

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number **20 - 903**

PHARMACOLOGY REVIEW(S)

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Executive CAC Recommendations and Conclusions:

It was the conclusion of the committee, based on the positive genotoxic effects seen with ribavirin in multiple assay systems, the lack of adequate in vivo rodent carcinogenicity data and, the extended period of human drug exposure (treatment regimen of 6 months duration), that:

- a) the product label for ribavirin could indicate that it may be a potential human carcinogen,
- b) the sponsor, as part of a Phase 4 Post-Marketing Agreement,

~~Joseph DeGeorge, Ph.D.
Chair, Executive CAC~~

5/28/98 - revised for
types

cc:\

/Division File, HFD-530
/JFarrelly, HFD-530
/DMorse, HFD-530
/ASeifried, HFD-024

DIVISION OF ANTIVIRAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20,903 CHEMISTRY REVIEW #: 1 REVIEW DATE: 5-26-98

<u>SUBMISSION TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
Original	12-3-97	12-5-97	12-9-97
Amendment BC	2-16-98	2-17-98	2-19-98
Amendment BC	3-3-98	3-4-98	3-9-98
Amendment NC	4-14-98	4-15-98	4-22-98
Amendment BL	4-24-98	4-27-98	5-1-98
Amendment BC	5-8-98	5-12-98	5-20-98
Amendment BL	5-12-98	5-12-98	5-20-98

NAME & ADDRESS OF APPLICANT:

Schering Corporation
2000 Galloping Hill Road
Kenilworth, NJ 07033

DRUG NAME:

Proprietary:

- 1) Rebetol™ (Schering), Virazole (ICN)
- 2) Intron A® (Schering)

Nonproprietary:

- 1) Ribavirin (USAN and generic),
Tribavirin (BAN)
- 2) Interferon alfa-2b , recombinant
(USAN)

Code Names/#:

- 1) SCH 18908, ICN 1229, Vilona,
Viramid, Virazid

CAS Registry Number:

- 2) SCH 30500
36791-04-5

Chem. Type/Ther. Class:

- 3, P

PHARMACOLOGICAL CATEGORY:

- 1) Antiviral
- 2) Biological response modifier;
Antineoplastic
Chronic Hepatitis C

INDICATION:

DOSAGE FORM:

- 1) Capsule
- 2) Injectable solution

STRENGTH:

- 1) 200 mg/capsule
- 2) 3 million IU/0.5 mL

ROUTE OF ADMINISTRATION:

- 1) Oral
- 2) Subcutaneous injection

DISPENSED:

Rx

CHEMICAL NAME, STRUCTURAL FORMULA,

CONCLUSIONS & RECOMMENDATIONS:

The CMC for Intron A Injection (except secondary packaging components) are same as those approved by the CBER, FDA. The analytical methods validation for Rebetol capsules by the Philadelphia district laboratory is not completed at this time point. This NDA qualifies for an exemption from the EA requirement. Pre-approval inspection Schering's Kenilworth and Las Piedras sites were recommended as acceptable by the Office of Compliance. Based on the available real time stability data and statistical analysis, an expiration dating period of 18 months is recommended for Rebetol capsule bottles of 150 and 180 counts and for unit dose blisters and aluminum foil strips. In conclusion, the CMC section of the NDA, as amended, is recommended for **approval**.

Rao V. Kambhampati, Ph.D.
Reviewing Chemist

Concurrence:
HFD-530/Chem. TL/SMiller

cc:
Original NDA #20-903
HFD-530/Chem. TL/SMiller
HFD-530/MO/RFleischer
HFD-530/CSO/TCrescenzi
HFD-530/Pharm/DMorse
HFD-530/Division File

HFD-530/Chem/RKambhampati
HFD-830/Director/CChen
HFD-530/MO/TNguyen
HFD-530/Micro/NBattula
HFD-530/Biopharm/PRajagopalan

DRAFT

**DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
MEDICAL OFFICER'S CONSULTING COMMENTS**

NDA: 20-903

SPONSOR: Schering-Plough

DATE OF REQUEST: 9 January 1998, 23 March, and continuing interactions, by R. Fleischer, HFD-530, to Dr. John Senior of HFD-180 for opinion on U.S. Study C95-144, of ribavirin or placebo added to interferon (INTRON A) for treatment of relapsed chronic hepatitis C, and other matters.

DRUG: Ribavirin (RABETROL®) tablets 200 mg

ROUTE OF ADMINISTRATION: Oral, 500-600 mg b.i.d. for 24 weeks, or placebo, added to INTRON A 3 million units subcutaneously (SC) three times/week for 24 weeks.

QUESTIONS: Please see list in Section III below.

MATERIAL REVIEWED: Schering report of Study C95-144 dated 27 October 1997; literature on interferon-induced depression to 25 March 1998; reports on Anti-Viral Drugs Advisory Committee meeting of 4 May 1998; and medical officer's review of International Study I95-145, received 15 May 1998.

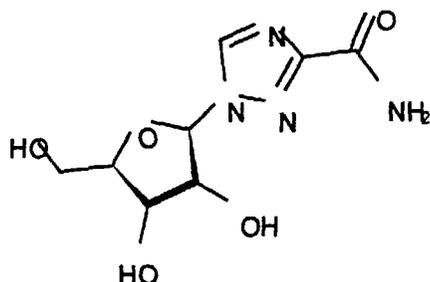
REVIEWER: John R. Senior, M.D., HFD-180; 18 May 1998.

I. Background and Introduction

Even before the identification in 1989 of the hepatitis C virus (HCV) and of specific tests for its detection, it had been recognized that a parenterally transmitted and apparently viral agent was the leading cause of post-transfusion chronic "non-A, non-B" (NANB) hepatitis. This NANB chronic hepatitis was usually insidious in onset, and slowly progressive, often to cirrhosis, liver failure, and sometimes to hepatocellular carcinoma. Its presence in blood donors was inferred by the poorly specific surrogate marker of viral hepatitis, elevated serum alanine aminotransferase (ALT) activity. Trials of interferon alpha (IFN α) treatment of chronic NANB hepatitis were started in 1986-7, and continued more actively after the reported discovery of HCV in 1989. These studies lead, after review by the Center for Biologic Evaluation and Research (CBER) at the Food and Drug Administration (FDA), to approval of two forms of IFN α : -2a (Roche, ROFERON-A) and -2b (Schering-Plough, INTRON A). Although IFN α 2a and 2b, and later variants, were the only known effective therapy for chronic hepatitis C, the initial normalization of ALT that was seen in 40-50% of patients was found to be followed by relapse in most of the patients after treatment was stopped, so that only 12-24% of IFN α -treated patients showed sustained normal ALT levels at 6 months after an initial 6-month course of IFN α at a dose of 3 million units three times/week for 24 weeks. Later, development of sensitive tests to detect and quantitate HCV in blood showed that the relapsed patients had persisting viremia.

In search of more effective treatment, the synthetic nucleoside 1- β -d-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide (virazole), which had earlier (1972-3) been

shown to have broad anti-viral activity, was developed as ribavirin (Schering 18908, RABETROL®) and was tested as an oral formulation in 1994-6 for treatment of chronic hepatitis C.



ribavirin (virazole)

$C_8H_{12}N_4O_5$; m.w. 244.21

Ribavirin as monotherapy failed to clear HCV from blood or improve liver histology in chronic hepatitis C. However, combined IFN α and ribavirin treatment in European pilot studies showed promising improvement in the numbers of patients who showed sustained remissions, by both ALT and PCR-HCV testing, at 6 months after a 6-month course of combined therapy. A phase II trial of ribavirin+IFN α combination in 100 patients with previously untreated chronic hepatitis C showed a significantly greater number of patients in sustained remission, 42%, compared to 20% in patients treated with IFN α alone (placebo). The addition of ribavirin to INTRON A appeared to cause no new or unexpected adverse effects beyond those noted with each agent alone, the anemia, fatigue, anorexia, and depression were more frequently seen with combined treatment than with IFN α only.

The question then was whether the incremental clinical benefit of more sustained viral remissions and histologic decrease in histologic inflammation after combined therapy, if confirmed, would outweigh the increased frequency or severity of those or other adverse effects, especially the hemolytic anemia and possibly enhanced neutropenia caused by ribavirin.

II. Study C95-144

A. Description

This study was initiated in April 1996 at 21 centers in the United States, and 154 patients were enrolled. These patients had been treated previously with one or two courses of from 5 to 18 months of IFN α (INTRON A, ROFERON-A, or WELLFERON) and had responded with ALT normalization at the end of treatment, but showed relapsed ALT elevation within a year. The relapse had to have been confirmed by detectable viremia (at least 100 copies/mL), by ALT elevation, and by liver biopsy consistent with chronic hepatitis C within 6 months of entry into this study. Patients were excluded if they had decompensated liver disease (ascites, bleeding varices, encephalopathy, serum albumin <3.5 g/dL, prothrombin time prolonged >2 seconds), anemia (hemoglobin [Hb] <13 g/dL in men or <12 g/dL in women), or significant cytopenia (white blood cells <3,000/ μ L, neutrophils <1,500/ μ L, or platelets <100,000/ μ L). They were also excluded if they were infected with human immunodeficiency

virus (HIV), had clinically significant cardiovascular, pulmonary, renal, gastrointestinal, metabolic, hematologic, rheumatic, immunologic, or central nervous diseases, or if they had other liver diseases, elevated serum levels of α -fetoprotein, history of alcohol or drug abuse, or had had a solid organ transplant.

The stated rationale for choosing the number of patients to be studied was based on assuming that addition of ribavirin to INTRON A treatment had a 90% power to detect a gain in sustained response from 15% to 40% at α error = 0.05, two-sided. The treatment doses and duration were based on the approved INTRON A regimen of 3 million units SC three times/week for 24 weeks, given openly to all patients, and pilot data for ribavirin at 1000 mg /day for patients weighing <75 kg and 1200 mg for those weighing 75 kg or more, in two divided oral doses for 24 weeks. Assignment to equally sized, blinded treatment groups was made at a central randomization center, with balancing across and within study sites of cirrhosis (present/absent), HCV genotype (Type 1/other Types), and level of viremia (at least 2 million copies/mL/less than 2 million copies/mL). The follow-up period of 24 weeks after completion of treatment was based on pilot studies of ribavirin plus IFN α , literature reports on relapse at 6 months after 6 months of treatment using IFN α alone, and confirmed later in March 1997 by the Consensus Conference on Management of Hepatitis C sponsored by the National Institutes of Health.

The primary outcome measure of response to treatment was BOTH the sustained (48-week) loss of detectable viremia testing AND histologic improvement in hepatic inflammation (reduction by at least 2 grades by the Knodell Hepatic Activity Index [HAI] score for items I, II, and III). The hepatic biopsy specimens were all evaluated at a central pathology laboratory and clinical laboratory tests were done definitively at a central laboratory.

