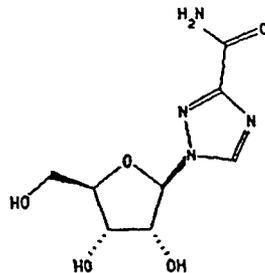
PHARMACOLOGY REVIEW(S)

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-511, Original
Sequence number/date/type of submission: 3 June 2002
Information to sponsor: Yes (X)
Sponsor and/or agent: Hoffmann-La Roche Inc, 340 Kingsland Street, Nutley, New Jersey 07110
Manufacturer for drug substance: Hoffmann-La Roche Inc; 340 Kingsland Street, Nutley, New Jersey

Reviewer name: Hao Zhang, M.D
Division name: Division of Antiviral Drug Products
HFD #: HFD-530
Review completion date: September 23, 2002

Drug:
Trade name: COPEGUS™
Generic name (list alphabetically): Ribavirin
Code name: Ro 20-9963/000
Chemical name: 1-(beta)-D-ribofuranosyl-1 H -1,2,4-triazole-3-carboxamide
Synonyms: ICN-1229, RTCA, Varazid, Viramid, Virazole
CAS registry number: 36791-04-5
Molecular formula/molecular weight: C₈H₁₂N₄O₅; 244.21
Structure:



Relevant INDs/NDAs/DMFs: INDs _____, _____, and 58,827 (Roche Inc) .
NDAs 18-226 (ICN), 18-859 (ICN), _____
_____ and 20,903 (Schering Corp); BB-INDs
_____ and 7823 (Roche Inc); BLA 103964/0 (Roche Inc)

Drug class: Antiviral Nucleoside
Indication: Indication: Treatment of HCV
Clinical formulation: COPEGUS™ Tablets 200 mg for oral administration; Each tablet also contains: pregelatinized starch, sodium starch glycolate, corn starch, microcrystalline cellulose, magnesium stearate, _____ triacetin, hydroxypropyl methyl cellulose, talc, titanium dioxide, synthetic yellow iron oxide, and synthetic red iron oxide.

Route of administration: Oral
Proposed use: COPEGUS™ (ribavirin) in combination with PEGASYS® (peg-interferon alfa-2a)

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability

The sponsor is requesting approval to market ribavirin tablets (COPEGUS™; 800-1200 mg/day in two divided doses for a 6-month treatment duration; PO) in combination with Peginterferon alfa-2a (PEGSYS®; 180 µg; QD; SC Inj.) for the treatment of patients with chronic hepatitis C. The drug product, ribavirin tablet or COPEGUS™, is approvable in the perspective of Pharmacology and Toxicology.

B. Recommendation for Nonclinical Studies

As part of a Phase 4 Agreement, the following non-clinical studies are recommended for the drug product in the pharmacology and toxicology perspective.

- It is recommended that the sponsor consider conducting ~~_____~~
- As part of a Phase 4 Post-marketing Agreement, it is understood that the sponsor should be required to submit the currently on-going 2-year rat carcinogenicity study report to the division for review by the CDER-CAC, when the study is completed.

C. Recommendations on Labeling

- Minor label revisions are recommended in the Carcinogenesis, Mutagenesis, and Impairment of Fertility Section (See Appendix 1, Page 73).
- Based on the positive genotoxic effects seen with ribavirin in the mouse lymphoma assay, and the extended period of human exposure (treatment regimen of ≥6 month duration), it is concluded that:
 - As part of a Phase 4 Post-marketing Agreement, it is recommended that the sponsor should complete and submit the 2-year rat carcinogenicity study (currently on-going) report to the agency for review by the CDER-CAC.
- It is recommended that the sponsor include the following published toxicological data in the COPEGUS labeling.
 - Published literature data show a relationship between chronic ribavirin exposure and increased incidences of vascular lesions (microscopic hemorrhages) in mice at 20-75 or 10-40 mg/kg/day, respectively, for 18 to 24 months (estimated human equivalent doses of 1.7-6.3 and 1.4-5.7 mg/kg/day, respectively, based on body surface area adjustment for a 60 kg adult; approximately 0.1-0.4 × the maximum human daily dose of ribavirin).

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings

General Toxicology Study Findings:

Results of the sponsor-conducted studies (mice, rats, and dogs) demonstrated that ribavirin has significant adverse effects on rapid proliferating tissues with high metabolic rate (lymphoid,

mucosa, bone marrow, intestines, testes, spleen, liver and skin). Previous human experience indicated that ribavirin induces a significant degree of anemia (due to a direct hemolytic effect and suppression of the bone marrow). Preclinical toxicology data showed anemia, reticulocytosis, and lymphoid atrophy in rats and dogs following repeat-dose oral administration. In general, the anemia and lymphoid effects are reversed within 6-week following the cessation of ribavirin administration.

The administration of ribavirin was also associated with increased gastrointestinal toxicities in dogs. Increased intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum was seen in dogs following 20 mg/kg/day treatment of ribavirin for 6 months. In addition, skin ulceration was seen in rats following ≥ 35 mg/kg/day treatment of ribavirin for 6 months. These changes had reversed following the 6-week recovery period.

In rats following 6-month administration of 10, 35 or 70 mg/kg/day of ribavirin, respectively, exposures (AUC_{0-24hr}) at Day 182 in the animals were 2.3, 7.9 and 19.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$ /ml. The NOAEL for ribavirin was considered to be less than 10 mg/kg/day in rats following a 6-month repeat-dose administration. Exposures (AUC_{0-24hr}) of 10.4, 23.1 and 40.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$ were achieved at treatment termination in dogs following administration of dose levels of 5, 10 and 20 mg/kg/day, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$ in the human. The no-effect level (NOAEL) for ribavirin in dogs after 26 weeks of daily administration is considered to be 10 mg/kg/day.

Reproductive Toxicity Study Findings:

Ribavirin's teratogenic and/or embryocidal effects in mice, rats, hamsters, and rabbits have been reported. Teratogenic effects were seen after daily oral doses of 0.3 to 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. In general, the incidence and severity of the teratogenic effects increased with increases of the drug dose. Viability of the fetuses and offspring is also reduced.

In a segment I study in the CD-1 mouse (published literature data), ribavirin produced significant dose and time dependent toxic responses in the testes in mice, including decreases in spermatid concentration, increases in abnormal sperm morphology, and germinal epithelia necrosis. The sponsor-conducted a fertility (segment I) study in CrI:BR rats, however, revealed no significant effects of ribavirin on the fertility indices and the reproductive function of the treated animals at doses up to 100 mg/kg/day. No adverse effects on sperm count or any other parameters of male reproductive performance were evident following a 9-week recovery period. In the study, the male rats were dosed for 9 weeks prior to mating and throughout the 2-week mating period, or, dosed for 11 weeks and then held off treatment for 9 weeks prior to mating. In contrast, the female rats were dosed for 4 weeks prior to mating, throughout the mating period, and until gestation Day 7, or dosed for 4 weeks and then held off treatment for 6 weeks prior to mating. Nevertheless, increased pre-implantation loss and resorption rates were observed in the litters of females at 30 mg/kg/day of ribavirin or greater in the study, which were reversed following a 6-week recovery period.

In a peri- and postnatal exposure study with ribavirin, treatment at doses up to 1 mg/kg of ribavirin was without significant adverse effects on pregnant SD rats or their offspring when exposure began after the period of organogenesis and continued through weaning.

Clinical studies with ribavirin administration to pregnant women have not been conducted. It should be assumed that ribavirin may cause fetal harm in humans.

Genotoxicity and Carcinogenicity Study Findings:

Ribavirin (Ro 20-9963/000) was evaluated as negative for inducing reverse mutations in the *Salmonella-Escherichia coli* reverse mutation assay conducted with four tester strains of *S. typhimurium* (TA1535, TA1537, TA98 and TA100) and *E. coli* WP2uvrA, using non-activation and activation conditions. However, ribavirin was evaluated as positive for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells using non-activation and activation conditions, with a clearly less positive response in the presence of S9 metabolic activation. In a confirmatory non-activation assay at concentrations from 7.85 to 2500 µg/ml, concentrations from 125 µg/ml and higher induced a 3 to 4-fold increase in mutant frequencies. In contrast, under activation conditions, there were 2.2 to 2.7-fold increases in mutant frequencies.

Ribavirin did not induce clastogenic or spindle-damaging effects in mouse bone marrow cells in CD-1 mice at dose levels up to 2000 mg/kg/day for days (the estimated human dose equivalent: 42 – 168 mg/kg, based on body surface area adjustment for a 60 kg adult). Ribavirin did not induce neoplastic lesions when administered to p53(+/-) mice by gavage at dose levels of 10, 50, or 100 mg/kg/day for 26 weeks.

Other Findings: Results from studies published in the literature show a relationship between chronic ribavirin exposure and increased incidences of vascular lesions (microscopic hemorrhages) in mice at 20-75 or 10-40 mg/kg/day, respectively, for 18 to 24 months (estimated human equivalent doses of 1.7-6.3 and 1.4-5.7 mg/kg/day, respectively, based on body surface area adjustment for a 60 kg adult; approximately 0.1-0.4 × the maximum human daily dose of ribavirin).

B. Pharmacologic Activity

Ribavirin is a nucleoside drug with antiviral activity, both *in vitro* and *in vivo*, against a wide range of RNA and DNA viruses. Ribavirin inhibits HCV RNA replication *in vitro* in human hepatoma cells, with an IC₅₀ of 11-21 µM. Additionally, ribavirin 5'-triphosphate inhibits HCV RNA-dependent RNA polymerase (NS5B), which could contribute to the inhibition of HCV RNA replication by ribavirin *in vitro*. Ribavirin inhibits cellular IMP dehydrogenase (IMPDH) and results in a depletion of intracellular GTP pools, which could contribute to the anti-proliferation effect of ribavirin. In addition, ribavirin increases Type 1 (Th1) cytokine and decreases Type 2 (Th2) cytokine secretion from stimulated human T cells *in vitro*.

C. Nonclinical Safety Issues Relevant to Clinical Use

Hematological toxicity: Ribavirin induces a significant degree of anemia (↓RBC, ↓Hb, ↓Hct, reticulocytosis, and extramedullary hematopoiesis), decreased thymic weights, thymic lymphoid depletion and lymphoid atrophy in rats or/and dogs following repeat-dose administration of ribavirin up to 6 months. In general, the anemia is reversed within 6-weeks.

Gastrointestinal toxicity: In the four-week toxicity study in mice, crypt cell necrosis and regenerative hyperplasia in the small and large intestine were seen in mice that died at ≥200 mg/kg/day. In the 26-week toxicity study in dogs, increased intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum were seen in dogs at 20 mg/kg/day, with increased inflammation in all sections of the small intestine.

Skin lesions: In the 13-week oral gavage toxicity study in rats, skin lesions (ulceration, dermis and epithelial sclerosis) were seen in rats at 40 mg/kg/day or greater. In the 26-week toxicity study in dogs, skin ulceration was seen at ≥35 mg/kg/day. The skin lesions were reversed within 6-weeks following the cessation of ribavirin in rats and dogs.

Liver toxicity: Liver centrilobular necrosis and vacuolar degeneration was seen in rats at 160 mg/kg/day in the 13-week oral toxicity study.

Vascular lesions: A relationship between chronic ribavirin exposure and increased incidences of vascular lesions (microscopic hemorrhages) was seen in mice at 20-75 or 10-40 mg/kg/day, respectively, for 18 to 24 months (estimated human equivalent doses of 1.7-6.3 and 1.4-5.7 mg/kg/day, respectively, based on body surface area adjustment for a 60 kg adult; approximately 0.1-0.4 × the maximum human daily dose of ribavirin) (published data in the literature).

Reproductive toxicity: Ribavirin administration for a pre-mating treatment period of 4 weeks to female rats resulted in increased pre-implantation loss and resorption rates in the litters of females at 30 mg/kg/day of ribavirin or greater. Ribavirin administration for at least 9 weeks to male rats marginally reduced the mean sperm count in the male rats at 100 mg/kg/day, which was reversed following a 6-week recovery period. In contrast, ribavirin produced significant dose and time dependent decreases in relative (to body weight) testes/epididymis weights in the surviving male mice at 200 mg/kg/day, accompanied by the treatment-related histopathologic changes in the epididymis (↓spermatid concentration, ↑abnormal sperm morphology, and ↑germinal epithelia necrosis). Ribavirin has demonstrated significant teratogenic and/or embryocidal potential in the mice, rat, rabbits and hamsters. Teratogenic effects have been seen after daily oral doses of 0.3 to 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. In general, the incidence and severity of the teratogenic effects increased with increases of the drug dose. Viability of the fetuses and offspring is also reduced. In a peri- and postnatal exposure study with ribavirin, treatment at doses up to 1 mg/kg of ribavirin was without significant adverse effects on pregnant SD rats or their offspring when exposure began after the period of organogenesis and continued through weaning.

Carcinogenicity: Ribavirin is a nucleoside analog that has produced positive findings in multiple *in vitro* and *in vivo* genotoxicity assays, although it does not possess oncogenic potential in the P53 (+/-) mouse carcinogenicity assay. A two year study in rats in progress.

III. Administrative

A. Reviewer signature: Hao Zhang, M.D.

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

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PHARMACOLOGY/TOXICOLOGY REVIEW**I. PHARMACOLOGY:****Primary pharmacodynamics:**

Ribavirin is a nucleoside drug with antiviral activity, both *in vitro* and *in vivo*, against a wide range of RNA and DNA viruses, including HCV. Ribavirin inhibits HCV RNA replication *in vitro* in human hepatoma cells, with an IC_{50} of 11-21 μ M. Additionally, the intracellular metabolite of ribavirin, ribavirin 5'-triphosphate, inhibits HCV RNA-dependent RNA polymerase (NS5B), which could contribute to the inhibition by ribavirin *in vitro*.

Mechanism of action:

The exact anti-HCV mechanism of action of ribavirin is unknown. Ribavirin inhibits HCV RNA replication *in vitro* in human hepatoma cells (in an HCV replicon assay). Inhibition of HCV RNA-dependent RNA polymerase (NS5B) by ribavirin triphosphate could contribute to the inhibition by ribavirin in the HCV replicon assay.

Drug activity related to proposed indication:

Ribavirin is a nucleoside drug with antiviral activity, both *in vitro* and *in vivo*, against a wide range of RNA and DNA viruses, including HCV. Ribavirin inhibits HCV RNA replication *in vitro* in human hepatoma cells, with an IC_{50} of 11-21 μ M. Additionally, the intracellular metabolite of ribavirin, ribavirin 5'-triphosphate, inhibits HCV RNA-dependent RNA polymerase (NS5B), which could contribute to the inhibition of HCV RNA replication by ribavirin *in vitro*. Ribavirin increases Type 1 (Th1) cytokine and decreases Type 2 (Th2) cytokine secretion from stimulated human T cells *in vitro*, in addition to inhibiting T cell proliferation.

Secondary pharmacodynamics:

Ribavirin inhibits cellular IMP dehydrogenase (IMPDH) via ribavirin 5'-monophosphate and results in a depletion of intracellular GTP pools, which could contribute to the anti-proliferation effect of ribavirin.

Pharmacology summary:

Ribavirin is a nucleoside drug with antiviral activity, both *in vitro* and *in vivo*, against a wide range of RNA and DNA viruses, including HCV. The anti-HCV mechanism of action of ribavirin is likely to involve multiple mechanisms. Ribavirin inhibits HCV RNA replication *in vitro* in human hepatoma cells. Inhibition of HCV RNA-dependent RNA polymerase (NS5B) by ribavirin triphosphate could contribute to the inhibition by ribavirin in the HCV replicon assay. Ribavirin increases Type 1 (Th1) cytokine and decreases Type 2 (Th2) cytokine secretion from stimulated human T cells *in vitro*, in addition to inhibiting T cell proliferation. Ribavirin inhibits cellular IMP dehydrogenase (IMPDH; K_i vs. ribavirin 5'-monophosphate: 250 nM) and results in a depletion of intracellular GTP pools, which could contribute to the anti-proliferation effect of ribavirin.

Pharmacology conclusions:

Ribavirin is a nucleoside drug with antiviral activity, both *in vitro* and *in vivo*, against a wide range of RNA and DNA viruses, including HCV. The anti-HCV mechanism of action of ribavirin is unknown. However, the anti-HCV mechanism of action of ribavirin is likely to involve multiple mechanisms.

In vitro pharmacology studies:**1. Study Title: Studies on Inhibition of HCV Replicon by Ribavirin and Possible Mechanisms of Action (Research Report No.: 1003775)****Methods**

Effects of ribavirin (ICN-Virazid, Sigma-R9644, and Synte-Ro 100-8541/007, and Roche (Ro 20-9963), mycophenolic acid (MPA; \rightarrow), interferon alfa-2a (Ro 22-8181; Lot No. R177993-01; Specific activity: 2.94×10^8 IU/mg) and pegylated interferon alfa-2a (Ro 25-8310; Lot No. R187096-01; Specific activity: 0.65×10^7 U/mg protein) were each evaluated in an HCV subgenomic replicon assay and a _____ which was developed by the sponsor. The

_____ assay was also performed to _____

To elucidate whether the mechanism of inhibition might be mediated through IMPDH or HCV RNA-dependent RNA polymerase, the study was carried out with ribavirin (or mycophenolic acid, a known IMPDH inhibitor) in the presence and absence of exogenous guanosine. Additionally, *in vitro* HCV RNA-dependent RNA polymerase (RdRp) studies were carried out to investigate the role of HCV RNA-dependent RNA polymerase in the presence of ribavirin triphosphate

Results

Interferon alfa-2a, pegylated interferon alfa-2a, MPA and ribavirin (_____) all showed inhibitory activity, determined as a reduction in replicon RNA normalised to that of b-actin, although with different potencies. Interferon alfa-2a and pegylated interferon alfa-2a showed IC₅₀ values of 1 IU/ml (0.003 ng/ml) and 0.6 ng protein/ml (4 U/ml), respectively. MPA had an IC₅₀ value of 0.5 μ M. Ribavirin (ICN) showed an IC₅₀ of 12 μ M (n=3). Ribavirin (Sigma) showed an IC₅₀ of 21 μ M (n=1) and ribavirin (Syntex, Ro 100-8541/007) an IC₅₀ of 15 μ M (n=1). Less than 50% cytotoxicity was observed at 100 μ M ribavirin, the highest concentration tested.

Under the same experimental conditions, ribavirin also inhibited expression of the HCV specific proteins, NS3, NS4A, NS5A and NS5B showing a good correlation between inhibition of both RNA and protein synthesis in the absence of cytotoxicity. Additionally, ribavirin triphosphate inhibits HCV RNA-dependent RNA polymerase (NS5B) with approximately 50% inhibition at 100 μ M (the highest concentration tested). Addition of an excess of GTP (1 mM) to either the heteropolymeric or homopolymeric assays results in a reversal of the inhibition observed, suggesting that ribavirin triphosphate competes with GTP at the level of the NS5B protein.

