RIBAVIRIN – DOSE AND
CONCENTRATION IN TREATMENT OF
CHRONIC HEPATITIS C INFECTED
PATIENTS

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ABSTRACT

Chronic hepatitis C infection is a global health problem with 170 million patients infected. Hepatitis C virus (HCV) infection is a leading cause of end stage liver disease. Current antiviral treatment options available are not optimal either regarding cure of disease or risk of side effects. The aim of the studies in this thesis was to investigate the pharmacokinetic-pharmacodynamic relationships of ribavirin in order to optimise ribavirin treatment for chronic HCV patients. In our first study a population pharmacokinetic analysis was performed using a non-linear mixed modelling evaluating population factors such as age, gender, body weight, serum creatinine and estimated GFR in patients with a wide range of renal function. Ribavirin clearance was found to be dependent on renal function and body weight gave a minor contribution to the final model. Hence, ribavirin dosing should not be based on body weight alone. A formula is presented, which can be used to estimate a ribavirin dose to reach a desired target concentration of ribavirin. The most frequent and serious side effect in ribavirin treatment is anaemia. In our second study we investigated which factors influences ribavirin induced anaemia. Ribavirin plasma concentration and dose per kg body weight versus anaemia was analysed in a non-linear regression model and we found that ribavirin induced anaemia depends on ribavirin plasma concentration, not on dose per body weight. The relation between ribavirin concentration and treatment outcome is not well investigated. In our third study, observed ribavirin concentrations in chronic HCV patients treated with interferon and ribavirin was analysed regarding treatment outcome. In a regression model genotype non-1 infection was found to be a significant predictor of treatment outcome (p<0.001) while type of interferon (standard versus pegylated) and ribavirin plasma concentration were not. In genotype 1 infected patients there was a tendency that sustained viral response was achieved more often in patients with ribavirin concentration above the median concentration of 8.7µM, than below the median. Data are suggesting that higher ribavirin concentrations may lead to better treatment response in genotype 1 infected patients. In our fourth study we investigate safety and feasibility of high ribavirin doses. 10 patients were treated with ribavirin doses aimed to reach a steady state ribavirin plasma concentration above 15 µM. The dose-predicting model from the first study was used, but underestimated the ribavirin dose. After dose adjustments a mean dose was 2540 mg/day. Due to anaemia, 2 patients required blood transfusions, twice. 9 patients completed treatment and 9 patients were cured. In the dose-estimating model from the first study, constant absorption of ribavirin was assumed. Saturable absorption could explain the underestimated dose, and sample instability could have biased the model. In our fifth study relative bioavailability was estimated using the observed ribavirin plasma concentration, divided by the concentration predicted by the model in 24 patients. Higher doses of ribavirin were linearly associated with reduced bioavailability. Stability of ribavirin was investigated in whole blood samples and ribavirin was found to be stable when stored < 24 h in room temperature.

Keywords: ribavirin, pharmacokinetics, renal function, plasma concentration, anaemia, population pharmacokinetic analysis, peginterferon, sustained viral response.

“The concept of a single dose for everyone is pharmacologically naive. 
-Treat the individual!”

Professor Evan J. Begg

To my courageous patients
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1 GENERAL INTRODUCTION

The first reference to epidemic jaundice found in the literature is from Hippocrates (460-377 BC), the earliest record in Western Europe is in a letter written by Pope Zacharias in 751 AD (1).

The epidemic of hepatitis C is probably much more recent. In 1975 transfusion-associated hepatitis not due to viral hepatitis A or B was reported (2) and in the late 1980s the virus causing the majority of nonA-nonB hepatitis was identified and named hepatitis C (HCV) (3).

Today chronic hepatitis C is a major global health problem and the World Health Organization (WHO) estimates that 3% of the world’s population is chronically infected. Due to the high risk of developing liver cirrhosis and hepatocellular carcinoma (HCC), HCV is currently the dominating cause for liver transplantation both in Sweden and in the United States.
2 HEPATITIS C VIROLOGY

The hepatitis C virus is a member of the Flaviviridae family, and the genus hepacivirus. It is a spherical enveloped virus, approximately 50 nm in diameter. Its genome is a positive single stranded, linear RNA molecule, consisting of approximately 9500 nucleotides. The genome contains a large single open reading frame encoding for a polyprotein of 3000 amino acids, flanked by highly conserved untranslated regions (UTR) at both the 5’ and the 3’ termini (4, 5). The precursor polyprotein is processed by cellular and viral proteases into at least 10 different polypeptides and results in the production of a core protein (C), two envelope proteins (E1, E2), a small protein of unknown function (p7) and six non-structural proteins (NS2, NS3, NS4A, NS5A, and NS5B)(6).

![Fig 1](image.png)

Schematic figure of HCV RNA genome.

The genome of hepatitis C virus is characterized by significant heterogeneity, and the HCV isolates can be classified into six major genotypes and more than 50 subtypes (7). Within subtypes there is significant mutation activity resulting in quasispecies, which are thought to cause opportunities for HCV to evade host immune responses (8). Hepatitis C virus replicates at a rapid rate, producing approximately $10^{12}$ particles per day. The virions have an estimated half-life of 2.7 h (9).
3 DIAGNOSIS AND DIAGNOSTIC METHODS

In clinical practice indirect and direct tests are used for virological diagnosis and monitoring.

Indirect tests
Third generation enzyme immunoassays (EIAs) or enzyme-linked immunosorbent assays (ELISAs) detect mixed antibodies against HCV core, NS3, NS4 and NS5 antigens. The antigens are coated on microtiter plates, designed for automated devices. The specificity of current used EIAs is greater than 99 % (10). A fourth generation immunoblot assay (RIBA) can be used to exclude false positive tests. It is important to recognise that EIAs can be negative in haemodialysis patients and in very immunosuppressed patients despite ongoing replication (11, 12). Specific antibodies can be detected 7-8 weeks after infection, 50-70 % of patients have detectable anti-HCV antibodies on clinical onset of disease (13). In chronically infected patients, antibodies persist for life.