Local testing at each site was also carried out for interim evaluation of ALT and other tests as clinically required. Several secondary measures of efficacy were analyzed, including ALT normalization at 24 and 48 weeks, undetectable PCR-HCV at 24 weeks, reduction by at least one grade in hepatic fibrosis on biopsy at 48 weeks (Knodell HAI IV score), mean changes of HAI by treatment groups, as well as in proportions of individuals responding by predefined criteria.

B. Results

As the study was executed, 154 patients were enrolled into the study (one patient randomized to ribavirin quit the study before starting treatment, and one patient randomized to placebo addition received blinded ribavirin by mistake discovered later and was counted with the ribavirin group). There were 77 patients treated by ribavirin addition, and 76 by placebo addition to standard INTRON A retreatment. The mean age of the treated patients was 43.5 years, ranging from 28 to 67. They were mostly men (102/153, 67%), Caucasian (140/153, 92%), and most had been infected with HCV after parenteral injections of illegal drugs. Most had HCV Type 1 infections (115/153, 75%), and over 2 million copies/mL (132/153, 86%); liver biopsies pre-study showed average Knodell inflammation (I+II+III) score of 6.9, and fibrosis 1.4 (Knodell IV). The two randomized treatment groups were not significantly different in distributions by age, gender, racial origin, body weight, HCV genotype, PCR-HCV count, ALT activity, type or duration of previous IFN α treatment, or histologic scores.

Of the 153 treated patients, 64/77 (83%) on ribavirin addition and 67/76 (88%) on placebo addition (difference not significant) completed both 24 weeks of treatment and 24 weeks of follow-up. During treatment, 10/77 (13%) on ribavirin and 5/76 (7%) on placebo dropped out (difference not significant); reasons given for dropping out during treatment included adverse events in 7/77 (9%) on ribavirin, 3/76 (4%) on placebo. After treatment, 3 more of 77 (4%) who had been on ribavirin and 4 more of 76 (5%) on placebo discontinued study during follow-up.

At the end of the study (48 weeks), 34 of the 77 (44%) who had had ribavirin addition to

INTRON A treatment still had no detectable viremia, compared to only 3 of the 76 (4%) who had had placebo addition, a very significant ($p < 0.0001$) difference. End-of-study liver biopsies were available for 61 of the 77 on ribavirin and for 64 of the 76 on placebo addition (3 patients in each treatment group who finished the full study declined to have repeat biopsy done). Hepatic inflammation scores (sum of Knodell I+II+III scores) were "improved" (reduced by 2 or more grades) in 38 of 61 (62%) who had received ribavirin addition and in 27 of 64 (42%) who had had placebo addition, a significant difference ($p = 0.032$, by Fisher exact test), but were worse by at least 1 grade in 8/61 (13%) on ribavirin and in 17/64 (27%) on placebo addition. (not quite significant, $0.05 < p < 0.07$). Comparison of "overall response," defined as BOTH undetectable viremia AND reduced histologic inflammation, showed 25 of 61 (41%) on ribavirin addition *versus* only 2 of 64 (3%) on placebo addition to INTRON A retreatment; this was also highly significant ($p < 0.0001$). Normal ALT at end-of-follow-up was highly correlated with disappearance of viremia. There was no significant difference between treatment groups in the changes of hepatic fibrosis scores.

As had been known before, HCV genotype 1 was less responsive to therapy, and this was confirmed in this study. Of those who showed disappearance of detectable HCV at 48 weeks (24 weeks after ribavirin addition to INTRON A for the 24-week treatment period), 21 of 31 (68%) had HCV genotypes other than Type 1, compared to only 13 of 46 (28%) in those with Type I HCV. It was notable that there still was this degree of clearance of the Type 1 HCV sustained at 48 weeks, an improvement on previous finding with IFN α treatment alone. Although the viral load had some importance, 28 of 68 (41%) on ribavirin addition who had had 2 million or more copies/mL cleared their viremia, compared to 6 of 9 (67%) who had had less than 2 million copies/mL (not significant, but the number of patients in the latter group was relatively small). There were only 3 patients with cirrhosis, all assigned to placebo addition, and none of them cleared their viremia. However, 28 patients with "bridging fibrosis" (Knodell IV score of 3) were studied, of 6/12 (50%) on ribavirin addition and 0/16 (0%) on placebo addition cleared their HCVemia, a highly significant difference, even with such small numbers.

The safety of ribavirin addition to INTRON A treatment was considered carefully. As is well known, IFN α treatment causes some adverse effects in nearly all patients treated, including headaches, muscle aches, fatigue, anorexia, nausea, fever, chills, joint aches, insomnia, depression, irritability, abdominal pain, diarrhea, flu-like symptoms. This was found in 74 of the 76 (97%) of the patients on placebo addition to INTRON A retreatment, as well as in 76 of the 77 patients who also had ribavirin addition. The latter group had slight but not significantly more frequent percentages of patients with nausea (+12%), anorexia (+7%), dyspepsia (+7%), fatigue (+7%), rigors (+6%), dizziness (+5%), irritability (+5%), and dyspnea (+5%) than in the patients on placebo addition to INTRON A treatment. Other symptoms were equally or more frequent in those receiving placebo addition to INTRON A. None of the symptoms reported with increased frequency by patients on ribavirin addition were of severe intensity.

Because of the known tendency of ribavirin to induce hemolysis of red blood cells, special concern was focused on this adverse effect, and patients who might be at particular risk such as those with severe cardiovascular disease were excluded from this study. The protocol also included provisions for dose reduction if blood Hb fell below 10 g/dL, or discontinuation of study drug if below 8.5 g/dL. It was observed that Hb levels fell by an average of 2 g/dL in the patients who had received ribavirin in addition to INTRON A, consistently in the first 4 weeks of treatment, with attendant reticulocytosis and serum bilirubin increase, not seen in those who had placebo addition. Of those on ribavirin 8 of 77 (10%) had decreases of Hb to < 10 g/dL and 6 of them had dose reduction, at least until Hb levels rebounded. All 8 patients showed recovery of Hb by week 4, and none had to have study drug stopped. Decreases in total white blood cell and neutrophil counts were noted in both treatment groups, slightly but not significantly greater in those on ribavirin addition. Blood platelets were less reduced by ribavirin than by placebo addition to INTRON A. There were no cases of pancreatitis reported in this study, and no deaths.

In summary, the addition of ribavirin at oral doses of approximately 15 ± 5 mg/kg/day (depending on body weight, in two divided doses morning and evening) to a standard repeat regimen of INTRON A 3 million units SC three times/week for 24 weeks produced statistically significant and clinically important improvements in sustained suppression of viremia and reduction in histologic liver inflammation 24 weeks after treatment was stopped, compared to monotherapy with INTRON A alone (placebo addition). The patients in whom these results were observed had previously responded to IFN α alone by showing ALT normalization but had relapsed after treatment was stopped, showing both ALT rebound and persisting viremia. Patients with decompensated liver disease or possible hepatocellular carcinoma (by ultrasound scan or elevated serum α -fetoprotein) were not studied. Bridging fibrosis if present was also significantly improved by ribavirin. Data were obtained in 86% of the patients treated, who completed the 24 weeks of treatment, the 24 weeks of follow-up after treatment, and had both liver biopsy and serologic data at end-of-study.

Other notable and significant positive effects of ribavirin addition to INTRON A included more patients with normal ALTs, both at end-of-treatment at 24 weeks and at end-of study at 48 weeks, clearance of detectable viremia even of Type 1 HCV and when the viral load was over 2 million copies/mL of blood.

These improvements in the several indicators of response to ribavirin addition were realized with no serious increase in risk in these selected patients, although the expected hemolytic effects of ribavirin were seen and had to be managed. Study drug (ribavirin) dose reduction, and allowing time for increased red cell production, were sufficient in this study to manage the hemolytic effects. No patient had to have ribavirin stopped entirely, none required transfusion, and all patients showed return of Hb to pretreatment levels during the follow-up period.

Comment: Both red and white cell counts need to be closely watched during ribavirin treatment, and especially so if patients with cardiovascular or pulmonary problems are treated. It may be worth considering erythropoietin pretreatment, to stimulate increased red cell production to offset the hemolytic losses caused by ribavirin. This is speculative, but is likely to be tried, if it has not already been so.

III. Questions (from R. Fleischer)

1) Are there data to suggest that this response would be an expected response to repeated treatment with interferon in patients who had relapsed following interferon therapy?

A great many studies have been done using various interferon doses for various durations of treatment, repeat courses of treatment, and several variants of interferon. While there have been some slight improvements in "responses" (variously defined by ALT normalization, blood HCV disappearance, histologic reduction of inflammation) from more prolonged, more intense, or repeated treatment with IFN α alone, none have shown the impressive results here seen with the addition of ribavirin. The results of this study are substantially better than would have been expected from simply repeating another course of the standard regimen of INTRON A.

2) What is the clinical relevance/significance of such an improvement in liver histology, given that very few patients had either an ALT or viral response to INTRON A and placebo?

The initial enthusiasm over normalization of ALT during or at the end of treatment of chronic hepatitis C with IFN α gave way several years ago to great disappointment, as it became realized that most of those who had appeared to respond then relapsed within 6 months, and that viremia continued to be detectable. All of the measures used (ALT normalization, blood HCV disappearance, histologic reduction of inflammation) are at best only surrogates for the important

clinical benefits sought, the reduction in progression of chronic hepatitis C to cirrhosis, liver failure, hepatocellular carcinoma, and death/transplantation. Best of all would be cure: eradication of HCV not only from blood but also from liver and other tissues, healing of the inflamed liver, and permanent normalization of all abnormalities. This may or may not ever be achieved. Stopping or significantly slowing progression of the process may be the most that can be accomplished now or in the immediate future, but that would be a significant clinical benefit. All of the three surrogate indicators may be valid, but they measure different things that do not seem to correlate closely or reliably with each other; they all have their possible errors in both measurement and significance. It is not disturbing that there may have been observed an improvement in histologic inflammation without ALT or viral response following INTRON A treatment only. It will require years to establish whether or not prolonged suppression of viremia and hepatic inflammation will accomplish even the more limited clinical benefit of significantly slowing progression of chronic hepatitis C to the "hard" endpoints of cirrhosis, liver failure, liver cancer, death/transplantation.

3) *What is the clinical significance/relevance of improvements in single components of the Knodell score? How does one interpret a patient with a "virologic response" or "histologic response" but worsening fibrosis on biopsy?*

Cautiously. Liver biopsy is a very valuable tool, but has its limitations and is not infallible. The limitations include sampling error, interpretive error, and invasive risk that limit serial measures. The liver disease, specifically fibrosis, may not be uniform throughout the whole liver, and a 10-20 mg sample of an organ weighing 100,000 times as much must be viewed not only with caution but by someone who can interpret what is seen through the microscope both accurately and consistently. The opinions and scoring by a single, very experienced, and highly respected pathologist represent an effort to reduce at least that variable, but cannot remove the problem of sampling error. Repeated biopsies over time are limited by how many are safe to do and tolerated by patients. Correlations with viremia or ALT levels are not reliable, have not been validated, and discordances are not easily explained. The Knodell score for inflammation types I, II, and III are not independent variables, but are often highly correlated. Fibrosis is generally believed to be the result of prolonged inflammation and cell necrosis or apoptosis, but to what extent it may be reversible will require more information and understanding. Despite these drawbacks, liver biopsy is still the best way to assess the degree of damage already done and in process. Worsening fibrosis evokes concern, but needs to be confirmed by repeat biopsy or other measures.