Inhibition of IMPDH by mycophenolic acid is reversed by guanosine (200 μ M). Inhibition of IMPDH by ribavirin is partially reversed by guanosine (200 μ M). This result suggests that it is unlikely that IMPDH inhibition contributes to the inhibitory effect of ribavirin in the HCV replicon assay. In contrast, in the HCV replicon assay, guanosine (200 μ M) has no effect on inhibition of HCV subgenomic RNA replication by ribavirin.

Comments

- Ribavirin inhibited replication of subgenomic HCV RNA in _____ cells with an IC₅₀ of 11-21 μ M, dose-dependently with complete inhibition achieved at higher concentrations.
- IC₅₀ values for ribavirin triphosphate in the range of 50-150 μ M for inhibition of NS5B were reported in published literature. However, it has been reported that no effect of ribavirin on NS5B was found up to 40 μ M, the highest concentration studied.
- As a substrate for HCV RNA polymerase, ribavirin 5'-triphosphate can be incorporated into RNA. The pseudo base of ribavirin can pair equivalently with both cytosine and uracil, which provides a

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molecular basis for its incorporation into RNA and mutagenic potential. HCV RNA polymerase catalyses incorporation of ribavirin opposite cytidine and uridine with equal efficiency and, once incorporated, ribavirin impedes RNA elongation (to a greater extent than observed for poliovirus). Thus, ribavirin can exert a direct effect on HCV replication mediated by the HCV RNA polymerase through causing lethal mutagenesis of the viral genome, as well as premature termination of nascent RNA. In addition, inhibition of HCV RNA-dependent RNA polymerase (NS5B) by ribavirin 5'-triphosphate could contribute to the inhibition by ribavirin in the HCV replicon assay. A difference in IC50 values of ribavirin was observed between *in vitro* HCV polymerase assays and in the HCV subgenomic RNA replication assays (100µM versus 11µM).

- Ribavirin monophosphate is a competitive inhibitor of IMPDH, with a Ki of 250nM. IMPDH catalyses the rate limiting step in *de novo* biosynthesis of guanine nucleotides, and, in cells dependent on the *de novo* pathway, inhibition of IMPDH reduces intracellular GTP and dGTP pools. Thus, the inhibition of IMPDH may result in antiviral and antiproliferative effects. However, inhibition of IMPDH appears not to contribute to the inhibitory effect of ribavirin on the HCV subgenomic RNA replication, because the inhibition of IMPDH by ribavirin or MPA cannot be reversed by exogenous guanosine. Similarly, it has been reported that guanosine was unable to reverse the inhibitory effect of ribavirin against the HCV surrogate virus BVDV, vaccinia virus, and parainfluenza virus.

2. Study Title: Inhibition of PHA-induced T Cell Proliferation by Ribavirin (Research Report No. 1003810)

Methods

An *in vitro* assay (³H-thymidine incorporation assay) of human T cell proliferation was performed to examine the effects of D-ribavirin (4.8 to 16 µM; Roche and Sigma) and mycophenolic acid (70 to 90 nM) on phytohemagglutinin (PHA)-stimulated (5µg/ml for 72 hours) human PBMC cells, in the presence and absence of guanosine (10 µM). Membrane integrity and intracellular viability (MTT; 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl-blue) assays were also performed.

Results

Ribavirin and mycophenolic acid (MPA) inhibit PHA-stimulated T cell proliferation with IC50 values in the range 5-16 µM and 70-90nM, respectively. Inhibition by ribavirin was partially reversed in the presence of guanosine, as demonstrated by a shift to higher IC50 values. As expected the MPA exhibited potent inhibition of T cell proliferation that could be totally reversed in the presence of guanosine. Additional *in vitro* assays demonstrate that inhibition of T cell proliferation was not a result of cytotoxicity. Membrane integrity and intracellular viability assays confirmed lack of cytotoxicity by ribavirin at concentrations up to 100 µM. Similar results are obtained with ribavirin from either Roche Inc or Sigma, validating the use of ribavirin from different sources in these assays.

Comments

- Ribavirin inhibits proliferation of peripheral blood mononuclear cells, and this activity is at least partly due to inhibition of IMPDH. These effects can be partially reversed by addition of exogenous guanosine.
- The study confirms published data demonstrating the anti-proliferative effects of ribavirin on peripheral blood T cells. Inhibition of T cell proliferation by ribavirin could be involved in reducing the generalized pro-inflammatory response to HCV infection and thereby reducing bystander damage to hepatocytes. Inhibition of T cell proliferation by ribavirin could thus account for the transient decrease in ALT seen on ribavirin treatment of HCV patients.
- Ribavirin has also been reported to inhibit protein synthesis and secretion, as well as growth factor-stimulated proliferation of cultured primary hepatocytes.

3. Study Title: Effect of Ribavirin on Type-1 and Type-2 Cytokine Responses in Human Peripheral Blood Mononuclear Cells, *In Vitro* (Research Report No. 1003792)

Methods

In the present study, the ability of ribavirin to modulate Staphylococcal enterotoxin B (SEB) and phorbol myristate acetate (PMA) plus ionomycin (ION)-induced Th-1 (TNF-I, IFN-g and IL-2) and Th-2 (IL-4 and IL-10) cytokine responses were assessed in human peripheral blood mononuclear cells (PBMCs), *in vitro*. Peripheral blood mononuclear cells (PBMCs) were incubated for 24 hours and/or 48 hours with Staphylococcal enterotoxin B (400 ng, Sigma) or phorbol myristate acetate (10 ng, —, plus ionomycin (0.5 µg, —) in the presence of ribavirin (Roche or Sigma, 0 to 5 µM, final concentration). Additionally, ribavirin from Roche (Ribavirin Roche active substance) was compared with that from an external commercial source (Sigma).

Results

Ribavirin (0.3-5µM) significantly ($p < 0.05$, $n=3$) increased SEB-induced TNF- α , IFN- α and IL-2 release from PBMCs as compared to controls (SEB only). The lowest concentration of ribavirin used in these studies (0.3 µM) appears to show a near maximal response ($\uparrow 59\%$ increase from control in all Th-1 cytokines). Ribavirin had no significant effect on SEB-induced IL-10 levels. Similar results were obtained with Roche and Sigma ribavirin. Additionally, in a preliminary study ribavirin increased IL-2 secretion from human whole blood following incubation with SEB (50 % increase from controls with 0.5 µM).

Comments

- Ribavirin increases secretion of Th1 cytokines, IL-2, IFN and TNF (maximum effect at 0.3 µM) and decreases secretion of Th2 cytokines from stimulated human T cells *in vitro*.

II. SAFETY PHARMACOLOGY:

According to an Agreement between the agency (both CBER and CDER Pharmacology and Toxicology reviewers) and the sponsor (Re: IND 58,827, Minutes of Industry Meeting; Meeting Date: 20 December 1999) regarding the toxicology program, no new safety pharmacology studies with ribavirin alone were conducted. Thus, no safety pharmacology studies were included in this NDA submission.

Neurological effects: Repeat-dose toxicity studies with ribavirin in mice, rats and dogs do not reveal any particular potential for CNS. Specifically, observation data (clinical signs) from the Sponsor's repeat-dose toxicity studies in mice, rats, dogs and monkeys indicate no treatment-related effects on the CNS.

Cardiovascular effects: Repeat-dose toxicity studies with ribavirin in mice, rats and dogs do not reveal any particular potential toxicity for cardiovascular effects. ECG data, collected at Weeks 1, 3, 26 (study termination) and 32 (recovery animals) in the 6-month dog study, show no effects on heart rate or ECG changes. In the 4-week monkey study with PEG-IFN alfa-2a in combination with ribavirin, no treatment-related ECG abnormalities are observed at the time of peak exposure to both compounds.

Pulmonary effects: Repeat-dose toxicity studies with ribavirin in dogs do not reveal any particular potential pulmonary toxicity. However, labored respiration and hypoactivity were seen in mice and rats that died prematurely in the toxicity studies, which were consistent with those expected for moribund animals.

Renal effects: Renal effects: Repeat-dose toxicity studies with ribavirin in mice, rats and dogs do not reveal any particular potential for renal effects. Clinical pathology data from these repeat-dose studies in rats and dogs show no adverse effects on renal function.

Gastrointestinal effects: Repeat-dose toxicity studies with ribavirin in rats (13-week study) and dogs (26-week study) reveal ribavirin-related gastrointestinal effects. An increased occurrence of liquid or mucoid feces was seen in rats at ≥ 80 mg/kg/day and in dogs at 20 mg/kg/day, respectively. Intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum were evident in dogs at 20 mg/kg/day, with increased inflammation in all sections of the small intestine. Edema and erosion in the stomach, and hypertrophy, squamous metaplasia in the duodenum, jejunum and ileum were observed in rats at 160 mg/kg/day.

Abuse liability: not determined

Other: N/A

Safety pharmacology summary: Repeat-dose toxicity studies with ribavirin in mice, rats and dogs do not reveal any potential for CNS, renal or cardiovascular effects. However, repeat-dose toxicity studies with ribavirin in rats (13-week study) and dogs (26-week study) reveal an increased occurrence of liquid or mucoid feces in rats at ≥ 80 mg/kg/day and in dogs at 20 mg/kg/day, respectively. Intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum were evident in dogs at 20 mg/kg/day, with increased inflammation in all sections of the small intestine. Similarly, edema and erosion in the stomach, as well as hypertrophy and squamous metaplasia in the duodenum, jejunum and ileum were observed in rats at 160 mg/kg/day.

Safety pharmacology conclusions: No safety pharmacology studies conducted with ribavirin alone were included in this NDA submission. Repeat-dose toxicity studies with ribavirin in mice, rats and dogs do not reveal any potential for CNS, renal or cardiovascular effects.

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III. PHARMACOKINETICS/TOXICOKINETICS:

The sponsor did not include preclinical absorption, distribution, metabolism, and excretion (ADME) studies conducted with ribavirin in this NDA submission. However, the sponsor included published data on ADME and PK data from the following pharmacokinetics/toxicokinetics studies in this NDA submission:

- Single dose dog PK study (Schering's Rebetol vs. Roche's ribavirin).
- 4-week mouse
- 13-week rat
- 26-week rat plus 6-week recovery
- 26-week dog plus 6-week recovery

PK parameters: Exposure data from the TK studies in the human, monkey, mouse, rat, and dogs were summarized as follows:

Species	Dose (mg/kg/day)	C _{max} (µg/ml)	AUC _{0-24 h} ^a (µg•hr/mL)	Mean Blood (Conc.: ng/mL) ^a	Roche Study No
Human	16 ^b (1200 mg/day)	2.781 ^c	25.5 ^c	Not available	NV 15801
Mouse (4-week)	30 100 200 400	1.148 3.394 4.585 19.6	11.6 35.5 71.9 142.5	Not available	#07296
Rat (13-week)	10 40 80 160	0.13 0.54 0.95 2.8	1.9 9.0 16.8 46.3	24.0 119 244 936	#07320
Rat (26-week)	10 35 70	0.20 0.54 1.14	2.3 7.9 19.1	21.0 69.0 197.0	#07321
Dog (26-week)	5 10 20	1.60 3.70 6.14	10.4 23.1 40.1	97.7 174.5 358.0	#07322
Monkey (4-week) ^d	50 100	1.5 3.7	23.0 ^e 59.1 ^e	25.9 ^f 38.9 ^f	#07211

^a Obtained 24 hours after last dose of the study; ^b Based on a 75 kg body weight for the patient; ^c C_{max} and AUC values were calculated from data obtained after the morning dose only (i.e., 600 mg/patient); ^d Data obtained from the combination therapy groups on Day 29; this combination study was reviewed by Dr. Anne Pilaro at CBER; ^e AUC_{1-12h}; ^f C_{max}

Absorption: Published literature data for the mouse, rat and dog suggest that ribavirin was readily absorbed and slowly eliminated in mice, rats and dogs with an approximate bioavailability of 80%. The absorption of ribavirin from the gastrointestinal route was reduced at high doses, suggesting a dose-dependent saturation of the carrier transport. Ribavirin achieved maximal levels in the serum or plasma within 1-2 hours of dosing, and delayed with an initial half-life of between 4 to 10 hours.

Distribution: The uptake of ribavirin into RBCs is via a nucleoside transporter (es-transporter) which maintains intra- and extracellular ribavirin concentrations at equilibrium. Following administration of [¹⁴C] ribavirin to monkeys and humans, 20% to 30% of the total dose was retained in body tissue after 72 hours. After 2 hours, the concentration of radioactivity in the RBCs exceeded that in serum and continued to increase rapidly. Accumulation of ribavirin in serum was seen in mice, rats and cynomolgus monkeys after repeat oral administration, but not in dogs. In contrast, accumulation of ribavirin in RBCs was observed in all tested species (dogs, mice, rats and cynomolgus monkeys) after repeat oral administration. These results suggest that RBCs may serve as a drug (or the triazole carboxamide metabolites, and the phosphorylated drug metabolites) reservoir with delayed release following drug withdrawal. The accumulation of ribavirin nucleotide metabolites within the RBCs may contribute to the observed anemia in patients administered ribavirin in high doses or for prolonged periods of time. Compared to the human and monkey, the rat does not accumulate radioactivity in RBCs to the same

extent as the human and monkey. In monkeys and humans, the amount of parent drug in blood cells increased through 48 hours and remained stable for 72 hours, whereas in rats, the concentration of ribavirin decreased at a rate similar to the plasma disappearance curve. Serum drug levels were comparable for male and female animals.

Metabolism: Ribavirin has two pathways of metabolism intracellularly: (i) a reversible phosphorylation pathway in nucleated cells; and (ii) a degradation pathway involving deribosylation and amide hydrolysis to yield a triazole carboxylic acid metabolite. The enzymes responsible for the deribosylation and/or amide hydrolysis have not been definitively identified; however, production of these metabolites has been reported in the literature to be associated with liver cytosolic fractions. In human and monkey erythrocytes cells, ribavirin is phosphorylated to mono-, di-, and triphosphate nucleotides, with the major metabolite being the triphosphate nucleotide. Adenine kinase is the rate-limiting enzyme for the initial phosphorylation of ribavirin. RBCs are able to concentrate ribavirin triphosphate nucleotide due to the lack of dephosphorylation enzymes. Results of *in vitro* studies using both rat and human liver micromosome preparations indicated little or no cytochrome P450 enzyme mediated metabolism of ribavirin, with minimal potential for P450 enzyme-based drug interaction.

Excretion: The principal route of elimination is renal for both ribavirin and its triazole carboxamide and triazole carboxylic acid metabolites, with the majority of the drug being eliminated as metabolites rather than the parent compound. It has been reported that 50 to 100% of the administered radioactivity were eliminated in the urine in mice, rats, dogs, and monkeys, within 24 to 48 hours of dosing, and approximately 5 to 20% of the administered radioactivity was recovered in the feces after dosing (depending on the species). After oral administration of 600 mg of ¹⁴C-ribavirin in the human, approximately 61% and 12% of the radioactivity was eliminated in the urine and feces, respectively, in 336 hours. Unchanged ribavirin accounted for 17 % of the administered dose.

Other studies:

Protein binding: Published literature data showed that the majority of ribavirin in plasma is available as free drug and not bound to plasma proteins.

Analytical methods for ribavirin: ~~_____~~ methods with ~~_____~~ detection were used to determine unchanged ribavirin concentrations in serum and whole blood samples from studies conducted in the mouse, rat dog and monkey. All assays were validated according to GLP principles prior to sample analysis. These assays are reproducible, and possess sufficient sensitivity and precision to support the preclinical and clinical program. However, there are no validated method(s) for ribavirin 5' mono-, di-, and triphosphate measurement available at this time.

PK/TK summary: Ribavirin was readily absorbed and slowly eliminated in mice, rats and dogs with an approximate bioavailability of 80%. Ribavirin achieved maximal levels in the serum or plasma within 1-2 hours of dosing, and delayed with an initial half-life of between 4 to 10 hours. The uptake of ribavirin into RBCs is via a nucleoside transporter. A different accumulation of ribavirin in serum and RBCs was seen among mice, rats and cynomolgus monkeys after repeat oral administration. Metabolism of ribavirin occurs by two metabolic pathways: a reversible phosphorylation pathway (to mono, di and triphosphate metabolites) and a degradation pathway involving deribosylation to a triazole carboxamide. In human and monkey erythrocytes cells, ribavirin is phosphorylated to mono-, di-, and triphosphate nucleotides, with the major metabolite being the triphosphate nucleotide. Adenine kinase is the rate-limiting enzyme for the initial phosphorylation of ribavirin. RBCs are able to concentrate ribavirin triphosphate nucleotide. Ribavirin has minimal effect on P450s in either animals or humans. The principal route of elimination is renal for both ribavirin and its triazole carboxamide and triazole carboxylic acid metabolites. The primary route of drug elimination in rats, mice, dogs, and monkeys was in the urine, with 50 to 100% of

the administered radioactivity being eliminated within 24 to 48 hours of dosing. In general, ribavirin is excreted more rapidly in the urine and to a greater extent in rats than in monkeys and humans.

PK/TK conclusion: Ribavirin elicits significant toxicity on RBCs. Results of the pharmacokinetic and ADME studies of ribavirin suggested that the affected tissue is also the primary site of drug deposition after oral dosing. These results suggest that RBCs may serve as a drug (or the triazole carboxamide metabolites, and the phosphorylated drug metabolites) reservoir with delayed release following drug withdrawal. The accumulation of ribavirin nucleotide metabolites within the RBCs may contribute to the observed anemia in patients administered ribavirin in high doses or for prolonged periods of time. The pharmacokinetics of a tablet form of ribavirin (Roche Inc) and a capsule form of ribavirin (Schering) were equivalent in dogs. There was less than a 1% difference in the mean AUC values of ribavirin following administration in a tablet and capsule form.

Single-dose PK study:

1. Study Title: A Pharmacokinetics Study of Ribavirin in the Dog Following Single Oral Administration. Bioanalytical and Toxicokinetic Results

Sponsor: Hoffmann-La Roche, Nutley, NJ; Roche Study No.: 98-7099; Testing Facility: _____ Study Initiation Date: 17 September 1998; GLP: Yes (X); Formulation: ribavirin tablet (200 mg/tablet; Lot #: HA 28053-240) or Rebetol™ capsule (200 mg/capsule; Lot #: BRC 1338); Purity: not available

Methods

Two groups of fasted male beagle dogs (5/group, 9.1 – 10.8 kg at initiation of dosing, 5 months old) were orally administered a single dose (200 mg) of ribavirin tablet or capsule. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, and 72 hours postdose via the cephalic or jugular vein. Ribavirin was determined in dog serum using an _____ method. The limit of quantitation (LOQ) was defined to be _____

Results

The pharmacokinetic parameters of ribavirin following oral administration via a capsule or tablet are summarized below.