Direct tests
Highly specific molecular biology techniques are useful in detecting and quantifying viral genomes in body fluids by means of polymerase chain reaction (PCR). The most used qualitative RNA tests have a detection level of 50 IU/mL. These test are more sensitive than the quantitative HCV RNA tests. Target amplification and signal amplification can be used to determine viral load. The usual cut-off level of quantitative tests is 6000 IU/mL. WHO has established an international standard for HCV RNA units to facilitate comparison between studies, recommendations and clinical guidelines.

HCV RNA becomes detectable within 1-2 weeks after infection and the level of viral load is stable during chronic infection (14). The HCV RNA level does not correlate to severity of disease but is generally low in end-stage liver disease (15). In clinical practice, HCV RNA tests are used to confirm chronic disease and to detect early infection. Viral load is primarily used for monitoring during treatment.

Genotyping of HCV is performed by direct sequencing of the NS5B or E1 regions (10). The treatment response rate and the duration of treatment depend on the genotype.
4 HEPATITIS C DISEASE

4.1 EPIDEMIOLOGY

At least 170 million people worldwide are estimated to be infected with HCV(16). The global distribution varies strongly (fig.2), with a peak prevalence of 20-25 % in Egypt. This variation may not only depend on geographical factors but is also reported to correlate to ethnicity and socio-economic status (17). The reported prevalence of 0.2 % - 0.7 % in Sweden corresponds to approximately 40 000 chronically infected patients in Sweden (18-20).

![Prevalence of chronic hepatitis C worldwide.](image)

Fig 2
Prevalence of chronic hepatitis C worldwide.
From WHO weekly epidemiology record No. 6, 2002, with permission.

The distribution of genotypes varies geographically (fig 3). In Sweden, there is a different genotype distribution compared to other parts of the Western world. Genotypes 2 and 3 are predominant (50-56 %) for unknown reasons (21, 22).
4.2 TRANSMISSION ROUTES

HCV is spread primarily by blood and blood products. Blood transfusions and intravenous drug use have been the predominant modes of transmission for HCV infections in the Western world (23). In the early -90s mandatory screening of blood donors and blood products was introduced in Sweden. This virtually eliminated transfusion as a source for HCV transmission (24). Dialysis, solid organ transplantation and occupational exposure are other risk factors for HCV transmission. Nosocomial transmission has been reported and may be underestimated (25, 26). The risk for perinatal transmission from an infected mother to the newborn is less than 5 %, and the risk for sexual transmission very low (27).
4.3 NATURAL COURSE OF DISEASE

The natural history of chronic HCV infection varies greatly and is only partially understood. Few patients with acute infection develop symptoms (28). The rate of persistence of hepatitis C virus infection varies, 54 – 86 % has been reported (29). Usually the disease progresses rather slowly over decades. The individual variation of progression rate is strongly influenced by a number of co-factors, such as age at infection, alcohol intake, hepatic steatosis and obesity (30-32). Coinfection with chronic hepatitis B infection is also a strong predictor of rapid disease progression. HCV is a leading cause of cirrhosis. Seeff reported that the risk of developing cirrhosis in chronic HCV infected patients is 2-20% after 20 years of infection (29). It is estimated that up to 23 % of infected patients who are untreated ultimately develop hepatocellular carcinoma (HCC) (33).
5 TREATMENT AND DOSING

5.1 BACKGROUND

The rational for HCV treatment is to halt progression towards end-stage liver disease, and to decrease the risk of HCC. However, in recommendations and guidelines the treatment effect or sustained viral response is usually defined as undetectable HCV RNA by qualitative PCR (< 50 IU/mL) 24 weeks after treatment cessation. In 1986 the first report on antiviral treatment with interferon alpha, for chronic nonA-nonB hepatitis infected patients, was published (34). The moderate treatment outcome of 10-20 % in interferon treated patients improved to approximately 40 % when interferon was combined with ribavirin a decade later (35-37). Combination therapy also reduced fibrosis in the liver in patients with sustained viral response (37, 38).

5.2 PRESENT TREATMENT

More recently, standard interferon treatment has been pharmacokinetically modified by adding a polyethyleneglykol (Peg) molecule to interferon-alpha. This increases the half-life of the drug and it is therefore administered once weekly, resulting in a larger AUC and thereby a more efficient viral suppression. Two different Peg-interferons are currently approved, peg-interferon alpha2a (Pegasys®) which has a 40 kiloDalton (kD) branched peg-molecule attached to interferon, and peg-interferon alpha2b (PegIntron®), which has a 12 kD linear peg-molecule attached to interferon. The peg-interferon alpha2b is dosed according to body weight, whereas the peg-interferon alpha2a has a fixed dose. When peg-interferon was compared to standard interferon in clinical trials, end-of-treatment response was improved but a high relapse-rate was observed, especially in patients infected with genotype 1 (39, 40).

The sustained viral response was increased when peg-interferon was combined with ribavirin. In pivotal clinical trials approximately 80 % of genotype 2 or 3 infected patients and 50 % of genotype 1 infected patients will achieve a sustained viral response (41, 42). In current Swedish guidelines genotype 2 and 3 patients are recommended 24 weeks of combination treatment and genotype 1 patients are recommended a longer treatment of 48 weeks due to the lower treatment efficacy in this population (43). More recently an even shorter treatment of 14 weeks for genotype 2 and 3 patients has been proposed(44).

Other drugs have been tested in HCV therapy. Although amantadine seemed promising in early pilot studies (45, 46), it has not been shown to have an effect additive to combination treatment in a meta-analysis of 31 randomised clinical trials (47). A prodrug to ribavirin, viramidine, has been shown to have a safer toxicity profile than ribavirin (48). Currently viramidine is investigated in phase III trials in combination with peg-interferon.

A diverse array of novel therapies is under development for patients with HCV infection. These include HCV serine protease inhibitors, antisense oligonucleotides and
human monoclonal antibodies (49). Although they have been tested in small number of patients, data is insufficient to predict how they can be used in clinical practice.

5.3 RIBAVIRIN DOSING IN HCV TREATMENT

The first case report of ribavirin, as single drug treatment for chronic hepatitis C, was published in 1990 (50). A daily dose of 1000 mg had been reported earlier for treatment of Lassa fever and was used as the foundation for ribavirin dosing in chronic hepatitis C treatment. A weight-based dose of 1000 versus 1200 mg daily (for patients with a body weight of < 75 kg or > 75 kg respectively) or 16 mg/kg/day was used in the early pilot trials (35, 51, 52). A significant biochemical effect was seen, measured as ALT normalisation during treatment. However, there was no decrease in viral load during ribavirin mono therapy.