4) *Are the more dramatic improvements in Knodell score, for example 7 points, indicative of a significantly better response or prognosis than improvements of a smaller magnitude?*

On the face of it, one might think so, but it hasn't been proved. Normalization to a score of 0, that is reconfirmed and sustained would be most impressive, i.e., complete disappearance of all the histologic abnormalities. On the other side of the coin, however, it should be appreciated that patients who show reductions of 7 points or more were necessarily worse to start with than those with initial scores of 5 or 6 who show reductions to 0. Longer term study will be needed to establish whether this interesting question can be answered affirmatively.

5) *Some patients had improvement of component IV (fibrosis) of their Knodell score. What is the clinical relevance/significance of such improvement in component IV?*

Bearing in mind the sampling and interpretive errors of liver biopsy, and the comments on question 3 above, we must realize that not all hepatic fibrosis is the same. As collagen is laid down and then remains in place it gradually changes in its cross-linking and structure. The chemical type of collagen, the duration of its deposition, its resistance to dissolution, the pattern of cellular and lobular distortion are all important, but imperfectly understood. Some collagen can resorb, especially "new" collagen recently laid down. Reduction in fibrosis generally would

seem to be a good indicator, but requires confirmation and longer observation.

6) In general, what parameters (levels of ALT, HCV, other) are those useful to clinicians and their patients in following the progression of HCV infection?

Clinicians like numbers (ALT, HCV) for estimating disease progression or response to treatment. Patients are most interested in symptoms they can perceive or that affect their quality of life, effects that have large temporal and individual variance and are not sensitive measures until late in the course of disease. Serial liver biopsy has its risks and limitations. Clinicians therefore hope the numbers can be validated as measures that will predict the progression of disease at the tissue level (biopsy) and at the organ level (symptoms and signs of clinical complications). The ALT alone has not been proved to be reliable as a predictor, and at present the disappearance of detectable viremia, if properly done, seems the most useful measure.

IV. Other Information

A. The International Study I95-145

This study was reviewed by Dr. T. Nguyen of HFD-530; a copy of his review was received 15 May 1998, but not the sponsor's report or the study protocol. From the review, the design was very similar to that of the U.S. study C95-144. The international study was carried out in 31 centers in Europe, Canada, Australia, and Israel, and enrolled 195 patients, 192 of whom were started on randomized treatment, 96 in each arm. The patients treated were predominantly men (122/192, 64%), Caucasian (95%), and had acquired HCV infection presumably via parenteral use of illegal drugs (59%). The mean age of patients was 43.5 years, the prevalence of Type 1 HCV was 55%, and viremia of at least 2 million copies/mL was observed in 66%. The mean total Knodell score was 9.3 (inflammation sum of I, II, and III was 8.0, and fibrosis score IV was 1.3), with no significant differences in the two treatment groups. These characteristics were similar to those of patients in the U.S. study C95-144.

Results of the international study corroborated those of C95-144. Of the 192 patients randomized to treatment, 185 (96%) completed the 24-week blinded treatment period, and 181 (94%) also completed the 24-week follow-up phase, with no significant differences between treatment groups. Patients randomized to ribavirin addition to INTRON A showed significantly more clearance of viremia at 24 weeks: 80/96 (83%), compared to 43/96 (45%) in those on placebo addition. This difference was even more marked at week 48, 24 weeks after treatment ended, with 50/96 (52%) on ribavirin still negative for detectable viremia *versus* on 5/96 (5%) of those on placebo addition ($p < 0.0001$). Histology improvement by at least 2 grades in Knodell inflammation score was significantly more frequent in those on ribavirin (48/96, 50%) than in those on placebo (31/96, 32%). Again, there was no significant difference in fibrosis reduction. The overall response of both viral disappearance and reduced inflammation was very significantly more often seen in the ribavirin group (35/96, 35%) than in those on placebo addition (4/96, 4%).

The ribavirin-induced hemolytic effects were again seen in the first 4 weeks of treatment, but were managed without serious problems except in one 76-year-old woman who developed chest pain and dyspnea when her Hb dropped after 19 weeks on treatment. A 37-year-old woman on placebo addition died from illicit drug overdose in the 18th week of follow-up, after a long history of drug abuse.

B. Antiviral Advisory Committee

Review and discussion of the data by the expert panel of consulting advisors on 4 May 1998 (summarized in the Pink Sheet of 11 May, and a short draft of the minutes sent by telefax on 15

May 1998) resulted in a unanimous recommendation (vote 8-0) for approval of ribavirin addition to IFN α treatment as reasonably safe and effective for treatment of chronic hepatitis C that had initially responded but then relapsed after treatment with IFN α alone. Concerns about ribavirin-induced hemolysis were discussed, but it was agreed that these could be managed by sufficiently close monitoring and dose adjustment.

V. Summary and Conclusions

After review of these data on U.S. Study C95-144, perusal of the FDA medical review of the international Study I95-145, and considerations of the questions forwarded from HFD-530 by R. Fleischer, I concur in recommending approval of RABÉTROLO® (ribavirin, Schering-Plough) at doses of 500-600 mg b.i.d., in conjunction with INTRON A SC 3 million units three times/week for 24 weeks, for treatment of relapsed chronic hepatitis C after treatment with IFN α alone.

We look forward to clinical data on combined ribavirin+ IFN α treatment of naïve patients with chronic hepatitis C who have not failed initially on treatment with IFN α only, and to the optimization of regimens with respect both to daily dose and duration of treatment.

John R. Senior, M.D.
Medical Reviewer, DGCDP

date

cc:

NDA 20-903
HFD-530/RFleischer
HFD-530/CSO
HFD-180
HFD-180/LTalarico
HFD-180/HGalloTorres
HFD-180/JSenior
HFD-181/CSO

THIS SECTION
WAS
DETERMINED
NOT
TO BE
RELEASABLE

17 pages

PHARMACOLOGIST'S REVIEW

NDA: 20-903 Original

Date Submitted: 5 Dec. 1997

Date Assigned: 5 Dec. 1997

Date Review Completed: 8 May 1998

Reviewer: David E. Morse, Ph.D.

HFD-530: Div. of Antiviral Drug Products

SPONSOR: Schering Corporation
Galloping Hill Road
Kenilworth, NJ 07033 (908) 298-4000

DRUG: Ribavirin (Rebetol® and Virazole®)
1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide
and, Interferon alpha-2b (Intron A®; PLA No. 994)

STRUCTURE:

EMPIRICAL FORMULA: $C_8H_{12}N_4O_5$
MOLECULAR WEIGHT: 244.21
MELTING POINT: 166-168°C in aq. ETOH
174-176°C in ETOH
SOLUBILITY: 142 mg/ml (H₂O, at 25°C)
FORMULATION: Capsules, 200 mg each

INDICATION: Treatment of Hepatitis C infection

RELATED IND(s)/NDA(s)/PLA(s):

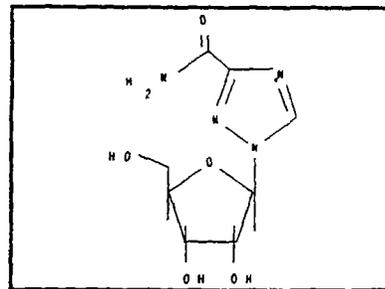
NDA's 18-266 (ICN); 18-859 (ICN); 19-705 (U.S. Army); 20-206 (ICN);

PLA's 994 (Schering Corp.)

DEFINITIONS:¹

INTRODUCTION

The sponsor has submitted a new NDA for the use of ribavirin capsules (REBETOL®; 1-1.2 g/day; PO) in combination with interferon alpha-2b (INTRON A®; 3 MIU TIW; SC Inj.) for the treatment of chronic active Hepatitis type C infection in patients who have relapsed following a previous course of interferon monotherapy. The proposed duration of combination drug administration in the treatment of Hepatitis type C infection is 6 months. Interferon alpha (INTRON A®, and related interferons: ROFERON®, WELLFERON®, INFERGEN®) has been previously approved for use at the proposed dose and route of administration for the treatment of Hepatitis type C infection. Since no change in the indication or use of interferon alpha has been proposed in this NDA, the reader is referred to the PLA review (No. 994) for a comprehensive review of the toxicology of INTRON A® brand of interferon alpha-2b. In contrast, ribavirin is currently only approved as an aerosol product (VIRAZOLE®; NDA 18-859) for use in the treatment of Respiratory Syncytial Virus (RSV) infection in the pediatric population. No oral ribavirin containing product is currently available for use in the US. Therefore, this NDA review will focus primarily on the repeat-dose toxicology, reproductive



Ribavirin

toxicology, genotoxicity and carcinogenicity, pharmacokinetic and ADME studies related to the use of ribavirin.

The following sections of this document contain reviews and/or summaries of the toxicology studies contained in this or related submissions. A discussion of additional toxicology requirements regarding the long-term human use of ribavirin is contained in the summary section of this document. Proposed product labeling for the combined use product (INTRON a and REBETOL) is contained in the last appendix to this document.

BACKGROUND

Interferon alfa-2b is obtained from bacterial fermentation of a genetically engineered strain of *Escherichia coli* containing the human interferon gene. Interferons are a class of related glycoproteins which are secreted by multiple body tissues in response to viral infection and various other inducers. Interferons bind to specific cell surface receptors and initiate intracellular changes which cause changes in cell proliferation, phagocytic and cytotoxic activity of lymphocytes, and inhibit viral replication. Interferon alfa-2b is currently approved for IV, SC or IM administration in the treatment of hepatitis B and C, NANB/C hepatitis, hairy cell leukemia, AIDS-related Kaposi's sarcoma, and external condylomata acuminata.

Ribavirin is a purine nucleoside analogue which has shown in vitro inhibitory activity against multiple DNA and RNA viruses. A proposed mechanism for the antiviral activity of ribavirin is through changes in the metabolism of guanosine and/or xanthosine. Ribavirin is currently approved only for the treatment of severe lower respiratory tract infections due to respiratory syncytial virus (RSV) in children. RSV is a member of the Paramyxovirus family, along with measles, mumps, para-influenza, and Newcastle disease viruses. All of the viruses in this family are RNA viruses, as is the causative agent in hepatitis C.

Ribavirin is approved only for aerosol administration by face mask, hood, tent or mechanical ventilator in the treatment of severe lower respiratory tract infections due to RSV in pediatric patients. There is no oral or intravenous formulation of ribavirin which is approved for use in the U.S. at this time.