Formulation	Tablet	Capsule
PK parameters		
T _{1/2} (hours)	10.5 ± 0.92	10.77 ± 1.01
T _{max} (hours)	0.7 ± 0.3	0.8 ± 0.3
C _{max} (ng/ml)	8760 ± 3480	9060 ± 1550
AUC _{0-∞}	51500 ± 6120	52800 ± 6750

Comments

The overall exposure to ribavirin and the terminal half-life of ribavirin in dogs following oral administration of ribavirin (200 mg) in a tablet or capsule form were similar. There was less than a 1% difference in the mean AUC values of ribavirin following administration in a tablet and capsule form.

Multiple-dose TK studies:

2. Study Title: Four-Week Oral Gavage Dose Range Finding and Toxicokinetic Study with Ro 20-9963/000 (Ribavirin) in C57BL/6 Mice (Report No 1002209; _____ Study No. 6131-296 and HLR Study No. 07296)

Sponsor: Hoffmann-La Roche, Nutley, NJ; Roche Study No.: 07296; Testing Facility: _____ Study Initiation Date: 16 December 1999; GLP: Yes (X); Drug and Drug Lot Numbers: Ro 20-9963/000 Lot No.: 990543; Purity: _____ Formulation: Ro 20-9963/000 (100 mg/ml) in sterile water for injection, USP

Methods

A 4-week toxicokinetics study of ribavirin was conducted in C57BL/6 mice. The animals (24/sex/dose group and 6/sex for the control group) were dosed once daily by oral gavage with vehicle (sterile water), or 30, 100, 200 or 400 mg/kg/day of ribavirin (Lot No. 990543) for 4 weeks. Blood samples were collected at selected time points on Days 12 (only for the high dose group), and 28, for analysis of ribavirin in serum and whole blood. Drug concentrations in serum and whole blood were measured using a validated _____ assay for ribavirin in mouse serum and whole blood. Erythrocyte drug concentrations were calculated in part from serum samples and whole blood concentrations of the drug.

Results

Serum exposure to ribavirin generally increased with the increase in dose level. The increase in C_{max} was not consistently proportional to the increase in dose level while the increases in AUC_{0-24} were approximately proportional to the increases in dose level. Serum T_{max} values for various dosages of ribavirin were observed between 0.5 and 8 hours postdosing. AUC_{0-24hr} values obtained at the end of 4 weeks following administration of 30, 100, 200 and 400 mg/kg/day were 11.6, 35.5, 71.9 and 142.5 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$. Because no anticoagulant was added to the samples processed for the determination of ribavirin in whole blood, the whole blood and erythrocyte results are considered unreliable for the accurate determination of whole blood and erythrocyte levels of ribavirin.

3. Study Title: 13-Week Oral Gavage Toxicity and Toxicokinetics Study with R0 20-9963/000 (ribavirin) in Wistar Rats (Report 1003487)

Sponsor: Hoffmann-La Roche Inc. Nutley, NJ 07110; Roche Study No: 07320; Testing Facility: _____
Date of Initiation: March, 2000; GLP Compliance: Yes (X); Drug Lot: 990543; Formulation: ribavirin
was dissolved in deionized water

Methods

A 13-week toxicokinetics study of ribavirin was conducted in CrI:WI (Glx/BRL/Han)IGS BR rats. The animals (15/sex/dose group and 6/sex for the control group) were administered once daily by oral gavage with vehicle (sterile water), or 10, 40, 80 or 160 mg/kg/day of ribavirin for 13 weeks. Blood samples were collected at 0.5, 1, 2, 4, 8, and 24 hours postdose on Days 1, 28, and 91 (treatment termination). Serum and whole blood levels of ribavirin were measured by a liquid chromatography method with UV detection. Ribavirin concentrations in RBCs were calculated, in part, from serum and whole blood ribavirin concentrations.

Results

Oral toxicokinetic data for ribavirin in the 13-week Wistar rat study were conducted to support the dose selection for the 2-year oral rat carcinogenicity study. Ribavirin was quantifiable in serum from males and females of all treated groups after a single (Day 1) and multiple (Days 28 and 91) doses. Peak serum ribavirin levels were reached by approximately 1 hour after dosing in both males and females at 10 mg/kg/day. However, peak serum ribavirin levels were reached by approximately 2-8 hours after dosing in both males and females at 40, 80 or 160 mg/kg/day. Serum Ribavirin concentrations were linearly proportional to dose in both sexes and at both Days 1, 29, and 91. Males had 1.4-fold higher mean serum ribavirin levels than females. Dose-related systemic exposures (AUC_{0-24h}) were demonstrated in all treatment groups. Serum ribavirin concentrations were 2.5 to 3-fold higher after 13-weeks of dosing compared with levels after the first dose, suggesting a ribavirin accumulation in tissues and in the serum. Systemic exposure to ribavirin was greater in males than in females on Day 1, but not on Days 28 and 91. Ribavirin whole blood concentrations generally increased with the increase in dose. Although the RBC data suggested a low distribution of ribavirin associated with RBCs at doses of 80 mg/kg/day or less, the calculated levels of ribavirin in the erythrocytes generally increased with the increase in dose and with time, indicating accumulation of ribavirin in the erythrocytes after multiple dosing. Gender differences

were noted at 160 mg/kg/day; at this dose level, females exhibited a 2-fold higher RBC level of ribavirin compared to males on Day 91 that correlated with the increased severity of anemia noted for females in the high dose group, as compared to males.

Pharmacokinetics of ribavirin in rats after 13-week oral doses of ribavirin (Ro 20-9963/000)

Ribavirin Dose (mg/kg)	SERUM RIBAVIRIN (PHAMACOKINETIC PARAMETERS)																	
	Day 1						Day 28						Day 91					
	C _{max} (ng/ml)		AUC _{0-24h} (ng•hr/ml)		T _{max} (hr)		C _{max} (ng/ml)		AUC _{0-24h} (ng•hr/ml)		T _{max} (hr)		C _{max} (ng/ml)		AUC _{0-24h} (ng•hr/ml)		T _{max} (hr)	
10	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
40	133	91	1114	973	1	1	157	133	2116	1821	1	2	122	142	1780	1941	1	0.5
80	434	292	6665	4585	1	4	538	539	9258	8103	8	8	465	610	8281	9622	4	8
160	811	589	11382	8478	4	4	857	859	15290	11877	2	2	961	933	16738	16946	8	8
	1346	1058	18887	15038	4	4	2070	1973	40400	31415	4	2	2927	2690	46633	46067	2	2

M: male; F: female

Comment:

Based on the MTD (less than 80 mg/kg) identified in the 13-week oral toxicity study in Wistar rats, systemic exposure in rats in the oral carcinogenicity study are expected to be 1.6-fold lower than the anticipated clinical oral dose for hepatitis in humans (ribavirin, 600 mg once daily for 14 weeks; multiple doses; AUC_{0-12hr}: 25467 ng•hr/mL; Study No. NV 15801)

4. Study Title: A 26-Week Oral Gavage Toxicity and Toxicokinetic Study with Ribavirin in Rats with a 6-Week Recovery

Sponsor: Hoffmann-La Roche; Study No. 07321; Study Initiation Date: November 11, 2000; Testing Facility:

GLP: Yes (X); Drug and Drug Lot Numbers: Ro 20-9963/000 Lot No. 990543;

Formulation: Ro 20-9963/000 in Sterile Water for Injection, USP (sterile water) (Lot Nos. 57-978-FW, and 61-900-FW).

Methods

A 26-week toxicokinetics study of ribavirin was conducted in Crl:WI (Glx/BRL/Han)IGS BR rats. The animals (15/sex/dose group and 6/sex for the control group) were dosed once daily by oral gavage with vehicle (sterile water), or 10, 30, or 70 mg/kg/day of ribavirin (Lot No. 990543) for 26 weeks. Blood samples were collected at selected time points on Days 1, 28, 91, 126 and 182 and once during recovery (week 33) for analysis of ribavirin in serum and whole blood. The concentration of ribavirin in RBCs was calculated, in part from serum and whole blood concentrations of ribavirin. Ribavirin concentrations in serum and whole blood were measured using a validated assay for ribavirin in rat serum and whole blood.

Results

In general, exposure to ribavirin increased as the dose increased from 10 to 70 mg/kg/day. The increases in C_{max} were not consistently dose-proportional to the increase in the dose level. The increases in AUC_{0-24hr} in rats were approximately dose proportional to the increase in the dose level. The C_{max} and AUC_{0-24hr} values on Days 28, 91, 126, and 182 were generally higher than on Day 1, indicating accumulation of Ro 20-9963 with time and after multiple dosing in rats. Ribavirin whole blood concentrations generally increased with the increase in dose level. The calculated erythrocyte levels varied across the different dose groups, but the data suggested a low distribution of ribavirin associated with RBCs within the tested dose range. Data collected on later days (Days 28, 91, 126, and 182) showed an increase in erythrocyte concentrations of ribavirin with increasing dose, which suggested an accumulation of ribavirin in RBCs over an extended period of drug treatment. Following 6-month administration of 10, 35 and 70 mg/kg/day of Ro 20-9963, respectively, exposures (AUC_{0-24hr}) at Day 182 were 2.3, 7.9 and 19.1 µg•hr/ml, as compared to the therapeutic AUC_{0-12hr} of 26.4µg•hr/ml/ml.

Toxicokinetic parameters following 26-weeks daily oral administration of ribavirin to rats

Dose (mg/kg/day)	Study Day	Serum C _{max} (ng/mL) M+F	Serum AUC _{0-24h} ng•h/mL M+F	Blood (ng/mL) M+F	RBC (ng/mL)	
					M	F
10	1	100	1162	8	10.2	10.7
	28	165	2170	18	3.4	8.5
	91	226	2259	21	5.3	22.7
	126	206	2260	23	10.9	16.2
	182	199	2264	21	1.68	9.5
35	1	324	4054	30	6.7	7.9
	28	457	6186	60	21.6	11.8
	91	490	7149	72	33.9	NR
	126	612	8521	89	47.2	22.4
	182	537	7925	69	18	5.1
70	1	663	8744	67	9.4	1.7
	28	887	13299	141	49.4	57
	91	1007	17244	205	74.2	41.9
	126	1297	21460	265	81.7	103
	182	1138	19155	197	30	71.8

NR= Not reported due to negative erythrocyte concentration of ribavirin.

5. A 26-Week Oral Gavage Toxicity and Toxicokinetic Study with Ribavirin in Dogs with a 6-Week Recovery (Report No. 1003489)

Sponsor: Hoffmann-La Roche; Study No.: 07322. Page 1 of 1151. Study Initiation Date: 9 September, 1998; Testing Facility: _____
 GLP: Yes (X); Drug and Drug Lot Numbers: Ro 20-9963/000 Lot No.: 990543; Purity: _____ Formulation: Ro 20-9963/000 in sterile water for injection, USP

Methods

A 26-week toxicokinetics study of ribavirin was conducted in Beagle dogs. The animals (7/sex/dose group and 6/sex for the control group) were dosed once daily by oral gavage with vehicle (sterile water), or 5, 10, or 20 mg/kg/day of ribavirin (Lot No. 990543) for 26 weeks. Blood samples were collected at selected time points on Days 1, 28, 91, and 182 for analysis of ribavirin in serum and whole blood. The concentration of ribavirin in RBCs was calculated, in part from serum and whole blood concentrations of ribavirin. Ribavirin concentrations in serum and whole blood were measured using a validated _____ assay for ribavirin in dog serum and whole blood.

Results

In general, ribavirin levels in the whole blood and RBC increased with the increase in the ribavirin dose level. The increase in C_{max} was proportional to the increase in dose level for males, but was slightly higher than dose proportional for females. The increases in AUC_{0-24hr} were generally proportional to the increase in the dose level. No accumulation of ribavirin in serum was seen in dogs after multiple dosing. Slight accumulation of ribavirin was seen in the RBCs of dogs. No gender differences were seen in dogs in the mean AUC_{0-24hr} values. Exposures (AUC_{0-24hr}) of 10.4, 23.1 and 40.1 µg•hr/ml were achieved at treatment termination following administration of dose levels of 5, 10 and 20 mg/kg/day, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 µg•hr/ml.

Toxicokinetic parameters following 26-weeks daily oral administration of ribavirin to dogs

Dose (mg/kg/day)	Study Day	Serum C _{max} (ng/mL) M+F	Serum AUC _{0-24h} ng•h/mL M+F	Blood (ng/mL) M+F	RBC (ng/mL)	
					M	F
5	1	1495	8315	37.9	NR	NR
	28	1180	8200	102.7	96.5	95.1
	91	1555	9795	73.1	1.8	58.3
	182	1505	10395	97.7	53.4	84.5
10	1	3170	17600	86.1	22.2	15.3
	28	2855	18500	142.5	61.0	81.9
	91	3405	19450	202.5	89.8	345
	182	3700	23100	174.5	74.8	78.5
20	1	7175	35700	236.5	18.2	205
	28	6888	42600	386	250	286
	91	7175	40450	273.5	NR	NR
	182	6145	40050	358	121	152

NR= Not reported due to negative erythrocyte concentration of ribavirin.

IV. GENERAL TOXICOLOGY:**List of General Toxicology Studies:****Mouse:**

1. 4-Week Oral Gavage Dose Range Finding and Toxicokinetic Study with Ro 20-9963/000 (ribavirin) in C57BL/6 Mice (— Study No. 6131-296 and — Study No. 07296).

Rat:

2. 13-Week Oral Gavage Toxicity and Toxicokinetic Study with Ro 20-9963/000 in Rats. April 3, 2001.
3. 26-Week Oral Gavage Toxicity and Toxicokinetic Study with a 6-Week Recovery Phase with Ro 20-9963/000 in Rats.

Dog:

4. 26-Week Oral Gavage Toxicity and Toxicokinetic Study with a 6-Week Recovery Phase with Ro 20-9963/000 in Dogs.

Acute Toxicology-Mouse, rats and dogs:

To support an NDA/BLA filing for PEG-IFN alfa-2a/ribavirin, the sponsor submits results from the non-clinical studies characterizing the acute and repeat-dose toxicities and the potential teratogenicity of ribavirin in rodent and non-rodent species to the division for review. The sponsor did not conduct acute toxicity studies with ribavirin, but provided published literature data from acute toxicity studies to the division for review. These results indicate that ribavirin is well-tolerated in rodents. Oral LD₅₀ values range from 2700 to 4116 mg/kg for male rats, 5827 to 6300 mg/kg for female rats and 2000 to 10,000 mg/kg for mice. In dogs, a single dose of 1500 mg/kg of ribavirin resulted in death.

Multiple-Dose Studies

To support an NDA/BLA filing for PEG-IFN alfa-2a/ribavirin, the sponsor submitted results from the non-clinical studies the repeat-dose toxicities of ribavirin in rodent and non-rodent species to the division for review.

Mice:

1. **Study Title: Four-week oral gavage dose range finding and toxicokinetic study with Ro 20-9963/000 (ribavirin) in C57BL/6 mice (Report No 1002209; — Study No. 6131-296 and — Study No. 07296)**

Sponsor: Hoffmann-La Roche, Nutley, NJ; Roche Study No.: 07296; Testing Facility: _____
Study No.: 6131-296; Study Initiation Date: 16 December 1999; GLP: Yes (X); Drug and Drug Lot
Numbers: Ro 20-9963/000; Lot No.: 990543; Purity: — Formulation: Ro 20-9963/000 (100 mg/ml) in sterile water for injection, USP

A 4-week oral gavage range-finding toxicity and toxicokinetic study was conducted in male and female C57BL/6 mice to characterize the toxicity of ribavirin (Ro 20-9963) after 4 weeks of administration and to determine dose levels for a subsequent 6-month carcinogenicity study in p53 knock-out mice. The mice received daily doses of 0, 30, 100, 200 or 400 mg/kg/day.

Key study findings:

- All main study animals and approximately one-half of the toxicokinetic animals at 400 mg/kg/day died or were sacrificed moribund by Study Day 12. At 200 mg/kg/day, three of 10 females (main study animals) and 5 of 24 males (toxicokinetic animals) died or were sacrificed moribund.

- Hypoactivity, labored respiration, hunched posture, thin appearance, cold to touch, reduced stools, and weight losses were seen in the mice that died or were sacrificed moribund prior to death.
- All animals that died or were sacrificed moribund showed ↓RBC, ↓Hct, and ↓Hb values; ↓leukocyte and ↓lymphocyte counts; and ↑platelet counts. Dose-related decreases in RBC counts were noted in males given 30 mg/kg/day and in both sexes given 100 and 200 mg/kg/day of ribavirin. Dose-related decreases in Hct and Hb were also noted in mice of both sexes administered 100 or 200 mg/kg/day of ribavirin. Platelet counts were increased in 100 mg/kg/day females and 200 mg/kg/day mice of both sexes. Total leukocyte and lymphocyte counts were decreased in males given 200 mg/kg/day of ribavirin.
- ALT values were decreased in male mice treated with ≥30 mg/kg/day and in female mice administered 100 or 200 mg/kg/day of ribavirin.
- A treatment-related increase in absolute and relative (to body and brain weights) spleen weights was noted for mice at 200 mg/kg/day. A statistically significant decrease in relative (to body weight) testes/epididymis weights was observed for surviving males at 200 mg/kg/day.
- Treatment-related histopathologic changes were seen in mice that died at ≥200 mg/kg/day, which included: crypt cell necrosis and regenerative hyperplasia in the small and large intestine; lymphoid depletion/necrosis in the thymus, spleen, and mesenteric lymph node; adrenal cortical hypertrophy, atrophy of salivary glands; and congestion and hypocellularity in the bone marrow and hypospermia in the epididymis. An increase in the incidence and severity of splenic extramedullary hematopoiesis was also seen in mice at ≥30 mg/kg/day. The severity of these findings was dose-related between 30 and 200 mg/kg/day.
- The NOEL for this study could not be determined, which was considered to be <30 mg/kg/day.
- The increase in C_{max} was not consistently proportional to the increase in dose level while the increases in AUC_{0-24} were approximately proportional to the increases in dose level. Serum T_{max} values for various dosages of ribavirin were observed between 0.5 and 8 hours postdosing. AUC_{0-24hr} values obtained at the end of 4 weeks following administration of 30, 100, 200 and 400 mg/kg/day were 11.6, 35.5, 71.9 and 142.5 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$.

Methods:**Dosing:****Species/strain:**

C57BL/6 strain mice

#/sex/group or time point (main study):

10/sex/treatment group-main study; 6/sex for the control group

Satellite groups used for toxicokinetics:

24/sex/treatment group-toxicokinetic analyses

Age:

7-8 weeks old;

Weight:

16-23.2 g for males, 13.9-19.59 g for females

Doses in administered units:

0 (vehicle control), 30, 100, 200 or 400 mg/kg/day x 4 weeks;

Route, form, volume, and infusion rate:

oral, by gavage; dosing volume-10 mL/kg/day; due to the excessive mortality noted at 400 mg/kg/day, all surviving main study and toxicokinetic animals in this group were sacrificed by Study Day 12.