In the first larger clinical trials of ribavirin in combination with standard interferon in the treatment of chronic hepatitis C patients, a dose of 1000-1200 mg daily was chosen, presumably based on the earlier ribavirin mono-therapy trials. This weight-based treatment regime for ribavirin was conserved in the peg-interferon trials, possibly because the primary objective was to explore dose related efficacy for peg-interferon. In one of the registration trial of peg-interferon alpha2b, 800 mg daily of ribavirin was used in one of three treatment arms (41). The low dose ribavirin was combined with the highest dose of peg-interferon (1.5 µg/kg). This is the basis for the affixed dose of 800 mg, which is currently the only ribavirin dose approved by the Food and Drug Administration in the United States.

Today, a weight based dosing regimen for ribavirin in combination with peg-interferon alpha2b is recommended by the manufacturer in Sweden. 800 mg is recommended for patients < 65 kg, 1000 mg for patients 65 – 85 kg and 1200 mg for patients > 85 kg (package insert Schering-Plough). This slightly different dosing is based on the manufacturers secondary analysis of the registration trial for peg-interferon alpha2b and a retrospective population pharmacokinetic pharmacodynamic model in an analysis by Jen et al (53).

Although no formal prospective dose-finding study has been published, a recent study by Hadziyannis et al showed increased SVR from 41 – 52 % for genotype 1 infected patients who received 800 mg compared to 1000-1200 mg of ribavirin (54). Increasing the dose of ribavirin did not improve treatment outcome for patients with genotype 2 or 3.

Adherence to therapy is recognized as an important factor for treatment outcome and increasing evidence indicates that the likelihood of treatment success in combination therapy for chronic hepatitis C is compromised by dose reduction of ribavirin (37, 41, 55-57).
6 RIBAVIRIN

Ribavirin is a guanosine analogue with a molecular weight of 244 g/mol, first synthesised in 1972 (58). It shows in vitro activity against a broad spectrum of RNA and DNA viruses, including herpes simplex 1 and 2, adenoviruses, cytomegalovirus, influenza A and B, parainfluenza, measles, rhinoviruses and more tropical viruses, such as Rift Valley fever and Lassa fever. Antiviral activity in animal models is also shown for herpes simplex, vaccinia, influenza, West Nile, RSV and Lassa fever viruses (59). In humans it has been tested for Lassa fever, respiratory syncytial virus (RSV) infections, hepatitis B and C as well as HIV and SARS (60-63). Today its most common use is for RSV infected immunosuppressed patients and in combination with interferon for chronic hepatitis C (64).

6.1 ANTI-VIRAL EFFECT IN HCV INFECTION

Intracellular phosphorylation to ribavirin triphosphate is believed to be required for ribavirin to exert its pharmacological activity, but the complete mechanism of antiviral activity is not known. Ribavirin mono therapy in HCV patients exerts a moderate (less than 0.5 log copies/mL reduction) and transient (on days 2 and 3 of administration) effect on hepatitis C virus replication. This effect has recently been shown to be associated with longer ribavirin clearance half-life and higher serum concentrations (65). It has also been suggested that ribavirin could accelerate the clearance of infected cells, given that it prevents relapse when added to interferons (66).

Currently there are several theories regarding indirect and direct anti viral effect in HCV treatment:

- Immunomodulatory effect
  A Th1 cytokine response is important in the early phase of viral infection, to control the infection and a dominant Th2 cell-response has been associated with the development of chronicity in a number of viral infections (67). It has been proposed that ribavirin could shift the host T-cell-response to an increased Th1/Th2 ratio (68). The greater efficacy of combination therapy with standard interferon might also be related to ribavirin ability to suppress HCV-specific IL-10 production (69).
• IMPDH inhibitor

Inosine monophosphate dehydrogenase (IMPDH) is the rate-limiting step when IMP is converted to GTP. Ribavirin competition with IMP is proposed to reduce the intracellular GTP pool, which could suppress RNA replication (70). However, other IMPDH inhibitors such as mycophenolic acid and VX-497, do not affect HCV replication in infected patients (71).

• Polymerase inhibitor

Antiviral effect is presumed to arise from the inhibition of viral RNA polymerase or from 5’ capping of viral mRNA by ribavirin triphosphate. This could have a direct limiting effect on HCV replication, but data so far is incongruous (53, 72, 73).

• RNA virus mutagen

Ribavirin may induce lethal mutagenesis of the RNA genome in vitro by incorporating into newly synthesized genomes, generating a nonviable quasispecies population (74, 75). However, studies of human HCV infection have not shown acceleration of mutagenesis during ribavirin therapy (65).

These theories are not mutually exclusive and it is possible that ribavirin acts through multiple mechanisms.

### 6.2 PHARMACOKINETICS

#### 6.2.1 Bioavailability

Bioavailability of single dose ribavirin has been reported to be 40-50% (62, 76, 77). In a single dose study by Preston the absolute bioavailability of ribavirin was estimated 52% (77). Ribavirin is reported to be absorbed in the intestines, and was found to be transported across the cell membrane by the N1-sodium dependent purine transporting protein in an in vitro study by Patil et al (78). Possibly other absorption mechanisms are involved, since it appears to be a slow process with an average t½ of 2.3 hours (77).

The extent to which food affect ribavirin bioavailability differs between studies. It is also unknown whether any food effect might be altered by the type of meal consumed (e.g. high versus low fat content), if a food effect still would be evident upon multiple dosing and what clinical implications this might have (79).

#### 6.2.2 Elimination

Ribavirin has two proposed pathways of metabolism:

- reversible phosphorylation to mono-, di-, and triphosphate metabolites, and
- degradation involving deribosylation and amide hydrolysis to a triazole carboxamide,

reported in the literature (80-82).
There is no evidence that any cytochrome P450 enzymes are involved in these metabolic pathways (79).
Mass-balance data suggest that ribavirin is subject to first pass metabolism (77).
According to Preston et al approximately one third of total ribavirin clearance is renal (77). Ribavirin is not removed in important quantities by haemodialysis (83).