NON-CLINICAL TOXICOLOGY

The following sections contain summaries of the major toxicology and pharmacokinetic data submitted as part of the NDA. The individual study reviews the proposed package insert for the combination drug preparation are included in appendices A-E of this document.

Ribavirin: Summary of General Toxicology Findings

Pre-clinical toxicology data indicate that ribavirin induces a significant degree of anemia (due to direct hemolytic effects and suppression of the bone marrow), reticulocytosis, and lymphoid atrophy following high dose, acute administration or low dose, repeat administration. The anemia is generally reversed within a few weeks following the cessation of ribavirin administration. Study data suggest that accumulation of ribavirin occurs in body tissues during repeat dosing procedures, but that it generally stabilizes 1-3 weeks following the start of dosing (although more gradual accumulation may continue up to 6 months of dosing).

The results of multiple studies suggest that the administration of ribavirin may be associated with reductions in serum protein, albumin and ALT levels among dogs dosed at 20 mg/kg/day and among rats dosed at 160 mg/kg/day (smaller effects sometimes evident among animals dosed at lower levels; estimated human equivalent doses of 10 and 23 mg/kg/day [based on body surface area conversion]). Histologic changes evident in either the liver or kidneys were not consistent with these changes. The pattern of the data suggests that the changes in serum protein, albumin and ALT levels may have resulted from a ribavirin induced inhibition of the synthetic capacity of the liver. Further, the results of these studies (rat and dog) suggest that ribavirin, when given at relatively high doses, has significant adverse effects on rapidly proliferating tissues (lymphoid, mucosa and spleen) and/or those tissues with high cellular metabolism (heart, liver and secretory cells of the intestinal mucosa).

Ribavirin: Summary of Carcinogenicity Study Findings

The in vivo carcinogenicity studies performed with ribavirin were inadequately designed (drug doses too low), were not conducted in accordance with the study protocols or were incomplete, and were inadequately reported/documented. Thus, the results of the 2 oral gavage oncogenicity studies in the mouse and rat (18-24 months; doses of 20-75 and 10-40 mg/kg/day, respectively [estimated human equivalent doses of 1.67-6.25 and 1.43-5.71 mg/kg/day, based on body surface area adjustment for the adult]; approximately 1.3x and 0.2x the human systemic exposure to ribavirin [based on 24 hour AUC] at the maximum recommended human dose of 1.2 grams per day) are inconclusive as to the carcinogenic potential of ribavirin. However, the studies suggested that chronic ribavirin exposure might be related to an increased incidence of vascular lesions (microscopic hemorrhages in mice) and retinal degeneration (in rats). Furthermore, results of a chronic feeding study with ribavirin in rats, at doses of 16-100 mg/kg/day (estimated human equivalent of 2.3-14.3 mg/kg/day, based on body surface area adjustment), suggest that ribavirin may induce benign mammary, pancreatic, pituitary and adrenal tumors.

Ribavirin: Summary of Reproductive Toxicity Study Findings

Ribavirin has demonstrated significant teratogenic and/or embryocidal potential in all animal species in which adequate studies have been conducted. Teratogenic effects have been seen after daily oral doses of 0.3 and 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster². Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. The incidence and severity of the teratogenic effects generally increased with escalation (increases) of the drug dose. Viability of the fetuses and offspring is typically reduced.

The results of the segment I study in the CD-1 mouse, suggest that ribavirin may produce significant dose and time dependent toxic responses in the testes, including decreases in spermatid concentration, increases in abnormal sperm morphology, and germinal epithelia necrosis. However, fertility studies conducted in male and female SD rats revealed no significant effects of ribavirin on reproductive behaviors or any indices of fertility when the drug was administered for 2-12 weeks prior to mating (females and males; high doses of 10 and 160 mg/kg/day). In a peri- and post-natal exposure study, ribavirin administration at doses up to 1.0 mg/kg/day was without significant adverse effects on pregnant SD rats or their offspring when exposure began after the period of organogenesis and continued through weaning.

² Open literature reports.

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

Interferon alfa-2b: Summary of General Toxicology Findings As stated previously, interferon alfa-2b is currently approved for IV, SC or IM administration in doses ranging from 2-30 million IU 3x/week, for the treatment of hepatitis B and C, NANB/C hepatitis, hairy cell leukemia, AIDS-related Kaposi's sarcoma and, external condylomata acuminata. The adverse events most commonly associated with interferon use include; fever and flu-like symptoms (headache, nausea, myalgia, fatigue, anorexia and vomiting), granulocytopenia, cardiovascular effects (hypotension, arrhythmia and tachycardia), central nervous system effects (depression, confusion and other changes in mental status), pulmonary infiltrates and, hepatotoxicity (indicated by elevations in serum ALT and/or AST, bilirubin, LDH activity, alkaline phosphatase and prothrombin time, and by decreases in serum albumin and protein). The incidence and severity of the adverse effects appear to increase with the administered dose of interferon alfa-2b and increased duration of therapy.

In preclinical toxicology studies in golden Syrian hamsters and rhesus monkeys, administration of various of the interferons was associated with decreased body weight, decreased food consumption and bone marrow suppression. High-dose chronic exposure (up to 90 fold higher than the maximum recommended clinical dose given daily) in rhesus monkeys was not tolerated for greater than 1 month due to the development of vascular leak syndrome.

Interferon alfa-2b: Summary of Reproductive Toxicology Findings When administered to pregnant female rhesus monkeys, interferon alfa-2b had abortifacient effects at all doses tested (7.5-30 million IU/kg). Furthermore, in non-human primates, interferon administration resulted in alterations in the female menstrual cycle, changes in reproductive behaviors and reductions in serum sex steroid levels. Studies in pregnant rhesus monkeys and golden Syrian hamsters, demonstrated an increase in fetal loss in hamsters treated with Infergen (a genetically engineered consensus interferon) at doses of greater than 150 µg/kg/day, and in rhesus monkeys at doses of 3 and 10 µg/kg/day. The Infergen toxicity profile described is consistent with the known toxicity profile of other alfa interferons.

There have been no controlled clinical trials in pregnant women or in fertile men to determine the potential effects of interferon administration on human reproduction. It should however, be assumed that interferon may have adverse reproductive effects in the human.

NON-CLINICAL PHARMACOKINETICS AND ADME STUDIES

Ribavirin: The results of multiple pharmacokinetic and ADME studies conducted in the mouse, rat and dog, suggest that ribavirin when given orally either as a solution or in capsule form is well absorbed with an approximate bioavailability of 80% whereas bioavailability in humans has been estimated. The data for the 3 animal species, suggest that ribavirin reached maximal levels in the plasma or serum within 1-2 hours of dosing, and decayed with an initial half-life of between 4-10 hours. Drug levels were comparable for male and female animals, and for drug naive or previously drug treated animals (although slight changes in AUC values were evident in rats and dogs following repeat administration). After acute doses, C_{max} and AUC_{24} values increased in a nearly linear manner with increases in dose, although a slight reduction in systemic exposure was evident in the rat and dog at the highest

doses tested. The data suggest that absorption of ribavirin from the gastrointestinal tract may be reduced at high doses, possibly due to saturation of a carrier transport.

The sponsor has recently submitted the results of two toxicokinetics studies conducted in the mouse and rat. These studies were conducted in the same animal strains and at the same drug doses as were used in the oncogenicity studies of ribavirin. More importantly, however, was the fact that a newly developed more sensitive and more specific drug assay was used than in the previous studies (the new assay being specific to the parent drug structure versus the two primary metabolites [i.e., the deribosylated and triazole carboxamide metabolites]). The results of these studies suggest that in the previously reported oncogenicity studies of ribavirin, relative interspecies 24 hour systemic drug exposure levels (at the maximum doses tested in animals versus the recommended 1200 mg clinical dose) were approximately 130% and 20% in the mouse and rat, respectively.

Tissue levels of radioactivity were nearly identical for male and female animals of each species, and were generally much higher than levels noted in the plasma and/or serum. Tissue levels of radioactivity were highest in the gastrointestinal tract, liver and kidneys (apparently related to the organs of absorption, metabolism and excretion of ribavirin). However, an exception to this was evident in the reproductive tissues of both male and female animals, which showed particularly high levels of drug following acute or repeat dose administration (the highest levels of radioactivity detected [per gram of tissue] were in the prostate of the dog). The lowest levels of radioactivity were generally detected in brain tissue.

The primary route of drug elimination was in the urine, with 50-100% of the administered radioactivity being eliminated within 24-48 hours of dosing. Approximately 5-20% of the administered radioactivity was recovered in the feces, and 10% was retained in the carcass at 24 hours after dosing (depending on the species). The entrance of ribavirin into red blood cells was somewhat delayed versus distribution of radioactivity in the plasma, suggesting that the red blood cell membrane may be semi-permeable to the passage of ribavirin and that red cells may serve as a drug reservoir (with delayed release) following drug withdrawal.

Comment: As discussed in the toxicology section of this review, ribavirin has significant adverse effects on rapidly proliferating tissues (lymphoid tissues, mucosa, spleen and testes) and those tissues with high cellular metabolism (heart, liver and secretory cells of the intestinal mucosa). The results of the pharmacokinetic and ADME studies of ribavirin, suggest that the affected tissues are also the primary sites of drug deposition after oral dosing.

Interferon alfa-2b: Pharmacokinetic studies of various of the interferons have been conducted in golden Syrian hamsters, rhesus monkeys and other species. These studies demonstrated rapid absorption following SC injection. Peak serum concentrations were observed at 1 and 4 hours following administration in golden Syrian hamsters and rhesus monkeys respectively. Subcutaneous bioavailability was high in both species, averaging 99% in golden Syrian hamsters and 83-104% in rhesus monkeys. Clearance of drug was approximately 2.0 mL/minute/kg in golden Syrian hamsters and 0.7-0.9 mL/minute/kg in rhesus monkeys. Plasma/serum drug clearance was due predominantly to catabolism and excretion by the kidneys. The terminal half-life of the interferons following SC dosing was approximately 1.5 hours in golden Syrian hamsters and 3.5 hours in rhesus monkeys. Upon 7-day multiple SC dosing, no accumulation of serum levels was observed in golden Syrian hamsters. All interferons have been shown to be highly species specific.

SUMMARY

The sponsor is requesting approval to market ribavirin capsules (REBETOL®; 1-1.2 g/day; PO) in combination with interferon alpha-2b (INTRON A®; 3 MIU TIW; SC Inj.) for the treatment of chronic active Hepatitis type C infection in patients who have relapsed following a previous course of interferon monotherapy. The proposed duration of combination drug administration in the treatment of Hepatitis type C infection is 6 months.