Observations and times:**Clinical signs:** twice daily**Body weight:** weekly**Food consumption:** weekly (main study animals only)**Ophthalmoscopy:** not determined**EKG:** not determined

Hematology:	at study termination (surviving mice) and prior to death/moribund sacrifice
Clinical chemistry:	at study termination (surviving mice) and prior to death/moribund sacrifice
Urinalysis:	not determined
Gross pathology:	at sacrifice (selected organs were weighed, Appendix Table 1)
Histopathology:	at sacrifice (a comprehensive list of tissues was collected and microscopic examinations were performed on 42 tissues for each animals in the main study). In addition, bone marrow smears from the femurs of animals in the control and high-dose groups were evaluated at the scheduled sacrifice (Appendix Table 1).
Toxicokinetics:	Blood samples for the determination of serum concentrations of ribavirin were collected from toxicokinetic animals in the 30, 100 and 200 mg/kg/day groups at approximately 30 minutes and 1, 2, 4, 8, and 24 hours after oral dosing on Day 28 (treatment termination). Control toxicokinetic animals were bled 4 hours post-dose on Day 28. Blood samples for toxicokinetic analyses were obtained from mice in the 400 mg/kg group on Study Day 12. Ribavirin concentrations in whole blood were evaluated 24 hours post-dose on Day 28. Erythrocyte levels of ribavirin were calculated, in part, from serum and whole blood ribavirin concentrations.
Mortality:	The high dose of 400 mg/kg/day of ribavirin was not well tolerated. All main study animals and approximately one-half of the toxicokinetic animals died or were sacrificed moribund by Study Day 12. At 200 mg/kg/day, three of 10 females (main study animals) and 5 of 24 males (toxicokinetic animals) died or were sacrificed moribund. No mortality was observed in mice dosed with 30 or 100 mg/kg/day of the test article.
Clinical signs:	Hypoactivity, labored respiration, hunched posture, thin appearance, cold to touch, reduced stools, and weight losses were seen in the mice that died or were sacrificed moribund prior to death. No treatment-related clinical signs were observed in surviving mice at 200 mg/kg/day, or in mice in the 30 or 100 mg/kg/day groups.
Body weight:	No consistent treatment-related effects on mean body weights, weekly body weight gains, or overall (weeks 1-4) weight gains were seen in any dose group.
Food consumption:	No treatment-related effects on food consumption were noted in any dose group.
Hematology:	All animals that died or were sacrificed moribund exhibited a variety of hematologic findings including: decreased RBC, Hct, and Hb values; decreased leukocyte and lymphocyte counts; and increased platelet counts. Dose-related decreases in RBC counts were noted in males given 30 mg/kg/day and in both sexes given 100 and 200 mg/kg/day of ribavirin. Dose-related decreases in Hct and Hb were also noted in mice of both sexes administered 100 or 200 mg/kg/day of ribavirin. Platelet counts were increased in 100 mg/kg/day females and 200 mg/kg/day mice of both sexes. Total leukocyte and lymphocyte counts were decreased in males given 200 mg/kg/day of ribavirin.
Clinical chemistry:	AST values were decreased in the 200 mg/kg/day group. ALT values were decreased in male mice treated with 30, 100, or 200 mg/kg/day and in female mice administered 100 or 200 mg/kg/day of ribavirin.
Organ weights:	A treatment-related increase in absolute and relative (to body and brain weights) spleen weights was noted for mice at 200 mg/kg/day. A statistically significant decrease in relative (to body weight) testes/epididymis weights was observed for surviving males at 200 mg/kg/day.
Gross pathology:	There were no treatment-related gross findings observed at the terminal necropsy.

Histopathology: Treatment-related microscopic changes were seen in the terminal-sacrifice animals from all treatment groups. All mice that died exhibited microscopic changes in the spleen, intestines, lymphoid tissues, adrenals, salivary glands, bone marrow, and epididymis. These changes included: crypt cell necrosis and regenerative hyperplasia in the small and large intestine; lymphoid depletion/necrosis in the thymus, spleen, and mesenteric lymph node; adrenal cortical hypertrophy; atrophy of salivary glands; congestion and hypocellularity in the bone marrow; and hypospermia in the epididymis. An increase in the incidence and severity of splenic extramedullary hematopoiesis were also seen in the test article treatment groups. The severity of these findings was dose-related between 30 and 200 mg/kg/day. All other microscopic changes were considered spontaneous or incidental in nature and not treatment-related.

Toxicokinetics: Serum exposure to ribavirin generally increased with the increase in dose level. The increase in C_{max} was not consistently proportional to the increase in dose level while the increases in AUC_{0-24} were approximately proportional to the increases in dose level. Serum T_{max} values for various dosages of ribavirin were observed between 0.5 and 8 hours postdosing. AUC_{0-24hr} values obtained at the end of 4 weeks following administration of 30, 100, 200 and 400 mg/kg/day were 11.6, 35.5, 71.9 and 142.5 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$. Because no anticoagulant was added to the samples processed for the determination of ribavirin in whole blood, the whole blood and erythrocyte results are considered unreliable for the accurate determination of whole blood and erythrocyte levels of ribavirin.

Comments

The maximum tolerated dose (MTD) in the 4-week dose range finding study is considered to be 100 mg/kg/day. A dose level of 100 mg/kg/day was associated with target-organ toxicity (i.e., hematology changes indicative of anemia with compensatory extramedullary hematopoiesis in the spleen), and a dose level of 200 mg/kg/day elicited mortality in this study. Thus, a high dose level of 100 mg/kg/day was selected for the 6-month carcinogenicity study. Based on the slight changes noted at 30 mg/kg/day in the present study, a low dose level of 10 mg/kg/day was selected for inclusion in the 6-month carcinogenicity study in p53 knock-out mice. A mid-dose level of 50 mg/kg/day was selected for the 6-month carcinogenicity study to evaluate a possible dose-response relationship, which is 50% of the high dose level of 100 mg/kg/day. Note that the dose levels selected for the 6-month carcinogenicity study in p53 knock-out mice was submitted to and subsequently approved by the CAC-EC, CDER (CAC-EC fax of July 18, 2000)

Rats:

2. Study Title: 13-Week Oral Gavage Toxicity and Toxicokinetics Study with R0 20-9963/000 (ribavirin) in Wistar Rats (Report 1003487)

Sponsor: Hoffmann-La Roche Inc., Nutley, NJ 07110; Roche Study No: 07320; Testing Facility: _____
Date of Initiation: March, 2000; GLP Compliance: Yes (X); Drug Lot: 990543; Formulation: ribavirin was dissolved in deionized water

Key study findings

- Mortality was seen in rats at 160 mg/kg/day. (3M, 5F). Clinical signs included hunched posture, thin appearance, and hypoactivity at the high dose.
- Food consumption, mean body weights and body weight gains were reduced for animals at ≥ 80 mg/kg/day. Effects on the hematological parameters (RBC, Hb, Hct) were seen in rats at 160 mg/kg/day. Platelet counts were increased and lymphocyte counts were decreased generally at 160

mg/kg/day. Bone marrow smears revealed elevated myeloid-to-erythroid ratios and erythroid hypoplasia at 160 mg/kg/day.

- Clinical chemistry changes were observed mainly at 160 mg/kg/day and included lower total protein, albumin, globulin, cholesterol, and triglycerides and higher AST and phosphorus.
- Organ weight changes included increased heart, spleen and lung weights at the high dose and decreased thymic weights at ≥ 40 mg/kg/day. Decreased thymic weights were seen in rats at 40 mg/kg/day or greater, which were generally correlated with microscopic observations of thymic lymphoid depletion. Increased lung weights were noted in rats at 80 mg/kg/day or greater, which were correlated with an increase in the incidence and/or severity of alveolar and interstitial macrophage, or foamy alveolar macrophage infiltration in the lungs.
- Hepatocellular necrosis was seen in rats at 80 mg/kg/day. Liver centrilobular necrosis and vacuolar degeneration was seen in rats at 160 mg/kg/day. Increases in splenic weights were seen in rats at 160 mg/kg/day, which were associated with splenic extramedullary hematopoiesis, lymphocytic depletion, and hemorrhage. Additionally, depletion of thymic-dependent areas of lymph nodes and decreased numbers of erythroid and myeloid precursor in the bone marrow were noted in rats at 160 mg/kg/day. Skin lesions (ulceration, dermis and epithelial sclerosis) were also noted microscopically in one female rat at 40 mg/kg/day, and in both sexes at 80 mg/kg/day or greater.
- The NOAEL for ribavirin administered by oral gavage to Wistar rats once daily for 13 weeks was 10 mg/kg/day. Treatment-related effects in males and females at doses ≥ 80 mg/kg indicated that these doses exceeded the maximum tolerated dose (MTD).

Methods

Species:	CrI:WI(G1x/BRL/Han) IGS BR rats (_____ , main study: 12/sex/group; toxicokinetic study: 15/sex/ group)
Age, weight:	6-8 weeks old; 181-216 g for males, 137-156 g for females
Dosage:	0 (vehicle control), 10, 40, 80, or 160 mg/kg/day (5 mL/kg/day) for 13 weeks
Route:	oral, by gavage
Drug:	Ro 20-9963/000 (Lot No. 990543) in Sterile Water for Injection, USP
Clinical signs:	twice daily
Body weight:	weekly
Food consumption:	weekly
Ophthalmoscopy:	main-study - prior to treatment, during Weeks 12
Hematology:	at Weeks 2, 4, 8, and 13
Clinical chemistry:	at Weeks 2, 4, 8, and 13
Urinalysis:	at Weeks 2, 4, 8, and 13
Gross pathology:	at sacrifice; after a 13-week treatment period (selected organs were weighed, Appendix Table 1)
Histopathology:	at sacrifice; after a 13-week treatment period (a comprehensive list of tissues was collected and microscopic examinations were performed on 42 tissues for each animals in the main study). In addition, bone marrow smears from the femurs of animals in the control and high-dose groups were evaluated at the scheduled sacrifice (Appendix Table 1).
Toxicokinetics:	Blood samples were collected from toxicokinetic animals predose and at approximately 0.5 and 1, 2, 4, 8, and 24 hours postdose on Days 1, 28, and 91 (treatment termination). Serum and whole blood levels of ribavirin were measured by a _____ method with _____ Ribavirin concentrations in RBCs were calculated, in part, from serum and whole blood ribavirin concentrations.

Results

- Mortality:** Eight of 24 (33%) animals at 160 mg/kg/day in the main study group were found dead or sacrificed moribund during the study. In addition, 8 of 30 (26%) toxicokinetic animals at 160 mg/kg/day, 1 of 30 rats at 80 mg/kg/day in the toxicokinetic group, and 1 of 24 rats at 10 mg/kg/day in the main study group were sacrificed moribund.
- Clinical signs:** Clinical signs were seen at 80 mg/kg/day or greater, including sores, scabs, hunched appearance, hypoactivity, sensitive to touch, vocalization, nasal discharge, few-, liquid-and/or no feces, pale eyes and labored respiration.
- Body weights:** Mean absolute body weights (Week 1-13) reduced by 13% and 6% for males and females, respectively, at 160 mg/kg/day. Mean absolute body weights (Week 1-13) reduced by 11% and 4% for males and females, respectively, at 80 mg/kg/day. Mean absolute body weights (Week 1-13) were reduced by 3% and 2% for males and females, respectively, at 40 mg/kg/day. Mean body weight gains (Week 1-13) were significantly reduced in rats at 80 mg/kg/day (19-20%↓) and 160 mg/kg/day (25-26%↓). Body weight gains reduced by 4% and 12% for males and females, respectively, at 40 mg/kg/day. At 10 mg/kg/day, overall body weight gains were comparable to controls for males but reduced by 10% for females (Tables 1 and 2).
- Food consumption:** Food consumption was reduced in rats at 80 mg/kg/day or greater (Tables 3).
- Ophthalmology:** No treatment-related changes were seen.
- Hematology:** RBC, hemoglobin, and hematocrit were moderate-to-markedly reduced in rats at 160 mg/kg/day during the 13-week rat study. Mild decreases in these parameters were seen in rats at 80 mg/kg/day. Increases in reticulocyte counts and decreases in lymphocyte counts were seen in rats at 160 mg/kg/day. Increases in platelet counts were seen in rats at 80 mg/kg/day or greater. Slight decreases in hemoglobin and hematocrit were seen in rats at 10 mg/kg/day and 40 mg/kg/day (Table 4).
- Clinical chemistry:** Decreased creatinine, total protein, albumin, sodium, globulin, cholesterol, and triglycerides were seen in rats at 160 mg/kg/day. Decreased cholesterol was seen in females at 80 mg/kg/day (Table 5).
- Urinalysis:** Urinalysis test results were unaffected by administration of Ro 20-
- Organ weights:** Increased heart, lung, and splenic weights were seen in rats at 160 mg/kg/day. Decreased thymic weights were seen in rats at 40 mg/kg/day or greater, which were generally correlated with microscopic observations of thymic lymphoid depletion. Increased lung weights were noted in rats at 80 mg/kg/day or greater, which were correlated with an increase in the incidence and/or severity of alveolar and interstitial macrophage, or foamy alveolar macrophage infiltration in the lungs (Table 6). Increased absolute and relative heart weights were seen in rats treated with ribavirin, with no histopathological correlates for the cardiac weight differences.
- Gross Findings:** "Crusted area" in the skin was seen in rats at 40 mg/kg/day or greater, which was Ro 20-9963-related gross lesion.
- Histopathology:** Hepatocellular necrosis was seen in rats at 80 mg/kg/day. Liver centrilobular necrosis and vacuolar degeneration was seen in rats at 160 mg/kg/day. Increases in splenic weights were seen in rats at 160 mg/kg/day, which were associated with splenic extramedullary hematopoiesis, lymphocytic depletion, and hemorrhage. Additionally, depletion of thymic-dependent areas of lymph nodes and decreased numbers of erythroid and myeloid precursor in the bone marrow were noted in rats at 160 mg/kg/day. Skin lesions (ulceration, dermis and epithelial sclerosis)

were also noted microscopically in one female rat at 40 mg/kg/day, and in both sexes at 80 mg/kg/day or greater. Toxicology summary tables from the 13-week range-finding toxicity study in Wistar rats are included in Appendixes 1 and 2.

Comment

The NOAEL for ribavirin administered by oral gavage to Wistar rats once daily for 13 weeks was 10 mg/kg/day. Treatment-related effects in males and females at doses \geq 80 mg/kg indicated that these doses exceeded the maximum tolerated dose (MTD).

Toxicokinetics: Oral toxicokinetic data for ribavirin in the 13-week Wistar rat study were conducted to support the dose selection for the 2-year oral rat carcinogenicity study. Ribavirin was quantifiable in serum from males and females of all treated groups after a single (Day 1) and multiple (Days 28 and 91) doses. Peak serum ribavirin levels were reached by approximately 1 hour after dosing in both males and females at 10 mg/kg/day. However, peak serum ribavirin levels were reached by approximately 2-8 hours after dosing in both males and females at 40, 80 or 160 mg/kg/day. Serum Ribavirin concentrations were linearly proportional to dose in both sexes and at both Days 1, 29, and 91. Males had 1.4 -fold higher mean serum ribavirin levels than females. Dose-related systemic exposures (AUC_{0-24h}) were demonstrated in all treatment groups (Table 7). Serum ribavirin concentrations were 2.5 to 3-fold higher after 13-weeks of dosing compared with levels after the first dose, suggesting a ribavirin accumulation in tissues and in the serum. Systemic exposure to ribavirin was greater in males than in females on Day 1, but not on Days 28 and 91. Ribavirin whole blood concentrations generally increased with the increase in dose. Although the RBC data suggested a low distribution of ribavirin associated with RBCs at doses of 80 mg/kg/day or less, the calculated levels of ribavirin in the erythrocytes generally increased with the increase in dose and with time, indicating accumulation of ribavirin in the erythrocytes after multiple dosing. Gender differences were noted at 160 mg/kg/day; at this dose level, females exhibited a 2-fold higher RBC level of ribavirin compared to males on Day 91 that correlated with the increased severity of anemia noted for females in the high dose group, as compared to males.

Comment:

- Based on the results from this study, the dose levels selected for the 2-year carcinogenicity study in rats were 10, 30 and 60 mg/kg/day. A dose level of 160 mg/kg/day was considered too high for inclusion in the 2-year carcinogenicity study in rats. Additionally, the 20% reduction in overall body weight gain at 80 mg/kg/day suggested that a dose level of 80 mg/kg/day in the 2-year carcinogenicity study would likely exceed the MTD. A low dose level of 10 mg/kg/day was selected for the 2-year rat carcinogenicity study, as this dose level is not expected to produce overt toxicity. A mid-dose level of 30 mg/kg/day was selected to evaluate a possible dose-response relationship. Additionally, the mid-dose of 30 mg/kg/day is 50% of the high dose level of 60 mg/kg/day. Note that the dose levels selected for the 2-year carcinogenicity study in rats were submitted to and subsequently approved by the CAC.