6.2.3 Distribution

Ribavirin does not bind to plasma proteins (76). The apparent volume of distribution of ribavirin is estimated to > 1000L (77). It has been demonstrated that ribavirin is a transported permeant for the nitrobenzylthioinosine-sensitive (es)-nucleoside transporter of erythrocytes (84). Ribavirin movement via the es-transporter requires a concentration gradient and may be bi-directional; therefore extensive transport of ribavirin out of the extracellular blood compartment during absorption and distribution phases is likely (85).

Es-transporters are distributed in many cell types in animal models (85). If the same is true for human cells, this could account for the extensive distribution in all tissue compartments.

As a consequence of the relation \( t_{1/2} = \frac{V}{CL \times \ln 2} \), the large volume of distribution should result in a long half-life of the drug, which is also what has been found (76, 77, 79, 86, 87).

6.2.4 Steady state

The long half-life of ribavirin is clearly strongly dependent not only on the distribution volume, but also on clearance. This results in a slow rate by which steady state is reached. 4-5 \( t_{1/2} \) is needed to reach > 90% of the steady state concentration. The level at steady state, on the other hand, depends on clearance but not on the volume of distribution:

\[
C_{ss} = \frac{F \times D}{CL \times T}
\]

Thus, in clinical practice, dosage depends primarily on clearance while the time to steady state depends on both clearance and volume of distribution.

6.2.5 Accumulation in erythrocytes

Early pharmacokinetic studies have shown that ribavirin concentrations in erythrocytes exceed the plasma concentration 60-fold (62, 76). The high concentration in red blood cells is due to ribavirin being phosphorylated to ribavirin triphosphate (RTP), which accumulates.
This is proposed to occur due to insufficient dephosphorylation in erythrocytes and the es-transporters inability to bind to phosphorylated ribavirin, i.e. RMP, RDP and RTP (82).

Nucleated cells:

Non-nucleated cells (e.g. erythrocytes):

Fig 5
A model for ribavirin cellular transport and phosphorylation.

6.3 ANAEMIA

The major dose-limiting toxicity of ribavirin treatment is anaemia, which was recognized already in early mono-therapy studies (88). This toxic effect was observed to be reversible with dose reduction or discontinuation of the drug. Haematotoxic side effects are also frequent in interferon mono-therapy; anaemia is seen in standard interferon treatment as well as peg-interferon treatment. The mechanism behind interferon-induced anaemia is believed to be mainly due to bone marrow suppression.
Haematopoetic growth factors are increased, but not enough to overcome the suppression of the bone marrow (89).

In a meta-analysis of 26 studies published 1991-1999, ribavirin induced anaemia (defined as reduction in haemoglobin to less than 100 g/L) was found in 7-9% of patients treated with combination therapy including ribavirin dose of 1000 – 1200 mg daily (90). In another retrospective study of approximately 600 patients treated with combination therapy including standard interferon and ribavirin, more than 95% of experienced a >= 10 g/L drop in haemoglobin and approximately one third of the patients dropped 25% of their baseline haemoglobin level (91). In a recent study, reduction of health related quality of life estimated in patients treated with combination therapy, was most pronounced in the patients who experienced at least 15% drop in haemoglobin levels (92).

The exact mechanism of ribavirin-induced anaemia is unknown. In toxicological studies of ribavirin in rhesus monkeys and cats, a dose-relate toxic effect on bone marrow was seen, primarily on late erythroid precursors (93, 94). The most common hypothesis is haemolysis. This could be due to extensive phosphorylation of ribavirin producing a relative ATP deficiency, or a membrane oxidative damage, reducing cell viability and promoting premature erythrocyte phagocytic removal in the reticulendothelial system (95). It has also been suggested that ribavirin may independently suppress erythropoiesis via down-regulation of erythropoietin receptors (93, 95, 96). A theory why combination therapy so often leads to anaemia could be that the interferon induced bone marrow suppression in combination therapy could down-regulate the reticulocyte response and further aggravate ribavirin-induced anaemia (97).

Ribavirin-induced anaemia is typically managed by dose reduction or discontinuation of the drug, but transfusions with erythrocyte concentrates is occasionally necessary in cases of severe or symptomatic anaemia (98).

6.3.1 Erythropoietin

In order to maintain ribavirin dose, despite toxic side effects, erythropoietin (epo) is currently evaluated. The hypothesis is that epo could be beneficial, as it improves adherence and thereby treatment outcome. It is unclear if endogenous epo production is adequate in chronic hepatitis C patients undergoing combination therapy. Conflicting data is reported so far (99, 100). Erythropoietin substitution has been shown to improve haemoglobin levels, to maintain ribavirin dosing and to improve quality of life in recent randomised trials (101-103). An effect of epo substitution on sustained viral response-rate has, however, not been shown, and cost-benefit analyses are therefore needed.
7 AIMS OF THE THESIS

The overall aim was to study the pharmacokinetic and pharmacodynamic relationships of ribavirin in order to optimise the treatment of patients with chronic hepatitis C.

In detail, the aims were:

- To study the influence of different population factors on ribavirin plasma concentrations, in particular renal function, using a population pharmacokinetic analysis, and to propose a dosing schedule based on the findings (Paper I).

- To study the relation between ribavirin plasma concentration and anaemia (Paper II) and between concentration and treatment outcome (Paper III).

- To study tolerance to high concentration of ribavirin in patients with chronic hepatitis C, using the population pharmacokinetic model and therapeutic drug monitoring to estimate the ribavirin dose (Paper IV).

- To study if ribavirin absorption is saturable and to investigate the stability of ribavirin levels in blood samples (Paper V).
8 ETHICS

Written informed consent was given from all included patients and the studies were approved by the ethics committee at Karolinska University Hospital, Stockholm, Sweden. All studies were performed in accordance with the principles put forward in the Helsinki Declaration. Study IV was approved by the Swedish Medical Product Agency.
9 PATIENTS AND TREATMENTS

All patients had chronic hepatitis C. With few exceptions, patients infected with genotype 1 were treated for 48 weeks and patients infected with genotype 2 or 3 were treated for 24 weeks. All patients were treated with combination therapy with the exception of the 7 patients with renal insufficiency (Paper I), see below. Ribavirin doses were based on body weight (except for patients with impaired renal function in Paper I and the 10 patients in Paper IV and Paper V); 1000mg for patients < 75 kg and 1200 mg for patients > 75 kg. In some cases patients were started on a lower dose of 800 mg daily if anaemia was judged to be a risk, in accordance with the clinical practice at this time. Patients with renal impairment started on lower doses.