Interferon alpha (INTRON A®, and related interferons: ROFERON®, WELLFERON®, INFERGEN®) has been previously approved for use at the proposed dose and route of administration for the treatment of Hepatitis type C infection. Since no change in the indication or use of interferon alpha has been proposed in this NDA, the reader is referred to the PLA review (No. 994) for a comprehensive review of the toxicology of INTRON A® brand of interferon alpha-2b. In contrast, ribavirin is currently only approved as an aerosol product (VIRAZOLE®; NDA 18-859) for use in the treatment of Respiratory Syncytial Virus (RSV) infection in the pediatric population. No oral ribavirin containing product is currently available for use in the US. Therefore, this NDA review has focused on the repeat-dose toxicology, reproductive toxicology, genotoxicity and carcinogenicity, pharmacokinetic and ADME studies related to the oral administration of ribavirin (REBETOL®).

The sponsor has submitted results of multiple general toxicology, reproductive toxicology and pharmacokinetic studies in support of this NDA. The results of these studies clearly indicate that when ribavirin is administered at fairly high doses it has significant adverse effects on many rapidly proliferating tissues (lymphoid tissues, mucosa, spleen and testes) and/or those tissues with high cellular metabolism (heart, liver and secretory cells of the intestinal mucosa). Of special concern is an apparent inhibition of the protein synthetic and/or metabolic capacity of the liver, as demonstrated by reductions in multiple serum proteins, albumin and globulins, and elongations of PT and APTT times. Reductions in serum ALT levels evident in several species of non-hepatitis infected animals used in the toxicology studies, and as reported among the Hepatitis type C infected patients treated with ribavirin, may have been due to a toxic inhibition of liver function with resultant decreases in ALT synthesis and release. Results of the pharmacokinetic/ADME studies suggest that the effected tissues are also the primary sites of drug deposition following the oral administration of ribavirin.

Ribavirin demonstrated significant teratogenic and/or embryocidal potential in all animal species in which adequate reproductive toxicology studies were conducted. Teratogenic effects were seen after daily oral doses of 0.3 and 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster³. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. The incidence and severity of the teratogenic effects generally increased with escalation (increases) of the drug dose. Viability of the fetuses and offspring was typically reduced.

It appears likely that these potentially serious adverse effects of ribavirin have only now become evident, because of the poor study conduct (incomplete gross and/or microscopic pathology, and clinical chemistry/hematology) and the inappropriately low test doses (selected by the drug sponsor) used in previous toxicity studies of ribavirin. The newly submitted toxicology, pharmacokinetic and ADME studies of ribavirin, are in direct response to multiple previous recommendations and requirements made by agency personnel for the provision of additional safety and toxicity information for this drug product.

Based on the available toxicology study results, it is recommended that the Product Label (and Patient Information Sheet) for the combination drug product (i.e., INTRON A®/ REBETOL®), clearly reflect the potentially serious nature of the adverse reproductive and hepatotoxic effects observed in multiple animal species.

CONCLUSIONS

Based on the available animal toxicology and pharmacokinetic data for orally administered ribavirin, it appears relatively safe to approve the combination drug product (interferon alfa-2b [INTRON A] and ribavirin [REBETOL]) for the treatment of chronic active hepatitis due to infection with Hepadnaviridae Type C virus.

RECOMMENDATIONS

1) Based on the availability of a new and more specific assay procedure, which is capable of differentiating between the parent drug molecule and its two primary metabolites,

2) The in vivo carcinogenicity studies and in vitro/in vivo mutagenicity studies performed with ribavirin were reviewed by the CDER Executive CAC (Center for Drug Evaluation and Research - Carcinogenicity Assessment Committee) in a session on 28 April 1998. It was the conclusion of the committee based on the positive genotoxic effects seen with ribavirin in multiple assay systems, the lack of adequate in vivo rodent carcinogenicity data, and the extended period of human drug exposure (treatment regimen of 6 months duration), that: a) the product label for ribavirin should indicate that it may be a potential carcinogen (see the Carcinogenesis and Mutagenesis section of the proposed product label as contained in Appendix E of this document), and b) the sponsor, as part of a Phase 4 Post-Marketing Agreement, should be required to

(See Appendix F of this document for a prioritization of the Sponsor's Phase 4 Commitment).

3) Pre-clinical toxicology data indicate that ribavirin induces a significant degree of anemia (due to direct hemolytic effects and by mild to moderate suppression of the bone marrow), reticulocytosis, and lymphoid tissue atrophy following high dose acute administration or, low dose repeat administration. The anemia is generally reversed within a few weeks following the cessation of ribavirin administration. Similarly, the administration of interferon has been associated with anemia, due to suppression of the bone marrow (an effect which has been seen in multiple animal species and man).

Since both study medications are known to induce hematologic abnormalities with anemia (both by the same and complimentary modes of action), the potential for a synergistic toxicity (i.e., anemia or other blood dyscrasia with earlier onset or greater magnitude of effect) should be clearly identified in the product label. It is recommended that the product label clearly state the need for early and regular monitoring for an increased degree of anemia and/or the onset of delayed anemia.

4) The results of multiple studies suggest that the administration of ribavirin may be associated with reductions in serum protein, albumin and ALT

levels among dogs dosed at 20 mg/kg/day and among rats dosed at 160 mg/kg/day (smaller effects were sometimes evident among animals dosed at lower doses; estimated human equivalent doses of 10 and 23 mg/kg/day [based on body surface area conversion]). Histologic changes evident in either the liver or kidneys were not consistent with these changes. The pattern of the data suggested that the changes in serum protein, albumin and ALT levels may have resulted from a ribavirin induced inhibition of the synthetic capacity of the liver.

Hepatotoxicity and hepatic failure has been noted in patients being treated with interferon. The incidence and severity of the adverse effects appears related to increases in the administered dose and duration of treatment. Hepatotoxicity due to interferon alfa-2b is typically indicated by elevations in serum ALT and/or AST, bilirubin, LDH activity, alkaline phosphatase and prothrombin time, and by decreases in serum albumin and protein concentrations.

It is recommended that the product label clearly state the need for regular monitoring of hepatic functioning, but that the measurement of ALT and AST (as the most common LFT'S) may not accurately reflect liver functioning or injury since the concurrent use of ribavirin may artificially suppress these values.

5) Ribavirin has demonstrated significant teratogenic and/or embryocidal potential in all animal species in which adequate studies have been conducted. Teratogenic effects have been seen after daily oral doses of 0.3 and 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster⁴. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. The incidence and severity of the teratogenic effects generally increased with escalation (increases) of the drug dose. Viability of the fetuses and offspring is typically reduced. In addition, interferon demonstrated significant dose-related abortifacient effects when administered to pregnant rhesus macaques.

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

As noted in the Pharmacokinetics section of this review, ribavirin is concentrated in erythrocytes and other tissues, and undergoes extended elimination. Therefore, the minimum interval following exposure to ribavirin before pregnancy may be safely initiated in the human is unknown, although an interval of 3-5x the terminal elimination half-time should be considered (i.e., 3-5x the erythrocyte $t_{1/2}$ of 40 days = 120-200 days or approximately 6 months).

It is recommended that the product label clearly state the need for determining pregnancy status prior to the initiation of drug treatment, in addition to regular monitoring for pregnancy during treatment and for 6 months following treatment. The product label should clearly state the need for the use of effective contraception methods by both female and male patients being treated interferon and ribavirin.

6) The results of 2 multi-year studies of ribavirin conducted in mice and rats suggest that chronic drug exposure might be related to an increased incidence of vascular lesions (microscopic hemorrhages in mice) and retinal degeneration (in rats). It is recommended that this adverse animal finding be included in the combination product label, along with a recommendation for the regular monitoring of patients for neurologic status and retinal changes.

⁴ Open literature reports.

David E. Morse, Ph.D.
Reviewing Pharmacologist

Concurrences:

HFD-530/Dir./HJolson
HFD-530/ADDir./WDempsey *7/15/98*
HFD-530/TLPharm/JGFarrelly *7/12/98*
HFD-530/Pharm/DMorse

HFD-530/Disk Copy

cc:

HFD-530/NDA 20-903
HFD-530/Division File
HFD-340/
HFD-530/CSO/TCrescenzi
HFD-530/Pharm/DMorse
HFD-530/MO/RFleischer, TNguyen
HFD-530/Chem/
HFD-530/Micro/

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Appendix A: Acute - Chronic Toxicity Studies**Summary of General Toxicology Findings:**

Pre-clinical animal toxicology data and previous human experience indicate that ribavirin induces a significant degree of anemia (due to a direct hemolytic effect and suppression of the bone marrow), reticulocytosis, and lymphoid atrophy following high dose, acute administration or low dose, repeat administration. The anemia and lymphoid effects are generally reversed within weeks following the cessation of ribavirin administration. Study data suggest that accumulation of ribavirin occurs during repeat dosing procedures, but that it generally stabilizes 1-3 weeks following the start of dosing.

As noted in the sections which follow, the administration of ribavirin was associated with slight reductions in serum protein, albumin and ALT levels, among dogs dosed at 20 mg/kg/day and among rats dosed at 160 mg/kg/day (smaller effects were sometimes evident among animals dosed at lower levels; estimated human equivalent doses of 10 and 23 mg/kg/day [based on body surface area conversion]). The results of these studies, in conjunction with other submitted materials, suggest that when ribavirin is administered at fairly high doses it has significant adverse effects on many rapidly proliferating tissues (lymphoid tissues, mucosa, spleen and testes) and/or those tissues with high cellular metabolism (heart and liver). Of concern is a potential inhibition of the protein synthetic and/or metabolic capacity of the liver, as demonstrated by changes in multiple serum proteins, albumin and globulins, and elongations of PT and APTT times. Histologic abnormalities evident in either the liver or kidneys were not consistent with these changes. The data suggest that the changes in serum protein, albumin and ALT might be the result of decreased synthesis. Thus, while serum protein, albumin and transaminase levels generally remained near-normal throughout treatment, the observed decreases in serum protein, albumin and ALT levels may have resulted from a ribavirin induced inhibition of liver synthetic capacity.

Reductions in serum ALT levels evident in the rat and other species of non-hepatitis infected animals used in the toxicology studies, and as reported among Hepatitis "C" infected patients treated with ribavirin, may be due to a toxic inhibition of liver function with resultant decreases in ALT synthesis and release. Results of ADME studies in multiple species suggest that the affected tissues are also the primary sites of drug deposition following oral dosing with ribavirin. Based on these new results, it is recommended that the product label for the approved formulation of ribavirin reflect the potentially serious nature of the toxic effects observed in the liver of the rat and dog.

It appears likely that these potentially serious adverse effects of ribavirin have only now become evident, because of the poor study conduct (incomplete gross and/or microscopic pathology, and clinical chemistry/hematology) and the inappropriately low test doses used in previous toxicity studies of ribavirin. The newly submitted toxicology, pharmacokinetic and ADME studies of ribavirin, are in direct response to multiple previous recommendations and requirements made by agency personnel for the provision of additional safety and toxicity information for this drug product.