Table 1. Summary of absolute body weights from the 13-week range-finding study with ribavirin in Rats

Dose (mg/kg)	Mean Absolute Body Weight (g) (Male)					Mean Absolute Body Weight (g) (Female)				
	Week 1	Week3	Week 6	Week9	Week13	Week 1	Week3	Week6	Week9	Week13
0	195±18	270±23	333±31	373±37	412±42	148±9	181±12	209±14	221±16	223±19
10	196±19	267±17	334±22	373±27	411±30	146±9	176±10	198±12	210±16	222±17
40	196±18	265±20	326±29	363±33	400±29	147±10	174±13	196±15	208±16	219±17
80	195±18	254±21	308±30	339±33	367±37*	147±9	169±11*	191±12*	203±11*	215±12*
160	198±19	238±28*	270±41*	319±41*	355±41*	147±8	168±9*	185±13*	201±10*	211±13*

*P<0.05

Table 2. Summary of body weight gains from the 13-week range-finding study with ribavirin in Rats

Dose (mg/kg)	Mean Body Weight Changes (g) (Male)						Mean Body Weight Changes (g) (Female)					
	1-2	4-5	6-7	13-14	1-14	(↓%)	1-2	4-5	6-7	13-14	1-14	(↓%)
0	42±4	19±23	16±3	3±4	220±30	-	21±4	7±5	6±4	2±4	86±16	-
10	42±4	20±17	17±5	5±2	221±27	-	17±4	6±5	9±5	2±3	77±13	10
40	40±8	19±20	16±2	6±2	209±29	4	17±5	4±5	7±3	3±3	75±11	12
80	35±6	24±21	15±3	5±3	177±31*	19	12±5*	5±5	7±2	1±2	69±11*	20
160	24±7*	1±22*	19±10	4±5	162±41*	26	13±3*	-3±9*	7±10	2±2	64±16*	25

*P<0.05

Table 3. Summary of food consumption data from the 13-week range-finding study with ribavirin in Rats

Dose (mg/kg)	Mean Food Consumption (g) (Male)					Mean Food Consumption (g) (Female)				
	Week1	Week3	Week6	Week9	Week13	Week 1	Week3	Week6	Week9	Week13
0	167±15	177±16	177±19	169±18	169±17	125±10	129±12	127±10	123±13	122±14
10	159±12	170±17	166±11	169±14	167±11	117±7	121±7	125±11	121±14	116±12
40	158±15	164±15	168±20	161±19	160±19	115±8	118±10	119±11	115±12	114±9
80	152±13*	155±15*	157±14*	152±14*	146±15*	111±9	115±8	119±7	115±8	114±8
160	140±12*	144±27*	165±14	153±11	155±14	108±12	114±11	129±18	124±16	119±21

*P<0.05

Table 4a. Summary of clinical hematology data from the 13-week range finding study with ribavirin in rats

Dose (mg/kg)	Hematological Parameters (Week 2) (Male)							Hematological Parameters (Week 2) (Female)						
	RBC $10^6/\mu\text{L}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{L}$	Retic $10^3/\mu\text{L}$	Retic %	WBC $10^3/\mu\text{L}$	RBC $10^6/\mu\text{L}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{L}$	Retic $10^3/\mu\text{L}$	Retic %	WBC $10^3/\mu\text{L}$
0	7.6	14.9	44	946	216	2.9	7.6	7.9	15.3	45	899	160	2.0	6.4
10	7.4	14.6	43	933	177	2.4	7.3	7.8	14.7*	44	950	130	1.7	6.7
40	7.4	14.6	43	880	191	2.6	6.8	7.7	14.4*	43*	876	138	1.8	6.0
80	7.5	14.4*	43	972	172	2.4	6.6	7.3*	14.1*	42*	845	145	2.0	5.7
160	6.5*	13.1*	38*	1135*	122	1.9	5.9	6.9*	13.4*	40*	934	140	2.0	6.0

*P<0.05

Table 4b. Summary of clinical hematology data from the 13-week range finding study with ribavirin in rats

Dose (mg/kg)	Hematological Parameters (Week 4) (Male)							Hematological Parameters (Week 4) (Female)						
	RBC $10^6/\mu\text{L}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{L}$	Retic $10^3/\mu\text{L}$	Retic %	WBC $10^3/\mu\text{L}$	RBC $10^6/\mu\text{L}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{L}$	Retic $10^3/\mu\text{L}$	Retic %	WBC $10^3/\mu\text{L}$
0	8.6	16.1	47	977	92	1.1	8.0	8.2	15.5	44	903	115	1.4	6.8
10	8.2	15.4	45	955	94	1.1	7.9	8.2	15.0*	44	1031	142	1.7	6.7
40	8.2	15.1	44*	951	147	1.8*	7.3	8.0	14.3*	42*	986	113.8	1.4	7.1
80	8.0*	14.6*	43*	1055	128	1.6	6.9	7.6*	13.9*	41*	958	152.0	2.0	5.6
160	6.8*	13.6*	38*	1281*	142	2.1	5.9*	6.6*	12.3*	35*	1273	114	1.7	6.7

*P<0.05

Table 4c. Summary of clinical hematology data from the 13-week range finding study with ribavirin in Wistar rats

Dose (mg/kg)	Hematological Parameters (Week 8) (Male)							Hematological Parameters (Week 8) (Female)						
	RBC $10^6/\mu\text{L}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{L}$	Retic $10^3/\mu\text{L}$	Retic %	WBC $10^3/\mu\text{L}$	RBC $10^6/\mu\text{L}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{L}$	Retic $10^3/\mu\text{L}$	Retic %	WBC $10^3/\mu\text{L}$
0	8.9	16.3	47	924	113	1.3	7.0	8.6	16.0	46	862	70	0.8	5.8
10	8.7	15.4*	45*	970	127	1.5	7.8	8.5	15.0*	44*	965	94	1.1	5.8
40	8.6	15.0*	44*	922	104	1.2	6.7	8.4	14.2*	42*	990	106	1.3	5.6
80	8.4	15.0*	43*	1102*	138	1.6	7.0	7.9*	13.9*	41*	1065*	109	1.4	5.1
160	5.3*	11.5*	32*	1120*	153	2.9	4.3*	5.1*	10.8*	29*	1376*	138	2.8*	4.8

*P<0.05

Table 4d. Summary of clinical hematology data from the 13-week dose-rage finding study with ribavirin in Wistar rats

Dose (mg/kg)	Hematological Parameters (Week 14) (Male)							Hematological Parameters (Week 14) (Female)						
	RBC <i>10⁶/µL</i>	HGB g/dL	Hct %	Platele <i>10³/µL</i>	Retic <i>10³/µL</i>	Retic %	WBC <i>10³/µL</i>	RBC <i>10⁶/µL</i>	HGB g/dL	Hct %	Platele <i>10³/µL</i>	Retic <i>10³/µL</i>	Retic %	WBC <i>10³/µL</i>
0	9.1	15.7	46	870	146	1.6	4.0	8.5	15.2	45	819	149	1.7	3.0
10	8.8	14.7*	44*	938	162	1.8	4.8	8.6	14.3*	43*	973	162	1.9	3.0
40	8.7	14.3*	43*	899	178	2.1	4.4	8.3	13.6*	41*	999	148	1.8	3.1
80	8.3*	14.2*	42*	1078*	152	1.8	4.0	7.4*	13.0*	39*	989	167	2.3	2.7
160	5.7*	12.4*	35*	1055*	221	4.0	3.1	4.0*	8.7*	23*	1348*	202	5.1*	2.3

*P<0.05

Table 5. Summary of clinical chemistry data from the 13-week dose-rage finding study with ribavirin in Wistar rats

Dose (mg/kg)	Chemistry Parameters (Week 14) (Male)							Chemistry Parameters (Week 14) (Female)						
	ALB g/dL	GLOB g/dL	CHOL mg/dL	TRIG mg/dL	AST IU/L	ALT IU/L	PHOS mg/dL	ALB g/dL	GLOB g/dL	CHOL mg/dL	TRIG mg/dL	AST IU/L	ALT IU/L	PHOS mg/dL
0	5.0	2.1	64	85	93	32	5.8	5.5	1.9	49	45	90	30	5.1
10	5.0	2.0	69	77	88	27	5.7	5.4	1.8	56	33*	88	26	5.2
40	4.9	1.9	62	85	86	24	5.8	5.5	1.8	45	38	80	24	5.8
80	5.0	2.0	55	82	89	28	6.6*	5.4	1.8	45	37	89	24	5.7
160	5.0	1.8*	41*	57	108	29	6.8*	4.9*	2.0	31*	39*	125*	27	6.9*

*P<0.05; ALB: albumin; GLOB: globulin; CHOL: cholesterol; TRIG: triglyceride; PHOS: inorganic phosphate

Table 6a. Mean absolute organ weight and relative organ weight (relative to the body weight) in Wistar rats with 13-week oral administration of ribavirin

Dose (mg/kg)	Mean Absolute Organ Weight (g) (Male)							Mean Relative Organ Weight (%) (Male)					
	Spleen	Lung	Heart	Kidney	Liver	Thymus	Body Wt	Spleen	Lung	Heart	Kidney	Liver	Thymus
0	0.75	1.47	1.07	2.33	10.00	0.35	394.3	0.19	0.37	0.27	0.59	2.53	0.09
10	0.72	1.46	1.11	2.32	9.73	0.37	397.0	0.18	0.37	0.28	0.59	2.46	0.09
40	0.73	1.49	1.14	2.34	9.57	0.32	383.9	0.19	0.39	0.30	0.61	2.50	0.09
80	0.65	1.46	1.05	2.14	8.52*	0.13*	352.7*	0.18	0.41*	0.30*	0.61	2.41	0.04*
160	0.92*	1.67*	1.37*	2.19	8.69	0.08*	336.2*	0.28*	0.50*	0.41*	0.65	2.59	0.02*

*P<0.05

Table 6b. Mean absolute organ weight and relative organ weight (relative to the body weight) in Wistar rats with 13-week oral administration of ribavirin

Dose (mg/kg)	Mean Relative Organ Weight (g) (Male)							Mean Relative Organ Weight (g) (Female)					
	Spleen	Lung	Heart	Kidney	Liver	Thymus	Body Wt	Spleen	Lung	Heart	Kidney	Liver	Thymus
0	0.50	1.07	0.76	1.45	5.92	0.33	219.8	0.23	0.49	0.35	0.66	2.70	0.15
10	0.46	1.06	0.73	1.39	5.53	0.33	209.8	0.22	0.50	0.35	0.66	2.64	0.16
40	0.48	1.03	0.77	1.38	5.28	0.26	207.4	0.23	0.50	0.37*	0.67	2.53	0.13
80	0.49	1.15	0.80	1.42	5.65	0.19*	202.9*	0.24	0.57*	0.39*	0.70	2.78	0.09*
160	0.72*	1.50*	1.13*	1.55	6.08	0.06*	196.0*	0.37*	0.76*	0.58*	0.79*	3.09	0.03*

*P<0.05

Table 7. Pharmacokinetics of ribavirin in rats after 13-week oral doses of ribavirin (Ro 20-9963/000)

Ribavirin	SERUM RIBAVIRIN (PHARMACOKINETIC PARAMETERS)																	
	Day 1			Day 28			Day 91											
Dose (mg/kg)	C _{max} (ng/ml)	AUC (ng•hr/ml)	T _{max} (hr)	C _{max} (ng/ml)	AUC (ng•hr/ml)	T _{max} (hr)	C _{max} (ng/ml)	AUC (ng•hr/ml)	T _{max} (hr)									
	M	F	M	F	M	F	M	F	M	F								
10	133	91	1114	973	1	1	157	133	2116	1821	1	2	122	142	1780	1941	1	0.5
40	434	292	6665	4585	1	4	538	539	9258	8103	8	8	465	610	8281	9622	4	8
80	811	589	11382	8478	4	4	857	859	15290	11877	2	2	961	933	16738	16946	8	8
160	1346	1058	18887	15038	4	4	2070	1973	40400	31415	4	2	2927	2690	46633	46067	2	2

M: male; F: female

3. Study Title: A 26-Week Oral Gavage Toxicity and Toxicokinetic Study with Ribavirin in Rats with a 6-Week Recovery (pharmtox\tox\1003488.pdf)

Sponsor: Hoffmann-La Roche; Study No. 07321; Study Initiation Date: November 11, 2000; Testing Facility: _____
 : GLP: Yes (X); Drug and Drug Lot Numbers: Ro 20-9963/000 Lot No. 990543;
 Formulation: Ro 20-9963/000 in Sterile Water for Injection, USP (sterile water) (Lot Nos. 57-978-FW, and 61-900-FW).

Key Study Findings:

- Mortality was seen in rats at 70 mg/kg/day. There were no Ro 20-9963-related clinical signs for rats given 10 mg/kg/day (AUC_{0-24hr}: 2.3 µg•hr/ml), however, mild hematological toxicity was present in rats at 10 mg/kg/day. The ribavirin-induced hematological effects are generally reversible during the recovery period.
- Mean body weights were statistically significantly lower at 70 mg/kg/day during Weeks 3 through 27 for males and Weeks 13 and 15 through 27 for females when compared to controls. Test article-related decreases in mean food consumption for animals in the 70 mg/kg/day group were seen, which were correlated with the lower mean body weights and body weight gains for animals at this dose level.
- Treatment-related clinical chemistry changes included: decreased cholesterol levels in males at 70 mg/kg/day; increased inorganic phosphorus levels in females at 70 mg/kg/day; and slightly decreased ALT levels in males at 35 mg/kg/day or greater. These changes had reversed following the 6-week recovery period.
- Three rats at 70 mg/kg/day and one rat at 35 mg/kg/day exhibited crusted regions of the skin that correlated with skin ulceration for these rats. At the recovery sacrifice, there were no treatment-related macroscopic observations.
- Treatment-related decreases in thymic weights and thymic lymphoid depletion, hypercellularity of the femur marrow, increased splenic extramedullary hematopoiesis, hepatic pigment deposition; and skin ulceration were seen in rats treated with ribavirin. Thymic lymphoid depletion was seen at all dose levels, with a dose-dependent increase in the incidence and severity of this finding from 10 to 70 mg/kg/day. Hypercellularity of the femur marrow was seen in both sexes at 70 mg/kg/day and in females at 35 mg/kg/day. Increased splenic extramedullary hematopoiesis and hepatic pigment deposition were seen in females at 70 mg/kg/day. Skin ulceration was seen in females at 35 and for both sexes at 70 mg/kg/day. In general these microscopic findings were reversed at the end of the recovery period.
- In general, increases in AUC_{0-24hr} in rats were approximately dose proportional to the increase in the dose level. The C_{max} and AUC_{0-24hr} values on Days 28, 91, 126, and 182 were generally higher than on Day 1, indicating accumulation of Ro 20-9963 with time and after multiple dosing in rats. Ribavirin whole blood concentrations generally increased with the increase in dose level. Data collected on later days (Days 28, 91, 126, and 182) showed an increase in erythrocyte concentrations of ribavirin with increasing dose, which suggested an accumulation of ribavirin in RBCs over an extended period of drug treatment. Following 6-month administration of 10, 35 and 70 mg/kg/day of Ro 20-9963,

respectively, exposures (AUC_{0-24hr}) at Day 182 were 2.3, 7.9 and 19.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$ /ml.

- The NOEL in rats for Ro 20-9963 could not be established in this study. The NOAEL for Ro 20-9963 was considered to be less than 10 mg/kg/day in this study.

Methods

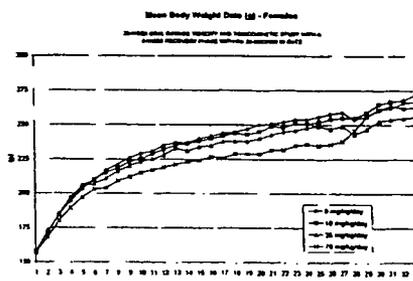
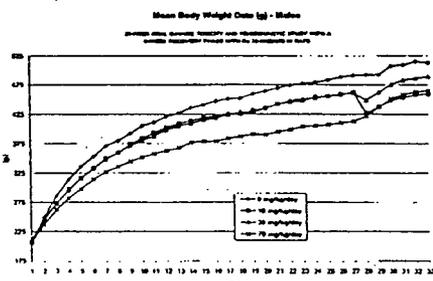
Species:	CrI:WI(G1x/BRL/Han) IGS BR rats: _____ _____, main study: 20/sex/group; toxicokinetic study: 15/sex/ group)
Age, weight:	48-54 days old; 168-241 g for males, 127 to 186 g for females
Dosage:	0 (vehicle control), 10, 35 or 70 mg/kg/day (5 mL/kg/day) for 26 weeks
Route:	oral, by gavage
Drug:	Ro 20-9963/000 (Lot No. 990543) in Sterile Water for Injection, USP
Clinical signs:	twice daily
Body weight:	weekly
Food consumption:	weekly
Ophthalmoscopy:	main-study - prior to treatment, during Weeks 13 and 26
Hematology:	main study - at Weeks 4, 18, 27, and 33; toxicokinetic study - 24 hours post-dose on Days 1, 28, 91, 126, and 182 and once during Week 33
Clinical chemistry:	during Weeks 4, 27, and 33 (following the 6-week recovery period)
Urinalysis:	during Weeks 27 and 33 (following the 6-week recovery period)
Gross pathology:	at sacrifice; after the 26-week treatment and the 6-week recovery period (selected organs were weighed, Appendix Table 1)
Histopathology:	at sacrifice; after the 26-week treatment and the 6-week recovery period (a comprehensive list of tissues was collected and microscopic examinations were performed on 42 tissues for each animals in the main study). In addition, bone marrow smears from the femurs of animals in the control and high-dose groups were evaluated at the scheduled sacrifice (Appendix Table 1).
Toxicokinetics:	Blood samples were collected from toxicokinetic animals predose and at approximately 0.5 and 1, 2, 4, 8, and 24 hours after oral dosing on Days 1, 28, 91, 126, and 182 (treatment termination). Additionally, blood was collected once during Week 33 for the determination of serum concentrations of ribavirin. Ribavirin concentrations in whole blood were evaluated 24 hours post-dose on Days 1, 28, 91, 126, and 182 and during Week 33. Intra-erythrocyte ribavirin levels were calculated, in part, from serum and whole blood ribavirin concentrations.

Results

Mortality: Three main study rats and two toxicokinetic rats died or were sacrificed due to poor health. The main study rats included one male at 10 mg/kg/day sacrificed during Week 23, one female at 70 mg/kg/day sacrificed during Week 20, and one female at 35 mg/kg/day that died during Week 5. Causes of these unscheduled deaths were pyelonephritis, skin ulceration, and mammary carcinoma, respectively. Thus, the deaths of rats given 10 and 35 mg/kg/day were not considered to be test article-related.

Clinical signs: Sores/scabs were noted in toxicity animals given 35 and 70 mg/kg/day. In addition, the incidence of audible respiration was increased for females given 70 mg/kg/day. These findings were attributed to effects of the test material. All other clinical observations were considered to be incidental. One toxicokinetic rat in the 35 mg/kg/day group exhibited clinical observations of skin sores and scabs. No test material-related clinical signs of toxicity were seen in rats at 10 mg/kg/day.

Body weights: There were decreases in mean body weights for males and females at 70 mg/kg/day. Mean body weights were statistically significantly lower at 70 mg/kg/day during Weeks 3 through 27 for males and Weeks 13 and 15 through 27 for females when compared to controls. There were no statistically significant differences in mean body weights for animals given 10 or 35 mg/kg/day. Mean body weights at Week 27 for animals given 10, 35, or 70 mg/kg/day were 94.1, 93.9, and 84.1% of controls, respectively, for males and 98.5, 96.1, and 91.9% of controls, respectively, for females.



Food consumption: Test article-related decreases in mean food consumption for animals in the 70 mg/kg/day group were seen, which were correlated with the lower mean body weights and body weight gains for animals at this dose level.

Ophthalmology: Animals selected for the study had no ocular lesions at the prestudy examination. No test material-related ophthalmic observations were noted at the Week 13 or 26 examination.

Hematology: Treatment-related hematology findings included decreased red blood cell counts for males and females at 70 mg/kg/day and decreased hemoglobin and hematocrit for males and females at all dose levels. Rats at 70 mg/kg/day also exhibited slightly increased reticulocyte counts at one or more time points during the treatment period. Increased platelet counts were seen in animals at all dose levels. Dose-related decreases in hemoglobin and hematocrit and increases in platelet counts were seen; the changes noted at the low dose of 10 mg/kg/day were relatively mild, while those seen at the high-dose of 70 mg/kg/day were generally of moderate severity. These changes were reversible at the end of the recovery phase. No treatment-related effects on myeloid-to-erythroid ratios or other bone marrow cytology parameters were seen in rats at 70 mg/kg/day at Week 27.