9.1 PAPER I

This study included 63 patients:

- 44 patients with normal serum creatinine treated with standard interferon alpha-2b, 3 MIU tiw and ribavirin, including 10 patients who were controls in a HPLC study (104) and 34 consecutive patients undergoing standard combination treatment at the outpatients clinic, Department of Infectious Diseases at Karolinska University Hospital at Huddinge, Stockholm.

- 19 patients with impaired renal function:
  7 haemodialysis patients treated with standard interferon and ribavirin,
  12 patients with renal impairment, including 7 patients treated with ribavirin monotherapy.

9.2 PAPER II AND III

A total of 117 consecutive patients were included (Paper III), with an initial ribavirin dose of 800-1200 mg daily, at the outpatients clinic, Department of Infectious Diseases between 1999-2001. 108 of these patients were included in paper II.

- 82 patients were treated with standard interferon alpha -2b or natural human leukocyte interferon, 3 -5 MIU tiw
- 36 patients were treated with peginterferon alpha-2b, 1.0 µg/kg once weekly

1 patient was treated twice during the study period, the first time with interferon alpha-2b, 3 MIU tiw and the second time with peginterferon-2b, 1.0 µg/kg weekly.
36 patients in this population had genotype 1,
74 patients had genotype 2 or 3 and
7 patients were non-typable.

9.3 PAPER IV

In this study 10 treatment naïve patients with genotype 1 and a viral load of > 800 000 IU/mL were prospectively recruited. They received combination therapy with peginterferon alpha-2a, 180 µg weekly, and concentration-controlled (target > 15µM) individually high-dosed ribavirin.
9.4 PAPER V

This study includes the 10 patients from paper IV and 14 patients from the study population in paper II and III, who were selected to cover different doses.

Figure 6
Patients included in the thesis.
10 METHODS

10.1 SAMPLING COLLECTION

383 plasma samples for ribavirin concentration analysis were collected from 63 patients in Paper I. An additional 233 samples for ribavirin concentration were collected from 117 patients (Paper II, III), including a few samples used in Paper I.

An additional 152 samples were collected from another 10 patients (Paper IV) including 4 samples used for the stability analysis in Paper V.

All samples were collected approximately 12 hours after dose administration, except for the patients with renal impairment in the pharmacokinetic analysis. Time of dose, concomitant medication and dose adjustments prior to sampling were recorded.

No dose adjustments 3 weeks prior to sampling were allowed to ensure steady state conditions, except for some samples in Paper I and IV where plasma concentrations were used for drug monitoring. The initial whole blood samples were centrifuged at 3000 rpm for 10 minutes; plasma was then separated and frozen at -70°C.

10.2 ANALYSIS OF RIBAVIRIN IN PLASMA

Ribavirin plasma concentrations were measured by solid phase extraction, followed by HPLC (high-performance liquid chromatography) and UV detection by a method previously published (104) (Paper I-V).

10.3 VIROLOGY

HCV RNA was analysed using a PCR test (Cobas Ampliprep/Cobas Amplicor HCV test version 2.0, Roche Molecular Systems) with a sensitivity of approximately 50 IU/mL (ref). Viral load was analysed using a quantitative PCR test (Cobas Ampliprep/Cobas Amplicor HCV Monitor version 2.0, Roche Molecular Systems) with a sensitivity of approximately 6000 IU/mL (ref).

HCV genotyping was made using an in-house method (105).

10.4 HISTOLOGY

A scoring system was used that ranked liver inflammation (grade) and fibrosis (stage) categorically on a scale of 0-4, according to a classification system that corresponds to Ludwig and Batts (38, 106). To summarise, grade 0 represents no or minimal inflammation and grade 4 severe inflammation. Stage 0 was classified as no fibrosis and stage 4 was equivalent with cirrhosis.

10.5 RENAL FUNCTION

Serum creatinine and weight was measured and registered in all patients at baseline and together with every ribavirin concentration sampling (Paper I-V). Glomerular filtration rate was estimated at baseline and in paper IV also at observation, for each patient using a formula (107), which is a modified version of the Cockroft-Gault equation(108):

for male subjects: $1.23 \times (140 – \text{age}) \times \text{weight}/\text{serum creatinine},$

for female subjects: $1.04 \times (140 – \text{age}) \times \text{weight}/\text{serum creatinine}.$
10.6 STATISTICAL ANALYSIS

The population pharmacokinetic analysis was performed using a non-linear mixed effect modelling programme, commercially available as NONMEM IV (Paper I). Linear regression and non-linear least squares regression were used to examine the correlation and fit to a concentration-effect curve using the Hill equation (Paper II). A linear regression model was used for continuous data (Paper V) and a logistic regression model was used for categorical data (Paper III).
11 PAPER I

11.1 BACKGROUND AND METHODS
Ribavirin dosing is based on body weight in two different dosing schedules (36, 37, 53). Ribavirin has been considered contraindicated in patients with renal insufficiency or haemodialysis because of reduced elimination rate. In a pharmacokinetic analysis of ribavirin (109), renal function estimates using creatinine clearance had been made. These estimates were excluded in the final model and only patients with normal serum creatinine were included in the model. The aim of this study was to investigate if ribavirin clearance is explained by estimated GFR in patients with a range of renal function during steady-state conditions. The aim was also to suggest a simple ribavirin dosing schedule based on the findings.

2-20 plasma samples were collected from each patient, for the purpose of the pharmacokinetic analysis. A basic pharmacokinetic two compartment model, with a first order absorption, first order elimination, was used. Non-linear mixed effect modelling was used and the modelling strategy was to test the dependency of ribavirin clearance on body weight, renal function, age and sex.

11.2 RESULTS
Ribavirin had a long half-life of around 100 h in patients with normal renal function and consequently much longer in dialysis patients. The volume of distribution was large and proportional to body-weight. Because of the long half-life and the large volume of distribution, time to steady state is long, especially in patients with renal impairment.