Toxicity Studies Summary:

MOUSE

- 1) A 90-Day Oral Gavage Range-Finding Study In Mice With Ribavirin, GLP,

RAT

- 2) Ribavirin - 30 Day Dietary Toxicity Study In Rats, Study No. 451199, GLP,
Initiation: 21 Jan. 1993, Ribavirin Lot# 05500787 (R-17),
3) A 90-Day Oral Gavage Range-Finding Study In Rats With Ribavirin, GLP,
4) Ribavirin: 52 Week Dietary Toxicity Study In Rats With 26 Week
Interim Kill, Study No. 451204, GLP,
5 May 1993, Ribavirin Lot# BR-17,

DOG

- 5) Ribavirin - 30 Day Pilot Oral (Gavage) Toxicity Study In The Beagle
Dog, Study No. 895/001, GLP, Study
Initiation: 15 Jan. 1993, Ribavirin Lot# 05500787 (R-17),
6) Ribavirin: 52 Week Oral (Gavage) Toxicity Study In The Beagle Dog
With An Interim Necropsy After 26 Weeks, Study No. 895/002, GLP,
23 March 1993,
Ribavirin Lot# BR-17,

Toxicity Study Reviews:

MOUSE

- 1) A 90-day oral gavage range-finding study in mice with ribavirin (GLP study conducted

This was a dose-finding study for a subsequent mouse carcinogenicity study. Six groups of CD-1 mice (10/s/g) were dosed daily with vehicle, 35, 75, 150, 300 or 600 mg/kg ribavirin by gastric intubation for 3 months. Physical observations, body weights and food consumption were measured in all animals pretest and throughout the study. Hematology was performed on all animals surviving to the end of the study. Blood samples were also taken for pharmacokinetic data; these data were not included in this study. All animals were necropsied; histopathology was confined to examination of testes and epididymides of all males except the 35 mg/kg group.

Premature mortality approached 50% at 300 mg/kg (5/10 males, 4/10 females) and 100% at 600 mg/kg (all but one female). Most of the deaths occurred late in the study (weeks 12-13). Specific pathologic changes leading to death were not identified, but the mortality was considered by the sponsor to be related to ribavirin administration. Based on hematologic changes observed in survivors from the 300 mg/kg group (see below), a tentative diagnosis of severe anemia is plausible. An additional 35 mg/kg female was found dead during week 7. Cause of death was not immediately apparent, but the death was not considered by the sponsor to be drug-related.

General behavioral observations included scabbing, particularly of the ear region, and staining of fur at doses of 150-600 mg/kg. In the 600 mg/kg group, body weight gains were decreased by 9-12% beginning at week 4 and continuing to week 12, when the decrement reached up to 34%. Animals in the

300 mg/kg group had sporadic body weight deficits of similar magnitude during weeks 8-12. There no body weight changes at or below 150 mg/kg. Food consumption (g/kg body weight) was generally maintained at or above control levels in all groups.

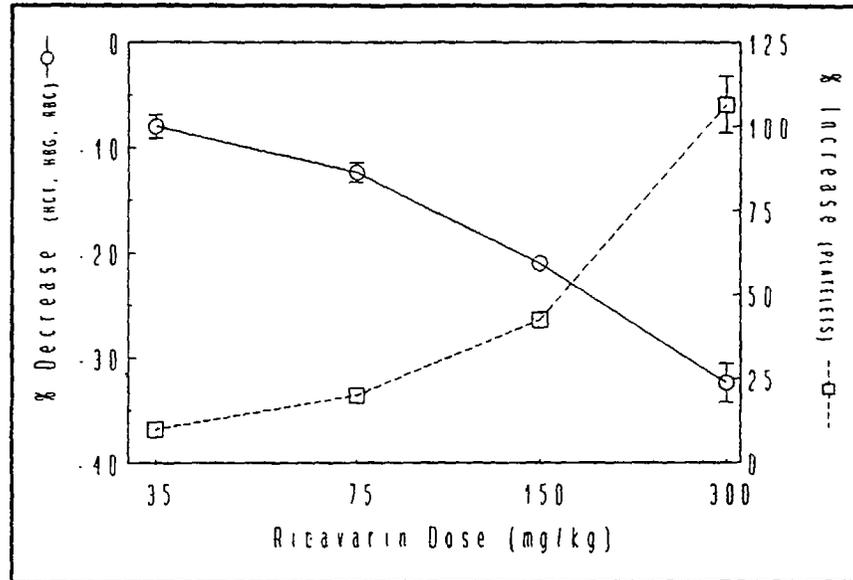


Figure 2 - Mouse Hematology Data

Anemia and thrombocytosis were observed at all ribavirin doses. There were dose-related decreases in hemoglobin, hematocrit and red cell counts and dose-related platelet increases which reached statistical significance in all but the 35 mg/kg group. Only one 600 mg/kg female was available for hematology; this animal was not included in the statistical analysis for obvious reasons. Mice treated with 300 mg/kg also had a 40% increase in reticulo-cyte counts and 10% increases in mean cell volume and hemoglobin. Differential counts appeared to be unaffected at 300 mg/kg, but these animals did have increases in nucleated red blood cells and poikilocytes (differential counts and morphology were performed only in the control and 300 mg/kg groups).

Spleen weights were increased 2 to 4-fold in the 300 mg/kg group and by 50-75% at 150 mg/kg ribavirin. Gross splenic enlargement was noted in 6 animals in the 300 mg/kg group. Testicular weights were decreased by 30 and 16% in males treated with 300 and 150 mg/kg, respectively. Histopathology revealed evidence of bilateral degeneration of the testicular germinal epithelium with epididymal oligospermia in the majority of males dosed with 150 mg/kg or more (vehicle 2/10, 75 mg/kg - 0/10, 150 mg/kg - 7/10, 300 mg/kg - 8/10, 600 mg/kg - 9/10 animals).

The lowest dose group (35 mg/kg) appeared to be near the no drug effect threshold. There was a trend in this group toward the same hematologic changes observed at the higher doses - loss of erythrocytes and increases in platelets. Testicular changes were observed beginning at 150 mg/kg. This group was probably close to the maximum tolerated dose, as higher doses produced severe anemia and unacceptable mortality.

RAT

2) Ribavirin - 30 Day Dietary Toxicity Study In Rats, Study No. 451199.

Status: GLP

Study Site:

Study Initiation: 21 Jan. 1993

Compound Tested: Ribavirin Lot# 05500787 (R-17),

Doses Tested: 0, 10, 40, 160 and 320 mg/kg/day

Dose Volume and Route: dietary admixture, oral

Solvent: ground rodent diet (Spec. Diet Services, Ltd.)

Species, Strain, Sex: male & female SD rats, age 6 weeks; weight range: male = 120-145 grams; female = 83-103 grams, 10 animals/sex/dose.

Test conditions: Animals were randomly assigned to treatments. Ribavirin was available continuously as a dietary admixture for a period of 30 days. Physical signs, mortality, body weight and food consumption were monitored regularly throughout the study. Hematologic analyses and clinical chemistries were performed after 31 days of drug administration. Ophthalmologic examinations were performed at baseline, and on treatment days 15 and 26. Gross and microscopic examinations of all standard tissues were performed at the termination of dosing.

Mortality: All animals from the high dose group (320 mg/kg/day) were sacrificed in extremis on day 10 of drug administration, with clinical signs of extreme weight loss, inappetence, pilo-erection and subdued behavior. An additional 3 males and 4 females from the high-intermediate dose (160 mg/kg/day) group died or were euthanized prior to the conclusion of the study. At necropsy, the most common abnormalities noted in these animals included a flaccid appearing heart, reddening and/or mottling of the lungs, fluid accumulation in the thoracic cavity, and enlargement of multiple lymph nodes.

There were no premature deaths among animals in the control, low or low-intermediate dose groups.

Clinical Signs: Other than signs noted among the moribund animals, surviving animals from the high-intermediate dose group displayed multiple signs including, ataxia, abnormal respiration, hunched posture, hypothermia and piloerection. There were no obviously drug related clinical signs noted among animals in the 2 lowest dose groups.

Body Weight and Food Consumption: Ribavirin caused a dose related reduction (group mean reductions in weight gain of 20-140% of the concurrent control) in body weight gain, or a loss of body weight, during the first week of dosing. Changes in weight gain were evident in both male and female test animals. Weight gain among all animals from the intermediate dose groups (i.e., 40 and 160 mg/kg/day) remained suppressed throughout the drug dosing interval, such that at termination, absolute body weight of the affected animals was moderately to markedly reduced (reduction in weight gain of 20-70%). Following the initial week of drug administration, weight gain among animals from the low dose group was comparable to the controls.

Among the drug treated animals, food consumption was reduced in a dose related manner which closely paralleled the changes noted in body weight gain. Food consumption was reduced throughout the dosing interval by an average of 30-50% among the animals treated at 160 mg/kg/day (versus the concurrent controls). Among the study animals dosed at 10 or 40 mg ribavirin/kg/day, average reductions in food intake were approximately 10-20% of control.

Dietary Drug Intake: The dietary intake of ribavirin for all dose groups was generally within 20% of the assigned dose (computation of drug intake and adjustment of drug-diet concentration were performed weekly). Drug

concentration was measured in specimens taken at the time of necropsy (time after lights-out was not specified), results indicating that plasma levels of ribavirin generally increased in a less than linear manner with the nominal dose.

Dose (mg/kg)	Plasma Drug Levels (μ Molar) at Necropsy				
	0	10	40	160	320
Sex					
♂	BLD	1.8	11.4	79.4	108.7
	N= (3)	(10)	(10)	(8)	(9)
♀	BLD	2.4	26.3	73.2	118.1
	N= (3)	(10)	(10)	(6)	(10)

BLD = Below Limit of Detection

Ophthalmoscopy: There were no apparent drug related effects.

Hematology and Clinical Chemistry: Among drug treated males, significant reductions (40% versus control) in mean hemoglobin concentration and hematocrit were evident at the conclusion of dosing. Reductions appeared dose related and were accompanied by a decrease in RBC counts (40%) in animals dose at 160 mg/kg/day, while animals dosed at 10 and 40 mg/kg/day displayed slight increases in MCH and MCV (5-10%). Similar reductions (40-50%) in hemoglobin, hematocrit and RBC counts were seen in surviving females from the 40 and 160 mg/kg/day treatment groups. However, in contrast to the effects seen in males, MCH and MCV were significantly reduced (5-10%) among female animals from the 10 and 40 mg/kg/day treatment groups.

Total white cell, lymphocyte and eosinophil counts were reduced (near 50%) among all surviving high-intermediate dose treated animals at the time of necropsy. The bone marrow appeared normal among all drug treated animals except 4 (2 male and 2 female) from the 160 mg/kg/day group, which showed moderately reduced cellularity of the marrow.

Changes in serum chemistry noted at the conclusion of dosing, included: a) decreases (10-25%) in serum protein, albumin, creatinine and calcium in females dosed at 160 mg/kg/day (high-intermediate dose), b) a reduction in serum ALT levels among males dosed at 160 mg/kg/day (approx. 50% versus the controls), and c) increases in serum AST levels among females dosed at 160 mg/kg/day (approx. 45% versus the controls). Prothrombin time was slightly increased (approx. 25% [mean of 4 sec.]) among the high-intermediate dose treated female animals. There were no other changes in any hematologic or serum chemistry parameter noted.