Clinical chemistry: Treatment-related clinical chemistry changes included: decreased cholesterol levels in males at 70 mg/kg/day; increased inorganic phosphorus levels in females at 70 mg/kg/day; and slightly decreased ALT levels in males at 35 mg/kg/day or greater. These changes had reversed following the 6-week recovery period.

Urinalysis: No treatment-related changes were seen in rats at any dose in this study.

Gross pathology: Small thymus was seen in two females at 70 mg/kg/day at terminal sacrifice. Three rats at 70 mg/kg/day and one rat at 35 mg/kg/day exhibited crusted regions of the skin that correlated with skin ulceration for these rats. At the recovery sacrifice, there were no treatment-related macroscopic observations.

Organ weights: Treatment-related decreases in thymic weights were seen in male and female rats at 70 mg/kg/day. At the recovery sacrifice, no treatment-related organ weight changes were seen in rats at any dose level.

Histopathology: At the terminal necropsy, treatment-related microscopic changes were seen in rats given ribavirin, which included thymic lymphoid depletion, hypercellularity of the femur marrow, increased splenic extramedullary hematopoiesis, hepatic pigment deposition; and skin ulceration. Thymic lymphoid depletion was seen at all dose levels, with a dose-dependent increase in the incidence and severity of this finding from 10 to 70 mg/kg/day. Hypercellularity of the femur marrow was seen in both sexes at 70 mg/kg/day and in females at 35 mg/kg/day. Increased splenic extramedullary hematopoiesis and hepatic pigment deposition were seen in females at 70 mg/kg/day. Skin ulceration was

seen in females at 35 and for both sexes at 70 mg/kg/day. At the recovery necropsy, there were no Ro 20-9963-related microscopic findings.

Toxicokinetics: In general, exposure to ribavirin increased as the dose increased from 10 to 70 mg/kg/day. The increases in C_{max} were not consistently dose-proportional to the increase in the dose level. The increases in AUC_{0-24hr} in rats were approximately dose proportional to the increase in the dose level. The C_{max} and AUC_{0-24hr} values on Days 28, 91, 126, and 182 were generally higher than on Day 1, indicating accumulation of Ro 20-9963 with time and after multiple dosing in rats. Ribavirin whole blood concentrations generally increased with the increase in dose level. The calculated erythrocyte levels varied across the different dose groups, but the data suggested a low distribution of ribavirin associated with RBCs within the tested dose range. Data collected on later days (Days 28, 91, 126, and 182) showed an increase in erythrocyte concentrations of ribavirin with increasing dose, which suggested an accumulation of ribavirin in RBCs over an extended period of drug treatment. Following 6-month administration of 10, 35 and 70 mg/kg/day of Ro 20-9963, respectively, exposures (AUC_{0-24hr}) at Day 182 were 2.3, 7.9 and 19.1 $\mu g \cdot hr/ml$, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu g \cdot hr/ml$.

Comments

- Oral gavage administration of Ro 20-9963 (ribavirin) once daily to male and female rats for at least 26 weeks was associated with toxicological effects on hematological and lymphoid tissues, as well as other rapidly proliferating cells/tissues. There were no Ro 20-9963-related clinical signs for rats given 10 mg/kg/day (AUC_{0-24hr} : 2.3 $\mu g \cdot hr/ml$), however, mild hematological effects and thymic lymphoid depletion were present in rats at 10 mg/kg/day. In the present study, the data suggested that the ribavirin-induced hematological effects are generally reversible during the recovery period. Thus, the NOEL in rats for Ro 20-9963 could not be established in this study. The NOAEL for Ro 20-9963 was considered to be less than 10 mg/kg/day in this study.

Tabulated Summary of Toxicity and Toxicokinetic Findings - Study No. 07321 In Rats

Dose (mg/kg/day)	Day of Sampling	Toxicokinetic Data					Treatment-Related Effects
		Ro 20-9963/000					
		Serum		Blood	Erythrocytes		
		C_{max} (ng/mL)	AUC_{0-24hr} (ng·hr/mL)	(ng/mL)	Males	Females	
10	1	100	1162	8	10.2	10.7	↓ Hemoglobin, ↓ hematocrit, ↑ platelet counts and thymic lymphoid depletion
	28	165	2170	18	3.4	8.5	
	91	226	2259	21	5.3	22.7	
	126	206	2260	23	10.9	16.2	
	182	199	2264	21	1.68	9.5	
35	1	324	4054	30	6.7	7.9	↑ Skin sores/scabs, ↓ hemoglobin, ↓ hematocrit, ↑ platelet counts, ↓ alanine aminotransferase activity, crusted regions of skin, skin ulceration (histologic), thymic lymphoid depletion, and hypercellularity of femur marrow
	28	457	6186	60	21.6	11.8	
	91	490	7149	72	33.9	NR	
	126	612	8521	89	47.2	22.4	
	182	537	7925	69	18.0	5.1	
70	1	663	8744	67	9.4	1.7	Mortality, ↑ skin sores/scabs, ↑ audible respiration, ↓ body weight, ↓ body weight gain, ↓ food consumption, ↓ erythrocyte counts, ↓ hemoglobin, ↓ hematocrit, ↑ platelet counts, ↑ absolute and relative reticulocyte counts, ↓ cholesterol, ↑ inorganic phosphorus, ↓ alanine aminotransferase activity, crusted regions of skin, skin ulceration (histologic), small thymus, ↓ thymus weights, thymic lymphoid depletion, hypercellularity of femur marrow, extramedullary hematopoiesis in spleen, and hepatic pigment deposition
	28	887	13299	141	49.4	57.0	
	91	1007	17244	205	74.2	41.9	
	126	1297	21460	265	81.7	103.0	
	182	1138	19155	197	30.0	71.8	

NR= Not reported due to negative erythrocyte concentrations of Ro 20-9963/000.

Dogs:

4. Study Title: A 26-Week Oral Gavage Toxicity and Toxicokinetic Study with Ribavirin in Dogs with a 6-Week Recovery (Report No. 1003489)

Sponsor: Hoffmann-La Roche Study No.: 07322. Page 1 of 1151. Study Initiation Date: 9 September, 1998; Testing Facility: _____
 GLP: Yes (X); Drug and Drug Lot Numbers: Ro 20-9963/000 Lot No.: 990543; Purity: _____ Formulation: Ro 20-9963/000 in sterile water for injection, USP

Key Study Findings:

- Decreases in RBCs, hemoglobin, hematocrit, and increases in reticulocyte counts were seen in dogs at 20 mg/kg/day. In general, erythrocyte parameters at recovery (Week 33) were comparable to those of control dogs, indicating the reversibility of changes in RBC parameters. Decreased lymphocyte

counts were seen in dogs at 5 mg/kg/day or greater at treatment Weeks 2, 6, 10, 14, 18, 27 and 33. These changes were only partially reversed at recovery Week 33.

- Clinical signs of toxicity, decreases in body weights and food consumption, and changes in clinical and histopathology parameters were observed in dogs at 20 mg/kg/day. An increase in the quantity of pigment in the red pulp and in Kupffer cells were seen in dogs at 20 mg/kg/day. Increased intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum were seen in dogs at 20 mg/kg/day, with increased inflammation in all sections of the small intestine.
- In general, ribavirin levels in the whole blood and RBC increased with the increase in the ribavirin dose level. The increase in C_{max} was proportional to the increase in dose level for males, but was slightly higher than dose proportional for females. The increases in AUC_{0-24hr} were generally proportional to the increase in the dose level. Exposures (AUC_{0-24hr}) of 10.4, 23.1 and 40.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$ were achieved at treatment termination following administration of dose levels of 5, 10 and 20 mg/kg/day, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$.
- The no-effect level (NOAEL) for ribavirin in dogs after 26 weeks of daily administration is considered to be 10 mg/kg/day.

Methods

Species:	Beagle dogs (7 dogs/sex/group for Groups 1 and 4; 2/sex/group for Groups 1 and 4 were designated as recovery animals after an additional 6-week recovery period)
Age, weight:	8 months old; 10.2-12.9 g for males; 7.9 to 11.2 g for females
Drug:	Ro 20-9963/000 (ribavirin)
Dosage:	0 (Sterile Water), 5, 10, or 20 mg/kg/day (dose volume: 1 mL/kg) for 28 consecutive days
Route:	oral
Mortality:	twice daily
Clinical signs:	weekly
EKG:	prior to treatment and during Weeks 13, 26, and 32
Ophthalmoscopy:	prior to treatment, during Weeks 13, 26 and 32
Body weight:	On Day 1 prior to treatment, weekly thereafter
Food consumption:	weekly
Hematology:	prior to treatment, and during Weeks 10, and 14
Clinical chemistry:	prior to treatment, and during Weeks 2, 6, 18, 27, and 33
Urinalysis:	prior to treatment, and during Weeks 2, 6, 18, 27, and 33
Gross pathology:	at sacrifice; Days 30 and 44 (selected organs, Appendix Table 1)
Histopathology:	at sacrifice; Days 30 and 44 (microscopic examinations were performed on 42 tissues for each animals in the control and high dose groups; the liver was also examined microscopically from each animal in the low- and mid-dose groups; Appendix Table 1)
Toxicokinetics:	blood samples collection for serum and erythrocytes (from the jugular vein) – collected on Days 1, 28, 91, and 182: predose, 1, 2, 6, 12, and 24 hours postdose;

Results

<u>Mortality:</u>	There were no unscheduled deaths in this study.
<u>Clinical signs:</u>	An increased occurrence of liquid and mucoid feces was seen in dogs at 20 mg/kg/day.

- Body weights:** Decreases in individual body weights were seen in male dogs (\downarrow 1%-13%; \downarrow 0.6 Kg/dog) and in female dogs (\downarrow 2%-28%; \downarrow 1.2 Kg/dog) at 20 mg/kg/day during Weeks 1 to Week 4. During Week 5 and continuing until study termination (including recovery period), all 20 mg/kg/day dogs were given moist dog food in addition to the standard diet. Thus, the body-weight comparison between the control and the 20 mg/kg/day groups after Week 4 is considered inappropriate.
- Food consumption:** Decreases in mean food consumption were seen in dogs at 20 mg/kg/day during treatment Weeks 1 to 4, which was related to body weight loss. All 20 mg/kg/day dogs were given moist dog food in addition to the standard diet, which helped stabilize their mean body weight. Due to the change in diet, food consumption data from the 20 mg/kg/day group was not analyzed from Week 5 on.
- EKG:** There were no treatment-related EKG findings.
- Ophthalmoscopy:** There were no treatment-related ophthalmic changes.
- Hematology:** The mean values for RBCs, hemoglobin, hematocrit were slightly decreased in dogs at 20 mg/kg/day. Erythrocyte parameters at recovery (Week 33) were comparable to those of control dogs. Reticulocyte counts were slightly and transiently elevated for females at Week 10 and for males at Week 6 and Week 20 in the 20 mg/kg/day group. However, both the absolute and relative reticulocyte counts for males and females in the 20 mg/kg/day dose group were slightly decreased at recovery (Week 33) compared to terminal sacrifice (Week 27). The mean values of lymphocyte counts in the 20 mg/kg/day dogs were generally lower than the control counts by approximately 1000 cell/ μ L throughout the treatment and recovery intervals. The decreases are attributed to ribavirin. Slightly decreased leukocyte counts were seen in dogs spuriously in the treated groups throughout the study, significantly for 20 mg/kg/day female at Week 2 and Week 18. There were no treatment-associated findings in the coagulation data and bone marrow evaluations. There were no treatment-related effects on the myeloid:erythroid ratio or other bone marrow cytology parameters at the treatment or recovery sacrifices.
- Clinical chemistry:** Slight and transient decreases in total protein levels and serum alanine aminotransferase (ALT) activity were seen in females at 20 mg/kg/day during the treatment phase. These decreases were not seen in dogs during the recovery period.
- Urinalysis:** Sporadic occurrences of blood in the urine were seen in dogs in all dose groups, including controls. Because no histopathological changes were seen in the kidneys or urinary tract of the treated or control animals, and blood in the urine was seen occasionally in the control group animals, this finding was not considered to be treatment-related.
- Gross pathology:** There were no treatment-related macroscopic findings noted at necropsy.
- Organ weights:** The relative spleen weights (spleen-to-body weight ratio) were statistically significantly increased in male dogs at 20 mg/kg/day at terminal necropsy. The increases in spleen weights may be related to the decreases in the terminal body weight noted for males at this dose level. There were no significant changes in organ weights between control and treated dogs at the end of the recovery period.
- Histopathology:** Treatment-related histopathological changes were seen in the spleen, liver and small intestine of dogs given 20 mg/kg/day and in the small intestine of one dog at 10 mg/kg/day at the terminal necropsy.

Spleen: An increase in the quantity of pigment in the red pulp was seen, which was of a moderate severity in three of 10 dogs.

Liver: Minimal or slight pigment was noted in Kupffer cells for two of 10 dogs.

Intestine: Increased intestinal crypt dilatation/necrosis in the duodenum was seen in two males and two females at 20 mg/kg/day. Erosion in the ileum was seen in one of these two males at 20 mg/kg/day, with increased inflammation in all sections of the small intestine. Increased inflammation in the small intestine was seen in the two females in this group that exhibited increased intestinal crypt dilatation/necrosis. Additionally, increased dilatation/necrosis of intestinal crypts in the duodenum was seen in one male dog at 10 mg/kg/day. At the end of the recovery period, increased dilatation/necrosis of intestinal crypts was present in the duodenum of one female dog in the 10 mg/kg/day group. However, intestinal sections from recovery animals in the high-dose (20 mg/kg/day) group were comparable to controls.

Toxicokinetics: In general, ribavirin levels in the whole blood and RBC increased with the increase in the ribavirin dose level. The increase in C_{max} was proportional to the increase in dose level for males, but was slightly higher than dose proportional for females. The increases in AUC_{0-24hr} were generally proportional to the increase in the dose level. No accumulation of the Ro 20-9963 in serum was seen in dogs after multiple dosing. Slight accumulation of ribavirin was seen in the RBCs of dogs. No gender differences were seen in dogs in the mean AUC_{0-24hr} values. Exposures (AUC_{0-24hr}) of 10.4, 23.1 and 40.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$ were achieved at treatment termination following administration of dose levels of 5, 10 and 20 mg/kg/day, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$.

Summary of Toxicity and Toxicokinetic Findings – Study No. 07322 in Dogs

Dose (mg/kg/day)	Day of Sampling	Toxicokinetic Data					Treatment-Related Effects
		Ro 20-9963/000		Blood (ng/mL)	Erythrocytes (ng/mL)		
		Serum			Males	Females	
		C_{max} (ng/mL)	AUC_{0-24hr} (ng·hr/mL)				
5	1	1495	8315	37.9	NR	NR	↓ Mean lymphocyte counts
	28	1180	9200	102.7	96.5	95.1	
	91	1555	9795	73.1	1.8	58.3	
	182	1605	10395	97.7	53.4	84.5	
10	1	3170	17600	86.1	22.2	15.3	↓ Mean lymphocyte counts, ↑ dilatation/necrosis of intestinal crypts (duodenum)
	28	2855	18550	142.5	61.0	81.9	
	91	3405	19450	202.5	89.8	345	
	182	3700	23100	174.5	74.8	78.5	
20	1	7175	35700	236.5	18.2	205	Fecal alterations, ↓ mean body weight (up to Week 4), ↓ body weight gain (up to Week 4), ↓ food consumption (up to Week 4), ↓ mean lymphocyte counts, ↓ WBCs, ↓ erythrocyte counts, ↓ hemoglobin, ↓ hematocrit, ↑ reticulocyte counts, ↓ alanine aminotransferase activity, ↓ total protein, ↑ pigment in the red pulp of the spleen, pigment deposition in Kupffer cells of the liver, ↑ dilatation/necrosis of intestinal crypts (duodenum), erosion of ileum, chronic inflammation of small intestine
	28	6888	42600	386	250	286	
	91	7175	40450	273.5	NR	NR	
	182	6145	40050	358	121	152	

NR= Not reported due to negative erythrocyte concentrations of Ro 20-9963/000.

Comments

- The no-effect level (NOAEL) for ribavirin in dogs after 26 weeks of daily administration was considered to be 10 mg/kg/day. At 20 mg/kg/day, ribavirin produced clinical signs of toxicity and changes in body weight, food consumption, and clinical and histopathology parameters. Note that thymic and tonsil lymphoid depletion was observed in dogs at 30 and 60 mg/kg/day following 4 weeks of treatment. Thus, it seems most likely that the 20 mg/kg/day tested in the present study were not high enough to elicit this adverse effect on the lymphoid system.

Summary of individual study findings:**Study Title: 4-Week Oral Gavage Dose Range Finding and Toxicokinetic Study with Ro 20-9963/000 (ribavirin) in C57BL/6 Mice**

A 4-week oral gavage range-finding toxicity and toxicokinetic study was conducted in male and female mice to characterize the toxicity of ribavirin (Ro 20-9963) after 4 weeks of administration. The mice received daily doses of 0, 30, 100, 200 or 400 mg/kg/day. All main study animals and approximately one-half of the toxicokinetic animals at 400 mg/kg/day died or were sacrificed moribund by Study Day 12. At 200 mg/kg/day, three of 10 females (main study animals) and 5 of 24 males (toxicokinetic animals) died or were sacrificed moribund. Hypoactivity, labored respiration, hunched posture, thin appearance, cold to touch, reduced stools, and weight losses were seen in the mice that died or were sacrificed moribund prior to death. All animals that died or were sacrificed moribund showed ↓RBC, ↓Hct, and ↓Hb values; ↓leukocyte and ↓lymphocyte counts; and ↑platelet counts. Dose-related decreases in ↓RBC counts were noted in males given 30 mg/kg/day and in both sexes given 100 and 200 mg/kg/day of ribavirin. Dose-related decreases in ↓Hct and ↓Hb were also noted in mice of both sexes administered 100 or 200 mg/kg/day of ribavirin. Platelet counts were increased in 100 mg/kg/day females and 200 mg/kg/day mice of both sexes. Total leukocyte and lymphocyte counts were decreased in males given 200 mg/kg/day of ribavirin. ALT values were decreased in male mice treated with ≥30 mg/kg/day and in female mice administered 100 or 200 mg/kg/day of ribavirin. A treatment-related increase in absolute and relative (to body and brain weights) spleen weights was noted for mice at 200 mg/kg/day. A statistically significant decrease in relative (to body weight) testes/epididymis weights was observed for surviving males at 200 mg/kg/day. Treatment-related histopathologic changes were seen in mice that died at ≥200 mg/kg/day, which included: crypt cell necrosis and regenerative hyperplasia in the small and large intestine; lymphoid depletion/necrosis in the thymus, spleen, and mesenteric lymph node; adrenal cortical hypertrophy, atrophy of salivary glands; and congestion and hypocellularity in the bone marrow and hypospermia in the epididymis. An increase in the incidence and severity of splenic extramedullary hematopoiesis were also seen in mice at ≥30 mg/kg/day. The severity of these findings was dose-related between 30 and 200 mg/kg/day. The NOEL for this study could not be determined, which was considered to be <30 mg/kg/day. The increase in C_{max} was not consistently proportional to the increase in dose level while the increases in AUC_{0-24} were approximately proportional to the increases in dose level. Serum T_{max} values for various dosages of ribavirin were observed between 0.5 and 8 hours postdosing. AUC_{0-24hr} values obtained at the end of 4 weeks following administration of 30, 100, 200 and 400 mg/kg/day were 11.6, 35.5, 71.9 and 142.5 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$.