Modelling ribavirin clearance as a function of renal function gave a significantly better fit than using body weight alone. Adding a body weight–dependent factor improved the model marginally but significantly.
A formula for individual dosing of ribavirin was suggested:

\[
\text{Ribavirin dose} = 0.244 \times C_{\text{target}} \times T \times (0.122 \times CL_{\text{creat}} + 0.0414 \times \text{body weight})
\]

were \( C_{\text{target}} \) is the intended concentration of ribavirin for a patient of given body weight (kg) and renal function (\( CL_{\text{creat}} \) = creatinine clearance in mL/min). \( T \) (h) is the dosing interval, 0.122 and 0.0414 are regression coefficients obtained from the regression analyses and 0.244 converts the dose to molar units.

A significant inter-individual variability in ribavirin clearance of 40 %, not dependent of renal function or body-weight was also found.
12 PAPER II

12.1 BACKGROUND AND METHODS
Ribavirin was found to be eliminated to a substantial degree by the kidneys and plasma concentrations were predicted primarily by renal function (Paper I). For this reason a dosing schedule mainly based on body weight is apparently not logical. In this study we hypothesised that the major side effect of ribavirin, anaemia, should be more strongly related to plasma concentrations than to dose per kg bodyweight. Ribavirin plasma concentration samples were collected from consecutive patients undergoing standard combination therapy with interferon and ribavirin, at treatment week 4, 8 and 12. Samples for haemoglobin and creatinine levels were collected at the same time-point. A non-linear regression model was used.

12.2 RESULTS
209 plasma samples from 105 patients were possible to analyse. The mean ribavirin concentration was 8.19 µM with a range of 0 – 17.7 µM. There was no apparent relation between the absolute or relative drop in haemoglobin and the dose per kg body weight. Ribavirin concentrations were non-linearly related to the relative haemoglobin drop as revealed by fitting a standard Hill dose response equation to the data. Also when ribavirin concentration data was subdivided into intervals, a clear haemoglobin drop-concentration relationship was found.
Fig 8
Relative drop in haemoglobin versus plasma concentrations.
The solid line is the Hill curve obtained by non-linear least squares modelling.

Fig 9
Relation between ribavirin plasma concentrations, grouped in intervals, and relative haemoglobin drop.
13 PAPER III

13.1 BACKGROUND AND METHODS
A crude dosing schedule for ribavirin based on body weight is used in most guidelines worldwide. The optimal dose or concentration of ribavirin is not known. This study was designed to explore the relationship between plasma concentration of ribavirin and treatment outcome based on the findings in Paper I and II. The hypothesis was that higher ribavirin concentrations could improve treatment outcome, especially in genotype 1 infected patients. 107 patients were possible to evaluate. Plasma samples were collected at treatment week 4, 8 and 12. The latest available sample for each patient was used in the analysis. Favourable treatment outcome was defined as a negative HCV PCR test 24 weeks after treatment cessation (SVR). Type of interferon treatment (standard versus pegylated), the natural logarithm of the latest plasma concentration and genotype (1 versus non-1) were the three independent variables chosen for analysis.

13.2 RESULTS
Upon visual inspection of plotted concentrations, there was a tendency towards higher ribavirin concentrations for genotype 1 infected patient who had a successful treatment outcome compared to genotype 1 patients who failed treatment. When the genotype 1 infected patients were divided into two groups, above and below the median concentration, there was a higher relative success rate in the group above the median plasma concentration. This was not seen in genotype 2 and 3 infected patients. In a logistic regression analysis genotype was a strong predictor for SVR (p<0.001) while neither interferon type (p=0.25) nor ribavirin plasma concentration (p=0.33) were found to be significant predictors.
Fig 10
Ribavirin plasma concentrations and treatment outcome in genotype 1 and genotype 2 +3 infected patients.

<table>
<thead>
<tr>
<th>Ribavirin concentration</th>
<th>&lt;= 8.7µmol/L</th>
<th>&gt; 8.7µmol/L</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1</td>
<td>4/20 (20%)</td>
<td>6/16 (38%)</td>
<td>10/36 (28%)</td>
</tr>
<tr>
<td>Genotype 2 or 3</td>
<td>29/34 (86%)</td>
<td>32/37 (86%)</td>
<td>61/71 (86%)</td>
</tr>
</tbody>
</table>

Table 1
Treatment success rate in 107 patients according to genotype and the level of ribavirin plasma concentration (median 8.7µM).
14.1 BACKGROUND AND METHODS
Although new HCV treatment regimens have improved viral response, patients infected with genotype 1 with a high viral load still have a SVR of less than 50%. Earlier data (109) imply that higher ribavirin plasma concentration could improve treatment outcome in this population. This study was designed as a pilot study including 10 treatment naïve patients, given individual concentration controlled doses of ribavirin using the dose predicting formula from paper I, together with standard dosed peginterferon for 48 weeks. Safety and tolerability was evaluated, using close visits and haemoglobin measurements. Therapeutic drug monitoring was used to maintain ribavirin concentrations of $> 15 \, \mu M$ over the entire treatment period. Erythropoietin, iron supplement and blood transfusion were used to control anaemia.

14.2 RESULTS
The individual dose, predicted by the formula in paper I, did not result in the intended target concentration in nine patients. Dose escalation using plasma concentration monitoring resulted in a mean daily dose of 2520 mg. The highest individual dose given was 4000 mg. All patients had moderate to severe anaemia during the treatment and were treated with erythropoietin and iron. Two patients were given transfusions on two occasions each, and ribavirin was discontinued for one week, to control anaemia. Fatigue, nausea and dermatological side effects were frequent and severe. All patients had a reduced working capacity. Eight patients completed 48 treatment weeks. One patient stopped treatment at week 39, mainly due to interferon associated side effects. One patient was taken off treatment at week 24 due to viral nonresponse. The remaining 9 patients had a sustained viral response 24 weeks after treatment cessation.
<table>
<thead>
<tr>
<th>Treatment week</th>
<th>0</th>
<th>4</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>≥72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily ribavirin dose mg, mean (range)</td>
<td>1520 (1200-2200)</td>
<td>1680 (1200-2200)</td>
<td>2520 (1600-3600)</td>
<td>2540 (1600-3600)</td>
<td>2325 (1600-3600)</td>
<td>n=8</td>
</tr>
<tr>
<td>Ribavirin concentration μmol/L, mean (range)</td>
<td>- 8.6 (6.0-15.7)</td>
<td>14.7 (10.8-18.7)</td>
<td>14.7 (7.8-22.0)</td>
<td>14.4 (11.9-17.0)</td>
<td>n=8</td>
<td></td>
</tr>
<tr>
<td>Viral load IU/mL, mean: 2.5 x10^6 range: 1.6-4.2</td>
<td>&lt;600 n=3</td>
<td>&lt;50 n=5</td>
<td>&lt;50 n=8</td>
<td>&lt;50 n=9</td>
<td>n=9</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin g/dL, mean (range)</td>
<td>14.4 (12.5-16.0)</td>
<td>12.4 (8.9-15.0)</td>
<td>11.1 (8.6-15.0)</td>
<td>9.7 (8.1-11.3)</td>
<td>9.9 (8.7-11.4)</td>
<td>n=8</td>
</tr>
<tr>
<td>Number of patients treated with Erythropoietin</td>
<td>0 8 10 10 8 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2
The ribavirin doses and corresponding ribavirin concentrations, viral load, haemoglobin levels and erythropoietin treatment in 10 patients with chronic HCV infection during and after ribavirin and peg-interferon treatment.
15 PAPER V