Gross and Microscopic Pathology: At necropsy, increases in the absolute and/or relative weight of the heart and lungs was seen in both male and female animals treated with ribavirin at 160 mg/kg/day. This effect was particularly pronounced in 5 males and 4 females treated at 160 mg/kg/day, but was also noted in 1 male and 3 female animals which had received ribavirin for 10 days at 320 mg/kg/day. Besides the increase in organ weight, the heart muscle of the affected animals appeared flaccid and dilated on gross examination. Microscopic examination of the tissues showed significant evidence of cardiomyopathy in 7 males and 6 females from the 160 mg/kg/day treatment group, and in all but 1 male (i. e., 9 males and 10 females) from the 320 mg ribavirin/kg/day group. More frequent and severe alveolitis was noted in these same animals than was evident in animals from any of the other treatment groups. Fluid was evident in the thoracic cavity of 3 male and 6 female animals treated at 160 mg ribavirin/kg/day.

Decreases in both absolute and relative weight of the thymus were noted in

male and female animals from the 160 mg/kg/day group, while spleen weight was increased. Spleen weight was also increased in male animals dosed at 10 and 40 mg/kg/day, the changes generally appearing dose related. Microscopic examination of multiple lymphoid tissues revealed generalized atrophy (thymus and lymph nodes) with loss of germinal centers, macrophage and other inflammatory cell infiltrates, and congestion. Extramedullary hemopoiesis was decreased or absent in the spleen of multiple animals dosed at 40 mg/kg/day and above.

Multifocal necrosis was seen in all segments of the intestines of animals from the two high dose treatment groups (i.e., 160 and 320 mg/kg/day). Necrosis was occasionally accompanied by mucosal atrophy and goblet cell hyperplasia in some animals (the effect being dose related). Mucosal cysts were evident in sections of the colon of 1 and 5 animals from the 160 and 320 mg/kg/day dose groups, respectively.

Microscopic examination of multiple other tissues showed evidence of drug related injuries, including: a) centrilobular necrosis of the liver (7 females dosed at 160 mg/kg, and 3 females and 1 male dosed at 320 mg/kg [for 10 days]), b) periportal fibrosis of the liver (4 males dosed at 160 mg/kg), c) atrophy of the salivary glands (4 males and 5 females dosed at 160 mg/kg, and 7 males and 9 females dosed at 320 mg/kg), d) decreased secretion of the seminal vesicals (2 and 4 males dosed at 160 and 320 mg/kg), and e) testicular tubular epithelial atrophy (1 and 5 males treated at 160 and 320 mg/kg).

All remaining gross and/or microscopic lesions appeared to be randomly distributed among the treatment and control groups.

- Comments:
- 1) Anemia and lymphopenia have been observed in nearly all animal species (including man) following acute or repeat dosing with ribavirin. These effects typically become apparent within 1-2 weeks of ribavirin administration. The degree of anemia and lymphopenia appears to be both dose and time dependent.
 - 2) The results of the present study suggest that ribavirin when given at relatively high doses (> 40 mg/kg/day) has significant adverse effects on rapidly proliferating tissues (lymphoid tissues, the gastro-intestinal mucosa, spleen, bone marrow, testes, seminal vesicals, and the salivary glands) and/or those tissues with the highest rate of cellular metabolism (heart, liver and secretory cells of the gastro-intestinal mucosa).

Significant dose related cardiomyopathy and periportal hepatic fibrosis (males) and/or necrosis (females) was evident in animals dosed at 160 mg/kg/day (estimated human equivalent dose of 22.8 mg/kg/day, based on body surface area dose adjustment), or above. These organs are likely to receive the highest levels of drug exposure following oral administration, due to the receipt of periportal and central venous blood flow.

Cardiac and hepatic injuries have not been noted in the previously submitted GLP toxicology studies. Therefore, it is recommended that all oral repeat dose clinical trials with ribavirin include follow-up evaluations of cardiac and hepatic function.

- 3) As noted above and in comments to the preceding study, the administration of ribavirin was associated with reductions in serum proteins and albumin and an increased prothrombin time among the female animals dosed at 160 mg/kg/day. ALT levels were reduced and AST levels increased (approx. 50% each) among the male and female animals, respectively. As in the dog study, no

abnormalities were noted that might suggest a mechanism of increased protein/albumin loss, therefore suggesting that the changes in serum protein/albumin might be the result of decreased synthesis in the liver. Additional markers of hepatic function including serum CPK levels and mitochondrial citrate synthase or cytochrome oxidase were not measured.

It should be remembered that the proposed mechanisms of action for ribavirin are through incorporation into, or inhibition of the synthesis of RNA, with a resultant failure in the RNA message. Incorporation of ribavirin in the cellular genome has not been reported. Thus, if the DNA remains intact, and messenger RNA turnover is high, the removal of ribavirin from the system should result in recovery of impaired functions (assuming that cellular function has not been so compromised as to preclude the minimal level of synthetic activity which is necessary for cellular survival).

4) The results of this study, suggest that the maximal survivable dose for a 12 month toxicity study in the SD rat likely falls between 40 and 160 mg/kg/day (when administered as a dietary admixture).

3) A 90-day oral gavage range-finding study in rats with ribavirin (GLP study conducted

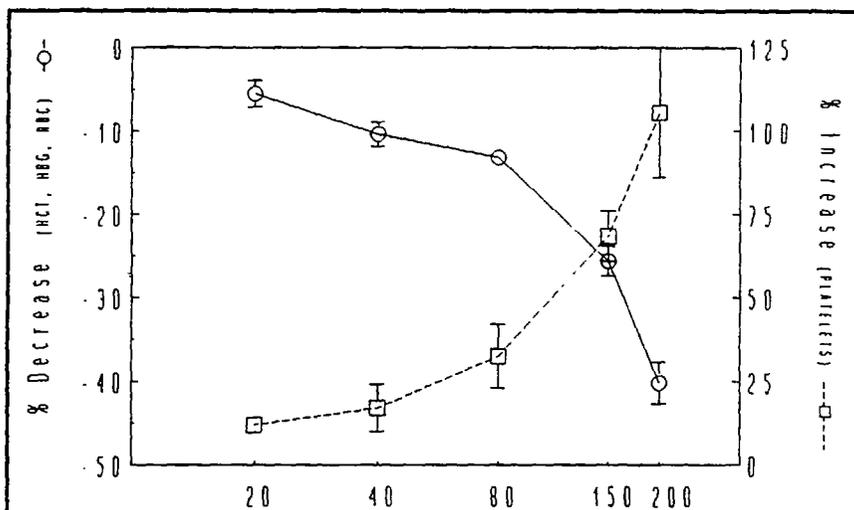
This was a dose-finding study for a subsequent rat carcinogenicity study. Six groups of Sprague-Dawley CD rats (10 animals /sex/group) were dosed daily with vehicle, 20, 40, 80, 150 or 200 mg/kg ribavirin by gastric intubation for 3 months. Physical observations, ophthalmoscopic examinations, body weights and food consumption were measured in all animals pretest and throughout the study. Hematology and clinical chemistry were performed on 5 animals/s/g at the beginning and end of the study. All animals were necropsied; histopathology was confined to examination of testes and epididymides in control and 200 mg/kg males.

There were 5 premature deaths: 1-150 mg/kg female was sacrificed moribund on day 16, 1-200 mg/kg female was found dead on day 78, 1-200 mg/kg male was sacrificed moribund on day 86 and 2 others were found dead; 1 each on days 32 and 83. Autopsy results were unrevealing as to cause of death and there were no other data for these animals (clinical chemistry, etc.) to aid in diagnoses.

Surviving 150 and 200 mg/kg animals displayed alopecia, scabs and sores, mostly of the mouth and dorsal cervical region. The frequency of these observations was increased with duration of treatment, so that these symptoms were present in the majority of animals in these groups by week 13. Body weights in the 200 mg/kg group were decreased by 12-22% relative to controls, beginning at study week 2 and continuing throughout the study. Body weights in the 150 mg/kg group were also sporadically decreased. Food consumption was affected in a parallel manner. There were no effects on body weight gain or food consumption at doses at or below 80 mg/kg. Ophthalmological examinations were unremarkable.

Hematologic measures indicated significant anemia and thrombocytosis at all ribavirin doses except 20 mg/kg, with small, statistically nonsignificant changes in the latter group. Decreases in hemoglobin, hematocrit and erythrocytes were dose-related, as were increased platelet counts. Total white cell counts were decreased by up to 50%, but only at 150-200 mg/kg. Differential counts appeared to be unaffected at 200 mg/kg, but these animals did have slight increases in the number of atypical red cells (nucleated,

poikilocytes, hypochromia - differential counts and morphology were performed only in control and 200 mg/kg groups). Serum potassium was increased by 8-16% at 150-200 mg/kg. SGPT and SGOT were elevated by ~50% at 200 mg/kg, but the changes were not statistically significant.



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The absolute and relative weights of the following organs were increased in 200 mg/kg animals of both sexes: adrenal (13-37%), heart (8-58%) and spleen (13-55%). Necropsy findings included the following: discolored lungs 7/10 males at 200 mg/kg vs. 0 control males, hepatomegaly 3/10 males at 200 mg/kg vs. 0 controls, liver discoloration 6/20 at 200 mg/kg vs. 0 controls, cardiac enlargement 2/10 males at 200 mg/kg vs. 0 controls. Microscopic examination of testes and epididymides from controls and 200 mg/kg males revealed no indications of ribavirin-related toxicity.

The data indicate 20 mg/kg as a no effect dose with the 40 mg/kg dose approaching the threshold for significant hematologic effects. The maximum tolerated dose appeared to be near 200 mg/kg, with weight loss, anemia and thrombocytosis evident at this dose level.

4) Ribavirin: 52 Week Dietary Toxicity Study In Rats With 26 Week Interim Kill, Study No. 451204.

Status: GLP

Study Site:

Study Initiation: 5 May 1993

Compound Tested: Ribavirin Lot# BR-17,

Doses Tested: 0, 1, 10, 30 and 90 mg/kg/day

Dose Volume and Route: dietary admixture, oral

Solvent: ground rodent diet

Species, Strain, Sex: male & female SD rats, age 6-7 weeks; weight range: male = 170-215 grams; female = 119-159 grams, 50 animals/sex/dose (an additional 10 animals/sex/dose were included in a satellite study for the periodic assessment of plasma drug levels).

Test conditions: Animals were randomly assigned to treatments. Ribavirin was available continuously as a dietary admixture for a period of 26 or 52 weeks. Physical signs, mortality, body weight and food consumption were monitored regularly throughout the study. Hematologic analyses and clinical chemistries were performed after 4, 13, 26, 39 and 50-52 weeks of drug exposure in a subset of animals. Ophthalmologic examinations were performed at baseline, and

at approximately 4-5 week intervals throughout the remainder of the study. Gross and/or microscopic examinations of all standard tissues were performed at the termination of dosing on all animals from the control and high dose groups and all premature deaths.