Study Title: 13-Week Oral Gavage Toxicity and Toxicokinetic Study with Ro 20-9963/000 in Rats

A 13-week oral gavage toxicity and toxicokinetic study was conducted in male and female Crl:WI (GlxBRL/Han) IGS BR rats to characterize the toxicity of ribavirin (Ro 20-9963) after 13 weeks of administration. The rats received daily doses of 0, 10, 40, 80 or 1600 mg/kg/day. Mortality was seen in rats at 160 mg/kg/day. (3M, 5F). Clinical signs included hunched posture, thin appearance, and hypoactivity at the high dose. Food consumption, mean body weights and body weight gains were reduced for animals at ≥80 mg/kg/day. Effects on the hematological parameters (RBC, Hb, Hct) were seen in rats at 160 mg/kg/day. Platelet counts were increased and lymphocyte counts were decreased generally at 160 mg/kg/day. Bone marrow smears revealed elevated myeloid-to-erythroid ratios and erythroid hypoplasia at 160 mg/kg/day. Clinical chemistry changes were observed mainly at 160 mg/kg/day and included lower total protein, albumin, globulin, cholesterol, and triglycerides and higher AST and phosphorus. Organ weight changes included increased heart, spleen and lung weights at the

high dose and decreased thymic weights at ≥ 40 mg/kg/day. Decreased thymic weights were seen in rats at 40 mg/kg/day or greater, which were generally correlated with microscopic observations of thymic lymphoid depletion. Increased lung weights were noted in rats at 80 mg/kg/day or greater, which were correlated with an increase in the incidence and/or severity of alveolar and interstitial macrophage, or foamy alveolar macrophage infiltration in the lungs. Hepatocellular necrosis was seen in rats at 80 mg/kg/day. Liver centrilobular necrosis and vacuolar degeneration was seen in rats at 160 mg/kg/day. Increases in splenic weights were seen in rats at 160 mg/kg/day, which were associated with splenic extramedullary hematopoiesis, lymphocytic depletion, and hemorrhage. Additionally, depletion of thymic-dependent areas of lymph nodes and decreased numbers of erythroid and myeloid precursors in the bone marrow were noted in rats at 160 mg/kg/day. Skin lesions (ulceration, dermis and epithelial sclerosis) were also noted microscopically in one female rat at 40 mg/kg/day, and in both sexes at 80 mg/kg/day or greater. The NOAEL for ribavirin administered by oral gavage to Wistar rats once daily for 13 weeks was 10 mg/kg/day. Treatment-related effects in males and females at doses ≥ 80 mg/kg indicated that these doses exceeded the maximum tolerated dose (MTD).

Study Title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study with a 6-Week Recovery Phase with Ro 20-9963/000 in Rats

A 26-week oral gavage toxicity and toxicokinetic study was conducted in male and female Crl:WI(G1x/BRL/Han) IGS BR rats with a 6-week recovery to characterize the toxicity of ribavirin (Ro 20-9963) after 26 weeks of administration. The rats received daily doses of 0, 10, 35, or 70 mg/kg/day. Mortality was seen in rats at 70 mg/kg/day. There were no Ro 20-9963-related clinical signs for rats given 10 mg/kg/day (AUC_{0-24hr} : 2.3 $\mu\text{g}\cdot\text{hr}/\text{ml}$), however, mild hematological toxicity was present in rats at 10 mg/kg/day. The ribavirin-induced hematological effects are generally reversible during the recovery period. Mean body weights were statistically significantly lower at 70 mg/kg/day during Weeks 3 through 27 for males and Weeks 13 and 15 through 27 for females when compared to controls. Test article-related decreases in mean food consumption for animals in the 70 mg/kg/day group were seen, which were correlated with the lower mean body weights and body weight gains for animals at this dose level. Treatment-related clinical chemistry changes included: decreased cholesterol levels in males at 70 mg/kg/day; increased inorganic phosphorus levels in females at 70 mg/kg/day; and slightly decreased ALT levels in males at 35 mg/kg/day or greater. These changes had reversed following the 6-week recovery period. Three rats at 70 mg/kg/day and one rat at 35 mg/kg/day exhibited crusted regions of the skin that correlated with skin ulceration for these rats. At the recovery sacrifice, there were no treatment-related macroscopic observations. Treatment-related decreases in thymic weights and thymic lymphoid depletion, hypercellularity of the femur marrow, increased splenic extramedullary hematopoiesis, hepatic pigment deposition and skin ulceration were seen in rats treated with ribavirin. Thymic lymphoid depletion was seen at all dose levels, with a dose-dependent increase in the incidence and severity of this finding from 10 to 70 mg/kg/day. Hypercellularity of the femur marrow was seen in both sexes at 70 mg/kg/day and in females at 35 mg/kg/day. Increased splenic extramedullary hematopoiesis and hepatic pigment deposition were seen in females at 70 mg/kg/day. Skin ulceration was seen in females at 35 and for both sexes at 70 mg/kg/day. In general these microscopic findings were reversed at the end of the recovery period. In general, increases in AUC_{0-24hr} in rats were approximately dose proportional to the increase in the dose level. The C_{max} and AUC_{0-24hr} values on Days 28, 91, 126, and 182 were generally higher than on Day 1, indicating accumulation of Ro 20-9963 with time and after multiple dosing in rats. Ribavirin whole blood concentrations generally increased with the increase in dose level. Data collected on later days (Days 28, 91, 126, and 182) showed an increase in erythrocyte concentrations of ribavirin with increasing dose, which suggested an accumulation of ribavirin in RBCs over an extended period of drug treatment. Following 6-month administration of 10, 35 and 70 mg/kg/day of Ro 20-9963, respectively, exposures (AUC_{0-24hr}) at Day 182 were 2.3, 7.9 and 19.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$. The NOEL in rats for Ro 20-9963 could not be established in this study. The NOAEL for Ro 20-9963 was considered to be less than 10 mg/kg/day in this study.

Study Title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study with a 6-Week Recovery Phase with Ro 20-9963/000 in Dogs

A 26-week oral gavage toxicity and toxicokinetic study was conducted in male and female dogs with a 6-week recovery to characterize the toxicity of ribavirin (Ro 20-9963) after 26 weeks of administration. The dogs received daily doses of 0, 5, 10, or 20 mg/kg/day. Decreases in RBCs, hemoglobin, hematocrit, WBC and lymphocyte counts, and increases in reticulocyte counts were seen in dogs at 20 mg/kg/day. Mean lymphocyte counts remained decreased for animals in all treated groups at recovery (Week 33) as did WBC counts for females at 20 mg/kg/day. In general, erythrocyte parameters at recovery (Week 33) were comparable to those of control dogs, indicating the reversibility of changes in RBC parameters. Decreased lymphocyte counts were seen in dogs at 5 mg/kg/day or greater at treatment Weeks 2, 6, 10, 14, 18, 27 and 33. These changes were only partially reversed at recovery Week 33. Clinical signs of toxicity, changes in body weights and food consumption, and clinical and histopathology parameters were observed in dogs at 20 mg/kg/day. An increase in the quantity of pigment in the red pulp and in Kupffer cells were seen in dogs at 20 mg/kg/day. Increased intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum were seen in dogs at 20 mg/kg/day, with increased inflammation in all sections of the small intestine. In general, ribavirin levels in the whole blood and RBC increased with the increase in the ribavirin dose level. The increase in C_{max} was proportional to the increase in dose level for males, but was slightly higher than dose proportional for females. The increases in AUC_{0-24hr} were generally proportional to the increase in the dose level. Exposures (AUC_{0-24hr}) of 10.4, 23.1 and 40.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$ were achieved at treatment termination following administration of dose levels of 5, 10 and 20 mg/kg/day, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$. The no-effect level (NOAEL) for ribavirin in dogs after 26 weeks of daily administration is considered to be 10 mg/kg/day.

Toxicology summary:

Preclinical toxicology data indicate that the hematological system, intestines and skin are the target sites of ribavirin. Ribavirin induces a significant degree of anemia, reticulocytosis, and lymphoid atrophy in rats and dogs following a 6-month, repeat-dose administration. The anemia is generally reversed within 6-weeks following the cessation of ribavirin. Increased intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum were seen in dogs at 20 mg/kg/day, with increased inflammation in all sections of the small intestine. Skin ulceration was seen in rats at ≥ 35 mg/kg/day. In addition, ribavirin causes clinical chemistry changes in rats at 70 mg/kg/day, which included decreased cholesterol levels in males, increased inorganic phosphorus levels in females, and slightly decreased ALT levels in males. These changes had reversed following the 6-week recovery period. Following 6-month administration of 10, 35 and 70 mg/kg/day of ribavirin, respectively, exposures (AUC_{0-24hr}) at Day 182 in rats were 2.3, 7.9 and 19.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$. The NOAEL in rats for ribavirin could not be established in the 6-month study. The NOAEL for ribavirin was considered to be less than 10 mg/kg/day in rats following a 6-month repeat-dose administration. Exposures (AUC_{0-24hr}) of 10.4, 23.1 and 40.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$ were achieved at treatment termination following administration of dose levels of 5, 10 and 20 mg/kg/day, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$ in the human. The no-effect level (NOAEL) for ribavirin in dogs after 26 weeks of daily administration is considered to be 10 mg/kg/day.

Toxicology conclusions: Preclinical toxicology data indicate that the hematological system and intestines, and skin are the target sites of ribavirin. Ribavirin induces a significant degree of anemia, reticulocytosis, and lymphoid atrophy in rats and dogs following a 6-month, repeat-dose administration. The NOAEL for ribavirin was considered to be less than 10 mg/kg/day (AUC_{0-24hr} : $< 2.3 \mu\text{g}\cdot\text{hr}/\text{ml}$) in rats following a 6-month repeat-dose administration. The no-effect level (NOAEL) for ribavirin in dogs after 26 weeks of daily administration is considered to be 10 mg/kg/day (AUC_{0-24hr} : $10.4 \mu\text{g}\cdot\text{hr}/\text{ml}$), as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$ in the human.

Appendix Table 1. Histopathology Inventory for General Toxicity Studies in rats, and dogs with oral administration of ribavirin (NDA #21-551)

Tissue and Organs	26-Month Rat (Han Wistar) Roche Study No. 07321	26-Month Dog (Beagle Dogs) Roche Study No. 07322	13-Weeks Rat (Han Wistar) Roche Study No. 07320	4-Weeks Mice (C57BL/6) Roche Study No. 07296
Adrenal (2)*	X	X	X	X
Brain*	X	X	X	X
Cecum	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis (2)*	X	X	X	X
Esophagus	X	X	X	X
Eyes (2)	X	X	X	X
Femur with bone marrow	X	X	X	X
Gallbladder	-	X	-	-
Harderian gland	X	X	X	X
Heart*	X	X	X	X
Ileum	X	X	X	X
Injection site	X	X	X	X
Jejunum	X	X	X	X
Kidney*	X	X	X	X
Lesions	X	X	X	X
Liver*	X	X	X	X
Lung*	X	X	X	X
Lymph node	X	X	X	X
Mammary gland (F)	X	X	X	X
Ovary (2)*	X	X	X	X
Pancreas	X	X	X	X
Pituitary gland* Prostate*	X	X	X	X
Rectum	X	X	X	X
Salivary gland (2)*	X	X	X	X
Sciatic nerve	X	X	X	X
Seminal vesicle (2)*	X	X	X	X
Skeletal muscle	X	X	X	X
Spleen*	X	X	X	X
Spinal cord (cervical, thoracic, and lumbar)	X	X	X	X
Sternum with bone marrow	X	X	X	X
Stomach	X	X	X	X
Testes (2)*	X	X	X	X
Thymus*	X	X	X	X
Thyroid (2) with parathyroid*	X	X	X	X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus with cervix (F)*	X	X	X	X
Vagina	X	X	X	X

*At each scheduled sacrifice, these organs were weighed; paired organ were weighed together. ** Tissues from each animal and the animal that died at an unscheduled interval were examined microscopically.

V. GENETIC TOXICOLOGY:

To support an NDA/BLA filing for PEG-IFN alfa-2a/ribavirin, the sponsor submitted results from the following genotoxicity studies characterizing the genetic toxicities of ribavirin to the division for review.

List of Genotoxicity Studies

1. E. Coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with Ribavirin (Ro 20-9963/000) (Study No. 07403)
2. L5178Y TK +/-Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with Ribavirin (Study No. 07404)
3. In Vivo Mouse Micronucleus Assay with Ribavirin (Ro 20-9963/000) (Study No. 07405)

Genotoxicity Study review:

1. Study Title: *Salmonella - E. coli/mammalian-microsome reverse mutation assay with a confirmatory assay with ribavirin (Ro 20-9963/000) (Study No. 07403, April 5, 2001; report 1003059)*

Sponsor: Hoffmann-La Roche; Roche Study No.: 07403; Study Initiation Date: 26 June 2000; Testing Facility: _____
Study No.: 21537-0-409OECD; GLP: Yes (X); Drug and Drug Lot Numbers:
Ro 20-9963/000 Lot No.: 990543; Purity: 100.2%; Formulation: Ro 20-9963/000 (100 mg/ml) in sterile water for injection, USP

Methods

Four tester strains of *S. typhimurium* (TA1535, TA1537, TA98 and TA100) and *E. coli* WP2uvrA were used in a standard plate incorporation Ames test. The tester strains were exposed to ribavirin, vehicle (Sterile Water) or positive control articles for approximately 48 hours in the absence and presence of an exogenous metabolic activation system (S9 mix) derived from the livers of Aroclor 1254 treated rats. The concentrations tested with the *S. typhimurium* tester strains in the definitive assay were 10.0, 33.3, 100, 333, 1000, and 5000 µg/plate in the presence of S9 and 3.33, 10.0, 33.3, 100, 333, 1000, and 5000 µg/plate in the absence of S9. The concentrations tested with the WP2uvrA tester strain in the definitive assay were 10.0, 33.3, 100, 333, 1000, and 5000 µg/plate in the presence and absence of S9.

Results

Cytotoxicity pre-screening in strains WP2uvrA and TA100 with and without S9 mix showed cytotoxicity with tester strain TA100 at 1000 µg/plate and greater in both the presence and absence of S9. No cytotoxicity was observed with tester strain WP2uvrA at the concentration up to 5000 µg/plate in the presence and absence of S9.

Ribavirin did not increase the number of revertant colonies in any strain, while data obtained from the positive controls verified the sensitivity of the assay and the activity of the metabolic activation system.

Comments

Ribavirin (Ro 20-9963/000) was evaluated as negative for inducing reverse mutations in the *Salmonella-Escherichia coli* reverse mutation assay using non-activation and activation conditions. Thus, under the described experimental conditions, ribavirin was not mutagenic in the Ames test.

2. Mammalian-Cell (L5178Y TK +/-) Gene Mutation Assay (Report No 1003060)

Sponsor: Hoffmann-La Roche; Roche Study No.: 07404; Study Initiation Date: 28 June 2000; Testing Facility: _____
Study No.: 21537-0-431 ICH; GLP: Yes (X); Drug and Drug Lot Numbers: Ro 20-9963/000 Lot No.: 990543; Purity: 100.2%; Formulation: Ro 20-9963/000 (100 mg/ml) in sterile water for injection, USP

Methods

The mutation activity of ribavirin (Ro 20-9963) was examined at the thymidine kinase (TK) locus of L5178Y mouse lymphoma cells with and without metabolic activation. Ribavirin was tested up to cytotoxic concentrations, both with and without S9 metabolic activation utilizing treatment periods for 4 hours (with and without S9). In the initial non-activation assay, concentrations of ribavirin ranging from 125 to 2500 µg/ml were employed. In the confirmatory non-activation assay, concentrations of ribavirin ranging from 7.85 to 2500 µg/ml was employed. In the initial activation assay, concentrations of ribavirin ranging from 62.5 to 2500 µg/ml were employed. In the confirmatory activation assay, concentrations of ribavirin ranging from 1000 to 2500 µg/ml and a 4-hour treatment period was employed.

Results

In the initial non-activation assay with a 4-hour treatment period, moderate to moderately high cytotoxicity and a treatment-induced increase in mutant frequencies was seen at all concentrations tested (125 to 2500 µg/ml). In a confirmatory non-activation assay at concentrations from 7.85 to 2500 µg/ml, concentrations from 125 µg/ml and higher induced a 3 to 4-fold increase in mutant frequencies.

In the confirmatory activation assay, eight concentrations of ribavirin (1000, 1500, 2000, 2100, 2200, 2300, 2400, and 2500 µg/ml) were assessed in an attempt to obtain highly cytotoxic treatments. Seven of these 8 concentrations induced a 2.2 to 2.7-fold increase in mutant frequencies indicative of a weak positive response (exceeds minimum criterion of 187×10^{-6}). However, under activation conditions, the positive response was clearly weaker than under non-activation conditions.

Data obtained from the positive control (methylcholanthrene: 2 and 4 µg/ml) showed positive results, which verified the sensitivity of the assay.

Comments

Ribavirin (Ro 20-9963/000) was evaluated as positive for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells using non-activation and activation conditions, with a clearly less positive response in the presence of S9 metabolic activation.

3. *In vivo* mouse micronucleus assay with ribavirin (Report No 1003058)

Sponsor: Hoffmann-La Roche; Roche Study No.: 07405; Study Initiation Date: 7 June 2000; Testing Facility: _____
Study No.: 21537-0-455OECD; GLP: Yes (X); Drug and Drug Lot Numbers: Ro 20-9963/000 Lot No.: 990543; Purity: 100.2%; Formulation: Ro 20-9963/000 (100 mg/ml) in sterile water for injection, USP

Methods

The bone marrow cells from CD-1 mice that had received 3 consecutive oral (gavage) doses of ribavirin (Ro 20-9963) at 500, 1000, or 2000 mg/kg were examined for induction of chromosome breakage or spindle disturbance via analysis for the presence of micronuclei. Ribavirin was tested for potential induction of chromosome breakage or spindle disturbance in CrI:CD-1(ICR) BR mouse bone marrow *in vivo* using the micronucleus test. Ribavirin was administered orally by gavage to 6 male mice/group, on 3 consecutive days, at dose levels of 0 (vehicle), 500, 1000, or 2000 mg/kg using a dose volume of 20 ml/kg. Bone marrow smears were evaluated from 5 mice/group which were sacrificed 24 hours after receiving the last dose for extraction of the bone marrow. The sensitivity of the assay was verified using cyclophosphamide (80 mg/kg administered as a single oral gavage dose) as the positive control.