15.1 BACKGROUND AND METHODS

The dose predicting formula proposed (Paper I) was used to estimate the individual dose of ribavirin in the pilot study (Paper IV). In nine of ten patients the dose was underestimated. One reason for this could be that ribavirin absorption is saturable and not constant as was assumed in the basic pharmacokinetic model in Paper I. Another possibility is that the regression model is biased e.g. by ribavirin instability in stored plasma samples.

Data on the 10 patients in Paper IV and 14 patients from the population included in Paper II was used. A sampling occasion for each patient was selected in steady state conditions. Relative bioavailability was estimated using the observed ribavirin plasma concentration, divided by the concentration predicted by the model. Stability of ribavirin was investigated in whole blood samples obtained from each of 4 patients in Paper IV, each sample divided into four portions stored under different conditions.

15.2 RESULTS

Higher doses of ribavirin are linearly associated with reduced bioavailability ($r^2 = 0.33$; $p<0.01$) with a maximum of 50 % reduction.

![Graph](image)

**y = -0.0007x + 1.2346**  
**$R^2 = 0.359$**

Fig 11  
The relative bioavailability of ribavirin at steady state in 24 patients undergoing combination therapy with interferon.

The ribavirin concentration greatly increased when whole blood samples were stored at longer time than 24 hours in room temperature before centrifugation, plasma separation and freezing.
16 GENERAL DISCUSSION

Ribavirin dosing in chronic hepatitis C treatment in Sweden is currently based on two different body-weight based schedules: either 1000mg daily for patients < 75 kg and 1200mg for patients > 75 kg, or 800mg for patients < 65 kg, 1000mg for patients 65-85 kg and 1200mg > 85 kg. These recommendations seem to be based, not on direct measurements of ribavirin concentrations, but on extrapolation of statistical relation between dose, body weight and response status. To do a clinically valid estimation of dosing it is important to evaluate all available factors that may influence the treatment response. No formal dose-finding studies have been made for ribavirin and the optimal dose is thus unknown. It is also unclear what the concentration–effect relationship of ribavirin is. Earlier pharmacokinetic studies have demonstrated renal elimination of ribavirin.

The overall aim of the studies in this thesis was to further investigate the pharmacokinetic-pharmacodynamic relationships of ribavirin and to use this knowledge to optimise ribavirin treatment for patients with hepatitis C.

16.1 PHARMACOKINETICS

16.1.1 Renal function as basis for ribavirin dosing

In a pharmacokinetic analysis of patients with apparent normal renal function (109), GFR was estimated but not included in the final model. The results in Paper I show that ribavirin clearance is strongly dependent of renal function and that estimated GFR is a better predictor of ribavirin clearance than body-weight alone. This has also been confirmed in studies of transplant patients and in Japanese patients (110, 111). The large volume of distribution has clinical implications, the half-life of ribavirin (100-500 hours depending on renal function) leads to slow effects of ribavirin dose alterations. This is especially important when monitoring the most important side effect of ribavirin: anaemia. This also highlights the importance of estimating renal function in chronic hepatitis C patients, not using serum creatinine levels, but preferably with GFR estimates.

Body weight gave a minor but significant contribution to the final model in Paper I, suggesting that minor modifications of the dose may be needed for gross deviations in body weight, especially in patients with impaired renal function.

In view of earlier data, in particular those published by Preston et al, the strong dependence on renal function is a little surprising since only 30 % of ribavirin clearance was found to be renal and the remaining portion was metabolic. It may well be that metabolic clearance is impaired in patients with reduced renal function. This issue merits further investigation.

The formula proposed in Paper I is currently used for dose estimations in clinical practice for dialysis patients and patients with reduced GFR undergoing treatment for hepatitis C at our hospital.
Because of a residual inter-individual variability in ribavirin clearance of 40 %, not dependent on renal function or body weight, dose monitoring with plasma concentrations is recommended in particular in patients with reduced renal capacity and those sensitive to anaemia.

16.1.2 Bioavailability of ribavirin

The finding of dose underestimation in Paper IV, by the dose-estimating formula from Paper I, was obvious in 9/10 treated patients. In Paper I constant absorption of ribavirin was assumed in the model. Saturable absorption of ribavirin could reasonably explain the underestimated dose.

Published data are conflicting. One single dose study claims that AUC is linearly related to dose while C_{max} displays signs of saturability (data cited by Glue) (79). In another study C_{max} is clearly saturable (76). Since higher doses than 1400 mg not have been given in clinical trials to our knowledge, saturable absorption has not been reported in this setting. Saturable absorption has been shown previously, for another guanosine analogue, acyclovir (112), hence, our findings are not entirely unexpected.

The main finding in Paper V is that bioavailability declines with increasing doses of ribavirin.

The probable mechanism is saturation of the N1-purine transporting protein, which is reported to transport ribavirin across cell membranes in the small intestine (78).

From a clinical perspective saturable absorption implies that high-dose ribavirin should be dosed more frequently than twice daily, despite its long half-life.