Results after 26 weeks of drug administration

Mortality: There were a total of 15 premature deaths (high dose: 8M/4F; high-intermediate dose: 1F; low-intermediate dose: 2M) during the 26 weeks of drug administration. Clinical signs noted among the affected animals included an increased incidence of peri-oral ulcers and scabbing, but did not include signs suggestive of a mechanism or cause of death.

There were no premature deaths among animals in the control or low dose groups.

Body Weight and Food Consumption: Ribavirin administration caused a dose related reduction in body weight gain (mean reductions in weight gain of 10-40% of the concurrent control) among males and females from the 2 highest dose groups. Among males and females from the high dose group, significant reductions in weight gain were evident after as little as one week of drug administration. Following the initial few weeks of drug administration, weight gain among animals from the high-intermediate dose group was generally comparable to the controls. Weight gain among animals from the low-intermediate and low dose groups was comparable to the controls.

Food consumption was reduced in a dose related manner among male and female animals from the 2 highest dose groups, which closely paralleled the changes noted in body weight gain. Food intake was reduced throughout the dosing interval by an approximate 10% among males and females from the high dose group. Among animals dosed at 30 mg ribavirin/kg/day, decreased food intake was evident during the early weeks of drug administration and sporadically thereafter.

Dietary Drug Intake: Dietary intake of ribavirin was within 20% of the nominal dose level set for each treatment group through-out the study. Drug intake was computed and adjustments of drug-diet concentration performed on a weekly basis.

Dose (mg/kg)	Plasma Drug Levels (µMolar)					
	0	1	10	30	90	
<u>Sex</u>						
♂	WK4	BLD	.028	.367	1.47	7.50
	WK13	BLD	.071	.325	1.46	7.72
	WK15	BLD	.084	.599	1.70	8.96
	WK26	BLD	.092	.740	2.23	10.99
	N=	10	10	10	10	10 or 9
♀	WK4	BLD	.026	.442	1.52	9.03
	WK13	BLD	.073	.454	1.64	9.07
	WK15	BLD	.059	.372	1.79	8.79
	WK26	BLD	.089	1.01	3.23	15.48
	N=	10	10	10	10	10

BLD = Below Limit of Detection

Ophthalmoscopy: There were no apparent drug related effects.

Hematology and Clinical Chemistry: The administration of ribavirin at a daily dose of 90 mg/kg, resulted in significant reductions (10-40% of the concurrent controls) in hemoglobin concentration, red blood cell counts, hematocrit and mean corpuscular hemoglobin concentration, which were evident after 4 weeks of drug administration and were maintained throughout the 26 week dosing interval. Similar, although somewhat smaller, reductions in red cell parameters were evident among males and female animals dosed at 10 or 30 mg/kg/day. The reductions in red cell parameters appeared dose related among animals dosed at 10-90 mg/kg/day, with animals dosed at 10-30 mg/kg/day showing slight increases in MCV (5-10%).

Total white cell, lymphocyte, basophil and/or eosinophil counts were reduced (up to 50%) among surviving animals dosed at 90 mg/kg/day throughout the drug administration interval. Sporadic reductions in most white cell parameters were also noted among animals treated with ribavirin at doses of 10-30 mg/kg. Despite the significant effects on circulating red and white blood cell parameters, the bone marrow appeared normal among all drug treated animals at the time of necropsy.

Changes in serum chemistry noted at multiple time-points during the drug administration interval, included: a) a reduction (20% versus the controls) in serum ALT levels among males and females dosed at 90 mg/kg/day (weeks 4, 13 and 26 of dosing), b) sporadic reductions in serum ALT levels among animals dosed at 30 mg/kg (males at week 13; females at weeks 13 and 26), c) increases in serum AST levels among male and female animals dosed at 90 mg/kg /day for 4 weeks (no significant effect thereafter), and d) decreases (up to 25%) in serum protein and fluctuations in serum albumin levels (increases and decreases) among males and females dosed at 30 mg/kg/day or greater.⁵ Among males, serum chloride levels were reduced slightly after 4 weeks of drug administration at levels of 10 and 30 mg/kg/day (but not at 90 mg/kg/day), while females dosed at 10-90 mg/kg/day for 26 weeks showed increases in serum phosphate levels (significant only among the high dose animals). Prothrombin time was not significantly altered by the administration of ribavirin at any dose tested. There were no other changes in any hematologic or serum chemistry parameter noted.

Gross and Microscopic Pathology: At necropsy, increases in the absolute or relative weight (covariate analyses) of the heart and lungs was seen in both male and female animals treated with ribavirin at 90 mg/kg/day. Thymus weights were significantly reduced among the high dose treated animals. Among females, the mean uterine weight was significantly decreased among animals dosed at 1, 30 or 90 (but not at 10) mg/kg/day. Sporadic, non-dose related, increases in the weight of the spleen, kidneys and liver were evident among ribavirin treated animals from all dose groups. Besides the increase in organ weight, the heart muscle of the affected animals appeared flaccid and dilated on gross examination.

⁵ The administration of ribavirin was associated with reductions in serum proteins and ALT levels, and sporadic increases in serum AST levels, in animals given ribavirin at > 30 mg/kg/day. Since no biochemical (i.e., creatinine, BUN or CPK) or histologic changes (i.e., renal or hepatic) were noted that might suggest a mechanism of increased protein loss, the data suggest that the changes in serum chemistry might be the result of decreased synthesis by the liver. However, this conclusion can not be confirmed based on the available study results.

As noted above, decreases in both absolute and relative weights of the thymus were noted in male and female animals dosed at 90 mg/kg/day, while spleen weight was increased. Microscopic examination of multiple lymphoid tissues revealed generalized atrophy (thymus and lymph nodes) with loss of germinal centers, macrophage and other inflammatory cell infiltrates, and congestion. Extramedullary hemopoiesis was decreased or absent in the spleen of multiple animals dosed at 30 mg/kg/day and above.

Microscopic examination of tissues from the lungs of animals dosed at 30 or 90 mg ribavirin/kg/day, revealed more frequent and severe alveolitis (with macrophage infiltration and pneumonitis) among these animals than was evident in animals from any of the other treatment groups. Hyperplasia of the pulmonary arterioles was also evident among 4/13 males from the high dose group.

All remaining gross and/or microscopic lesions appeared to be randomly distributed among the treatment and control groups.

Results after 50⁶-52 weeks of drug administration

Mortality: There were a total of 45 premature deaths (high dose: 27M/8F; high-intermediate dose: 2F; low-intermediate dose: 3M; low dose: 1M/1F; control: 3F) during the 52 weeks of drug exposure (which includes animals dyeing prior to the mid-study interim sacrifice). Similar to the findings after 26 weeks of drug administration, the only clinical signs noted among the effected animals included an increased incidence of peri-oral and peri-anal ulcers/scabbing and inflammation.

Body Weight and Food Consumption: Ribavirin administration was associated with a significant decrease in terminal body weight and weight gain (mean reductions in weight gain of 30-40%) of male and female animals from the high dose group. Among animals from the high dose group, significant reductions in weight gain were evident after as little as one week of drug administration and remained evident throughout the drug administration interval. Animals from the high-intermediate dose group showed a transient decline in weight gain immediately following dose initiation, although weight gain following several weeks of continuous drug exposure was generally comparable to control. Weight gain among animals from the low-intermediate and low dose groups was comparable to the controls throughout the study.

Food consumption was reduced in a dose related manner among male and female animals from the 2 highest dose groups, particularly early in the drug administration interval. However, by the conclusion of the 50-52 week drug administration period, only the animals from the high dose group continued to display reduced intake of food (5-10% reduction) as compared with control. The changes in food consumption seen among the high-intermediate and high dose animals closely paralleled the changes noted in body weight. Similar to the perturbations in body weight gain seen among the high-intermediate dose animals, decreased food intake among these animals was evident during the early weeks of drug administration and sporadically thereafter.

Dietary Drug Intake: The cumulative and weekly dietary intake of ribavirin was within 2 and 20% of the nominal dose level set for each treatment group in the study, respectively. Plasma and erythrocyte drug levels were measured in in-life and necropsy specimens and are reported in the following tables.

⁶ Due to excessive mortality the high dose treated male animals were terminated at week 50. All remaining study animals were terminated as scheduled during week 52.

		Plasma Drug Levels (µMolar) at Necropsy (data for weeks 4-26 are presented in a previous table)				
Dose (mg/kg)		0	1	10	30	90
Sex						
♂	WK26	BLD	.092	.740	2.23	10.99
	N=	10	10	10	10	9
	WK39	---	---	---	2.64	---
	N=	---	---	---	10	---
	WK50	BLD	.10	.97	4.38	13.32
	N=	10	10	9	10	7
♀	WK26	BLD	.089	1.01	3.23	15.48
	N=	10	10	10	10	10
	WK39	---	---	---	2.72	---
	N=	---	---	---	10	---
	WK52	BLD	.05	.86	3.56	16.65
	N=	8	8	10	9	8

BLD = Below Limit of Detection
 --- = No data reported

		Mean Erythrocyte Drug Levels (µMolar)				
Dose (mg/kg)		0	1	10	30	90
Sex						
♂	WK4	BLD	0.42	3.06	8.20	59.75
	WK15	BLD	BLD	BLD	1.86	12.97
	WK26	BLD	BLD	0.21	2.21	9.39
	WK39	BLD	BLD	2.85	7.45	12.66
	WK50	BLD	BLD	0.64	3.16	5.91
	N=	7-10	9-10	9-10	9-10	6-10
♀	WK4	BLD	BLD	1.65	8.83	59.09
	WK15	BLD	BLD	0.28	3.78	11.00
	WK26	BLD	BLD	0.71	6.43	16.24
	WK39	BLD	BLD	1.43	8.22	23.01
	WK52	BLD	BLD	0.69	7.33	17.95
	N=	8-10	7-10	8-10	9-10	6-10

BLD = Below Limit of Detection

Comments: 1) The data presented below suggest that in the rat, ribavirin is well absorbed after oral administration in the diet and plasma drug levels generally increase in an approximately linear manner with the nominal dose. The data also suggest that a slight increase in plasma drug levels may occur over an extended period of drug exposure. However, timing of sample acquisition is not included in the study report and would be expected to have a significant impact on measured drug levels (i.e., since the majority of diet consumption [and therefore drug intake] occurs shortly after the start of the dark period, samples drawn shortly after this time would be expected to be higher than samples taken at other times [based on a $t_{1/2}$ = 4 hrs]; it is therefore not clear whether the variation in the reported values is due to changes in physiologic disposition of the drug or to experimental artifact).

2) Drug levels in the erythrocyte appear to be more variable than levels in the plasma, and do not appear closely associated with the administered dose. The study data suggest that a gradual decrease in the intra-erythrocyte levels of ribavirin may occur during long periods of repeat exposure. While no data exist, the