Results

Ribavirin at 1000 and 2000 mg/kg resulted in clinical signs of toxicity (slight hypoactivity and/or rough hair coat). Additionally, a decrease in the PCE:NCE ratio was observed at all ribavirin doses tested indicating a cytotoxic effect of ribavirin to the bone marrow. The frequency of micronuclei in polychromatic erythrocytes (MN-PCE) was not increased after 24 hours for any of the three dose levels. Exposure to the positive control, cyclophosphamide, resulted in an increase in micronucleated PCEs.

Comments

- Under the experimental conditions described, ribavirin did not induce clastogenic or spindle-damaging effects in mouse bone marrow cells *in vivo* up to a dose level of 2000 mg/kg. In this study, cytotoxicity was observed as indicated by a decrease in the ratio of PCEs to NCEs (decreased percentage of PCEs), which indicates that ribavirin in this study reached the bone marrow.
- In contrast, a non-GLP study using a different strain of mice (Swiss albino mice) with the same doses, route of administration and dosing regimen showed that ribavirin was genotoxic, as reported in the published literature. In addition, another non-GLP study published in the literature using B6C3F1 mice and a different dosing regimen (200 mg/kg x 2 days, IP) showed that ribavirin was genotoxic.
- Mouse strain differences in the susceptibility to induction of micronucleated polychromatic erythrocytes (PCEs) by various compounds, including clastogenic nucleosides, have been reported. However, literature data show CD-1 mice to be an adequate strain for detecting micronucleated PCEs following administration of other purine nucleoside analogues (penciclovir, acyclovir and ganciclovir) in the micronucleus assay.

Summary of individual study findings:

- Ribavirin (Ro 20-9963/000) was evaluated as negative for inducing reverse mutations in the *Salmonella-Escherichia coli* reverse mutation assay conducted with four tester strains of *S. typhimurium* (TA1535, TA1537, TA98 and TA100) and *E. coli* WP2uvrA, using nonactivation and activation conditions. Ribavirin did not increase the number of revertant colonies in any strain for approximately 48 hours in the absence and presence of an exogenous metabolic activation system (S9 mix). Cytotoxicity pre-screening in strains WP2uvrA and TA100 with and without S9 mix showed cytotoxicity with tester strain TA100 at 1000 µg/plate and greater in both the presence and absence of S9. No cytotoxicity was observed with tester strain WP2uvrA at the concentration up to 5000 µg/plate in the presence and absence of S9. Thus, under the described experimental conditions, ribavirin was not mutagenic in the Ames test.
- Ribavirin was evaluated as positive for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells using non-activation and activation conditions, with a clearly less positive response in the presence of S9 metabolic activation. In a confirmatory non-activation assay at concentrations from 7.85 to 2500 µg/ml, concentrations from 125 µg/ml and higher induced a 3 to 4-fold increase in mutant frequencies. However, under activation conditions, the positive response was clearly weaker than under non-activation conditions. Seven of these 8 concentrations (1000, 1500, 2000, 2100, 2200, 2300, 2400, and 2500 µg/ml) induced a 2.2 to 2.7-fold increase in mutant frequencies indicative of a weak positive response.
- When tested in the *in vivo* mouse micronucleus assay, ribavirin did not induce clastogenic or spindle-damaging effects in mouse bone marrow cells *in vivo* for any of the dose levels (from 500 to 2000 mg/kg/day x 3 days; the estimated human dose equivalent: 42 – 168 mg/kg, based on body surface area adjustment for a 60 kg adult). Additionally, a decrease in the PCE: NCE ratio was observed at all ribavirin doses tested indicating a cytotoxic effect of ribavirin to the bone marrow.

Study validity: The studies were adequately designed with appropriate positive controls.

Study outcome: The data were adequately reported and documented.

Genetic toxicology summary and conclusions:

- In the *in vivo* micronucleus assay in the bone marrow cells from CD-1 mice, ribavirin did not induce clastogenic or spindle-damaging effects in mouse bone marrow cells *in vivo* for any of the dose levels (from 500 to 2000 mg/kg/day x 3 days; the estimated human dose equivalent: 42 – 168 mg/kg, based on body surface area adjustment for a 60 kg adult). Additionally, a decrease in the PCE: NCE ratio was observed at all ribavirin doses tested indicating a cytotoxic effect of ribavirin to the bone marrow.
- In contrast, a non-GLP study using a different strain of mice (Swiss albino mice) with the same doses, route of administration and dosing regimen showed that ribavirin was genotoxic, as reported in the published literature. In addition, another non-GLP study published in the literature using B6C3F1 mice and a different dosing regimen (200 mg/kg x 2 days, IP) showed that ribavirin was genotoxic.
- Mouse strain differences in the susceptibility to induction of micronucleated polychromatic erythrocytes (PCEs) by various compounds, including clastogenic nucleosides, have been reported. However, literature data show CD-1 mice to be an adequate strain for detecting micronucleated PCEs following administration of other purine nucleoside analogues (penciclovir, acyclovir and ganciclovir) in the micronucleus assay.

Labeling recommendations:

- The sponsor-conducted *in vivo* micronucleus assay in CD-1 mice showed that ribavirin did not induce clastogenic or spindle-damaging effects in mouse bone marrow cells at any of the dose levels tested (from 500 to 2000 mg/kg/day x 3 days; the estimated human dose equivalent: 42 – 168 mg/kg, based on body surface area adjustment for a 60 kg adult). However, two non-GLP studies (data from the published literature) conducted in different strains of mice (Swiss albino mice or B6C3F1 mice) with the same or a different dosing regimen of ribavirin demonstrated that ribavirin was genotoxic.

VI. CARCINOGENICITY:

To support an NDA/BLA filing for PEG-IFN alfa-2a/ribavirin, the sponsor submitted results from a carcinogenicity study characterizing the potential carcinogenicity of ribavirin in p53 plus or minus C57BL/6 mice and rats to the division for review. A 6-month carcinogenicity study in p53 (+/-) C57BL/6 mice (p53 knock-out mice) with ribavirin was conducted and completed by the sponsor. Prior to the conduct of the 6-month carcinogenicity study, the CAC-EC reviewed and approved the dose levels and protocol design for this study (CAC-EC fax of July 18, 2000). A 2-year carcinogenicity study in the rat with ribavirin is currently on-going. This study was initiated on June 6, 2001. Prior to the initiation of the 2-year rat carcinogenicity study, the CAC-EC reviewed and approved the dose levels and protocol design for this study (CAC-EC fax of March 2, 2001).

1. Study Title: Twenty – six week gavage oncogenicity study with Ro 20-9963/000 (ribavirin) in p53(+/-) C57BL/6 Mice (Report 1003486)

Sponsor: Hoffmann-La Roche, Nutley, NJ; Roche Study No.: 07402; Vol.: 1 of 2; Pages: 1-832; Testing Facility: _____; Study No.: 6131-297; Study Initiation Date: 21 July 2000; GLP: Yes (X); Drug and Drug Lot Numbers: Ro 20-9963/000 Lot No.: 990543; Purity: 100.2%; Formulation: Ro 20-9963/000 in sterile water for injection, USP

Key study findings:

- Ribavirin did not result in neoplastic lesions when administered to p53(+/-) mice by gavage at dose levels of 10, 50, or 100 mg/kg/day for 26 weeks.
- The predicted early development of malignant lymphomas of thymic origin in positive control (MNU-treated) mice validates the use of the p53(+/-) model for oncogenicity evaluation.

Methods

Species:	C57BL/6TacfBR-(KO) p53 N5 heterozygous (+/-) mice for main study (20/sex/group); C57BL/6NTac strain mice (background strain) (66/sex/group, including a negative control group) were used for the toxicokinetic study ;
Age, weight:	C57BL/6TacfBR-(KO) p53 N5 heterozygous (+/-) mice: 7 weeks old; 15.1-25.5 for males, 16.2-21.1 g for females C57BL/6NTac strain mice (background strain): 8 weeks old; 13.3 -25 for males, 14.7-20.5 for females
Dosage:	0 (vehicle control), 10, 50 and 100 mg/kg/day x 26 weeks; In addition, C57BL/6TacfBR-[KO] p53 N5 heterozygous (+/-) strain mice (15/sex/group) were used for the positive control group and received methylnitrosourea (MNU; Lot#17H10211,) in citrate buffered saline at a dose level of 90 mg/kg once, on Day 1. The dosing volume for all groups, including controls, was 10 mL/kg/day.
Route:	oral, by gavage
Drug:	Ro 20-9963/000 (Lot No. 990543)
Clinical signs:	twice daily
Body weight:	once daily at approximately one hour post-dose; once weekly thereafter.
Food consumption:	weekly (main study animals only)
Ophthalmoscopy:	not determined
Hematology:	at study termination (surviving mice) and prior to death/moribund sacrifice
Clinical chemistry:	at study termination (surviving mice) and prior to death/moribund sacrifice
Urinalysis:	not determined
Gross pathology:	at sacrifice (selected organs were weighed, Appendix Table 2). Macroscopic lesions and the thymus from positive control animals were examined microscopically.
Histopathology:	at sacrifice (a comprehensive list of tissues was collected and microscopic examinations were performed on 42 tissues for each animals in the main study). In addition, bone marrow smears from the femurs of animals in the control and high-dose groups were evaluated at the scheduled sacrifice (Appendix Table 1).
Toxicokinetics:	Blood samples for the determination of serum concentrations of ribavirin were collected from toxicokinetic animals predose and at approximately 15 and 30 minutes and 1, 2, 4, 8, 12 and 24 hours after oral dosing on the first and last days of treatment. For the determination of ribavirin concentrations in whole blood, blood samples were evaluated 24 hours post-dose on the first and last days of treatment. Erythrocyte levels of ribavirin were calculated, in part, from serum and whole blood ribavirin concentrations.
Mortality:	An increased trend in mortality was seen in the main study male mice treated with ribavirin. No treatment-related mortality was observed in female mice. All positive control mice died during the study or were sacrificed by weeks 17 (females) or 25 (males).
Clinical signs:	No treatment-related clinical signs were observed. In the positive control group, clinical signs indicated a deteriorating general condition including hunched posture, tremors, hypoactivity, irregular and/or labored respiration, hypothermia, rough hair coat, and masses.
Body weight:	No effects on body weight or body weight gain were seen after administration of ribavirin. A decrease in body weight was observed in

the positive control group from week 14 (females) or week 16 (males) until study termination.

- Food consumption:** No effects on mean food consumption were seen after administration of Ribavirin. A decrease in food consumption was observed in the positive control group beginning at week 11 (females) or week 14 (males).
- Hematology:** Treatment-related hematological changes were observed in the 50 and 100 mg ribavirin/kg/day dose groups and consisted, in general, of decreased hemoglobin (↓2-6%), decreased erythrocyte indices (↓2-4%), decreased hematocrit (↓6%), and increased red cell distribution width (↑12-14%). The few samples collected from moribund positive control mice revealed a severe anemia and thrombocytopenia.
- Organ weights:** Treatment-related decreases in testis/epididymis weights (absolute and relative to brain and body weights) were seen in the 50 and 100 mg/kg/day dose group animals. A decrease in spleen weights for males and females were seen in the 100 mg/kg/day dose group (males: absolute and relative to brain and body weights; females: relative to body weights).
- Gross pathology:** No ribavirin treatment-related macroscopic changes were observed at the end of the treatment period. MNU-treated animals had an increased incidence of enlargement in various lymphoid organs (thymus, spleen, and selected lymph nodes).
- Histopathology:**
- Non-neoplastic:** A number of non-neoplastic findings were seen in the ribavirin treated groups in the liver and spleen in both male and females, as well as in the testes and epididymides at the low and the high doses.

Ribavirin-Related Non-Neoplastic Histological Findings						
Tissues	10 (mg/kg/day)		50 (mg/kg/day)		100 (mg/kg/day)	
	Male	Female	Male	Female	Male	Female
Spleen	Incidence					
Pigment		1/20	1/20		1/20	1/20
Lymphoid depletion					2/20	
↑Extramedullary hematopoiesis		1/20	3/20	3/20		1/20
Liver	Incidence					
Glycogen depletion	1/20	1/20			2/20	
Clear cell focus					1/20	
Focal necrosis					1/20	
↑Extramedullary hematopoiesis				2/20		
Testis/Epididymis	Incidence					
Degeneration/atrophy, unilateral			1/20		1/20	
Chronic inflammation			1/20			
Lung	Incidence					
Alveolar histiocytosis		1/19				2/20

Neoplastic:

No ribavirin-related neoplastic lesions were seen in this study. However, two male mice at 50 mg/kg/day dose had malignant lymphomas. The low incidence (within the current control range for compounds tested) and the lack of a dose-response relationship indicated that these lesions were not treatment-related.

Findings		Incidence of Tumors (Numeric)									
		Males					Females				
		Ribavirin (mg/kg/day)					Ribavirin (mg/kg/day)				
		0	10	50	100	90*	0	10	50	100	90*
Thymus	<i>M-lymphoma</i>	0/19	0/20	2/20	0/19	15/15	0/20	0/20	0/20	0/20	13/15
Lung	<i>M-lymphoma</i>	0/18	0/18	1/16	0/20	2/2	0/20	0/20	0/20	0/20	2/3
Spleen	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	8/8	0/20	0/20	0/20	0/20	6/6
Thyroid	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Liver	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	2/2	0/20	0/20	0/20	0/20	0/1
Kidney	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	2/2	0/20	0/20	0/20	0/20	1/2
Pancreas	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	1/1	0/20	0/19	0/20	0/20	0/2
Lymph Nodes	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	5/5	0/20	0/20	0/20	0/20	3/3
Harderian gland	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Skeletal muscle	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Sciatic nerve	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Prostate	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	—	—	—	—	—
Marrow (femur)	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Urinary bladder	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Hemato neoplasia	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	15/15	0/20	0/20	0/20	0/20	15/15
Stomach	<i>M-leiomyosarcoma</i>	0/20	0/20	1/20	0/20	1/1	0/20	0/20	1/20	0/20	1/1
Subcutaneous tissue	<i>M-sarcoma**</i>	0/20	1/20	0/20	0/20	1/1	1/1	1/1	5/5	—	—

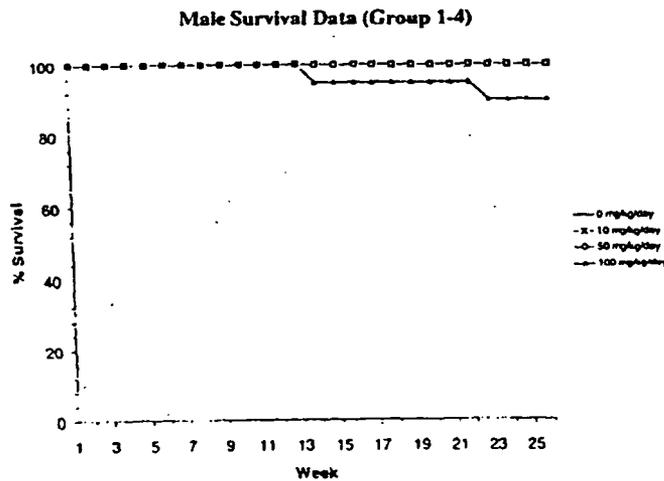
*:90 mg/kg methylnitrosourea, once, on Day 1. —: not examined. ** related to biomedic implant.

Note that malignant lymphomas are one of the most common neoplastic lesions observed in the p53 mouse model. The positive control group resulted in malignant lymphomas of thymic origin.

Toxicokinetics:

The serum exposure to ribavirin increased with increasing doses in C57BL/6Ntac mice. There were no consistent gender differences in C_{max} and AUC_{0-24hr} values although females had higher C_{max} and AUC_{0-24hr} values than males in the 50 mg/kg/day dose group on Day 183. The increases in C_{max} and AUC_{0-24hr} values were generally less than dose-proportional. C_{max} and AUC_{0-24hr} values were similar on Day 183 in comparison to Day 1, indicating no accumulation of ribavirin. Exposures (AUC_{0-24hr}) of 6.5, 23.2, and 33.8 $\mu g \cdot hr/ml$ were obtained after 26 weeks of treatment following administration of 10, 50 and 100 mg/kg/day, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu g \cdot hr/ml$. Although blood was collected for the analyses of the concentration of ribavirin in whole blood samples, no anticoagulant was added to prevent the collected blood samples from clotting. Consequently, the concentration of ribavirin in whole blood could not be reliably measured and the concentration of ribavirin in erythrocytes could not be calculated.

Figure 1. Survival Data – twenty-six week oral gavage oncogenicity study with Ro 20-9963/000 (ribavirin) in P53 (+/-) C57BL/6 mice



Comments

- Ribavirin did not result in neoplastic lesions when administered to p53(+/-) mice by gavage at dose levels of 10, 50, or 100 mg/kg/day for 26 weeks.
- The predicted early development of malignant lymphomas of thymic origin in positive control (MNU-treated) mice validates the use of the p53(+/-) model for oncogenicity evaluation.

Summary of Toxicity and Toxicokinetic Findings

Dose (mg/kg/day)	Toxicokinetic Data			Treatment-Related Findings
	Day of Sampling	Ro 20-9963		
		C _{max} (ng/ml)	AUC _{0.25-24 hr} (ng.hr/ml)	
Males 10	1	1240	5410	No treatment-related findings were observed
	183	902	6390	
50	1	2440	19400	↓ Hemoglobin; ↓ mean corpuscular hemoglobin; ↑ red cell distribution width; ↓ mean corpuscular hemoglobin concentration; ↓ testis/epididymis weight (absolute and relative)
	183	1570	17200	
100	1	3470	27800	↓ Hemoglobin; ↓ hematocrit; ↓ mean corpuscular volume; ↓ mean corpuscular hemoglobin; ↑ red cell distribution width; ↓ mean corpuscular hemoglobin concentration; ↓ spleen weight (absolute and relative); ↓ testis/epididymis weight (absolute and relative)
	183	2790	33200	
Females 10	1	1050	7200	No treatment-related findings were observed
	183	883	6650	
50	1	2560	23600	↓ Hemoglobin; ↓ mean corpuscular volume; ↓ mean corpuscular hemoglobin; ↑ red cell distribution width
	183	4790	29100	
100	1	3930	31000	↓ Hemoglobin; ↓ mean corpuscular volume; ↓ mean corpuscular hemoglobin; ↑ red cell distribution width; ↓ mean corpuscular hemoglobin concentration; ↓ spleen weight (relative)
	183	4830	34400	

↑ - Increased; ↓ - Decreased

a) An increased trend for mortality was observed in males.

Summary of individual study findings:

- This short-term oncogenicity study in mice (P53+/-) is adequate to assess the carcinogenicity of ribavirin in mice. The test model is appropriate. Dose levels of 0, 10, 50 and 100 mg/kg/day were used for this study and are supported by results from the 4-week dose range finding studies in mice, which was concurred by the CAC-Exec, CDER. The predicted early development of malignant