16.1.3 Instability during different storing conditions

In Paper V stability of ribavirin in whole blood samples was tested. Instability may bias the dose-predicting model in Paper I. However, in the 16 samples tested in Paper V, ribavirin concentration remained stable in whole blood during up to 24 hours storage in room temperature. When samples were stored for one week in room temperature considerable increase in concentration was seen. This is probably due to dephosphorylation of ribavirin triphosphate. The best practice for ribavirin sampling is to separate plasma and store samples frozen within 24 hours.

16.2 PHARMACODYNAMICS

16.2.1 Concentration-effect relationship

The dose predicting formula in Paper I focus on the question of optimal target concentration of ribavirin. In the study of Jen et al (109) the probability of response to treatment, defined as loss of detectable HCV-RNA 24 weeks after completion of treatment, increased with higher ribavirin concentrations. The slope of concentration-response curve for genotype 1 infected patients treated for 48 weeks increased for concentrations up to 16-20 µM, although few patients reached these concentrations.
Other retrospective and secondary analysis in clinical trials of genotype 1 infected patients undergoing combination therapy have shown trends of higher sustained viral response rates in patients with higher ribavirin doses and concentrations (41, 113). One must consider that the number of patients in these analyses is small.

More recently a randomised trial designed to assess the efficacy of low dose (800mg daily) versus standard dose (1000-1200 mg daily) of ribavirin was presented (54). Data clearly show that the response rate among patients with genotype 1 was higher in the group who received the higher dose of ribavirin.

In Paper III concentration-viral effect relationship was more directly investigated. The design of the study was to analyse observed ribavirin concentrations in a standard treated patient population and its effect on treatment outcome. Because of the diversity of clinical practice we used regression analysis to control for confounders such as different types of interferon (pegylated versus standard interferon) and also viral genotype (1 versus 2 and 3). The study was probably under-powered mainly due to the introduction of pegylated interferon which reduced the number of genotype 1 infected patients included, and the common practice to control anaemia with ribavirin dose reduction, resulting in a lower range of plasma concentrations. A tendency to better viral response in genotype 1 infected patients with higher ribavirin concentrations, though not significant, was found. 38 % of patients with ribavirin concentrations higher than the median concentration of 8.7 µM achieved sustained viral response compared to 20% of the patients with ribavirin concentrations lower than the median.

Recently, ribavirin concentrations at treatment week 4 and 8, as well as $C_{\text{max}}$ and AUC at steady state, were found to be significantly higher in genotype 1 infected patients with sustained viral response compared to patients with viral relapse or non-response (114).

There is apparently little to be gained from increasing the ribavirin dose in patients infected with genotype 2 or 3 (41, 54, 109). In the study of Jen et al (109) the slope of the concentration-response curve seems to level off in the higher range of concentrations in genotype non-1 infected patients. In Paper III there was a similar high response rate of 86 % both in patients with ribavirin plasma concentrations higher and lower than the median concentration. Because of this high response rate a much larger study, with a wider range of concentrations, is needed to define the concentration–effect relationship. In the randomized trial of Hadziyannis et al doses of 1000-1200 mg did not significantly improve viral response compared to a lower, fixed dose of 800 mg, for these genotypes(54).

In this population (genotype 2 and 3), the high response rate of more than 80 % raises the question if lower doses are sufficient, but this remains to be studied.

16.2.2 Anaemia

The findings in Paper II, that the most important side-effect of ribavirin is correlated to ribavirin plasma concentrations rather than dose per kg body weight, supports the hypothesis that ribavirin doses should be based on renal function and not body weight alone. Also others have been unable to correlate ribavirin dose to anaemia (96).
In Paper II, maximum effect on anaemia was obtained at approximately 8 µM. The dosing tradition at the time of the study possibly resulted in few patients achieving high ribavirin concentrations and dose reductions in patients non-tolerant to ribavirin-induced anaemia may have biased the slope of the curve.

The results in Paper II are congruent with an analysis of the incidence of haemolysis in liver transplanted patients undergoing combination treatment with interferon and ribavirin, where decreased creatinine clearance was significantly associated with haemolysis (115). The influence of renal function on ribavirin-induced anaemia has also been confirmed in a retrospective study by Sulkowski et al (91). In their analysis of 595 patients treated with standard interferon and ribavirin, lower baseline creatinine clearance, higher baseline haemoglobin levels and increased age were independently associated with increased risk of haemoglobin decrease of > 27.7 %.

The main finding of Paper II, that anaemia is related to ribavirin concentration, also points to the major limitation when investigating the concentration-effect relationship of ribavirin. In order to achieve a higher response rate in genotype 1 infected patients, anaemia can be perceived to be a severe risk. Previously, dose reduction has been recommended to manage ribavirin-induced anaemia. More recent data have found that adherence to therapy is a major factor contributing to sustained viral response (56) and that ribavirin dose reduction is associated with a decline in viral response rate (57). Thus, when investigating higher doses of ribavirin than currently used in clinical trials, plasma concentration monitoring must be performed.

Erythropoietin is an efficient method to control anaemia, and thus maintain ribavirin doses during hepatitis C treatment, but it is controversial considering the costs.

16.2.3 Safety and feasibility of high-doses ribavirin

To be able to study the concentration-effect relationship of ribavirin in patients infected with genotype I, higher doses resulting in higher plasma concentrations are needed. High viral load (> 2 milj copies/mL corresponding to approximately 800 000 IU/mL) is a factor associated with a reduced rate of sustained viral response in this population (54) and therefore we reasoned that this difficult-to-treat population could benefit from increased ribavirin doses. The basic pharmacokinetic model assumed in Paper I was used for the first time in chronic hepatitis C patients with normal renal function in Paper IV. Since this was a new dosing concept resulting in previously untested doses, 10 patients seemed a reasonably sized pilot population. Therapy adherence was important and therefore erythropoietin, blood transfusions and a high threshold for ribavirin dose reduction, was used. Cirrhotic patients were excluded for safety reasons, which of course reduces the scope of the results, especially in a subpopulation in such great need of curative treatment. The outcome of the study confirmed the high risk and magnitude of anaemia during treatment resulting in higher concentrations of ribavirin. This potentially life-threatening side effect must be emphasised. However, anaemia has been found to be associated with reduction in quality of life estimations reported by the
patients (92), which makes it even more interesting that the adherence to therapy in this study was the same (90%) as in the two large multicenter registration trials (86% versus 90%) (41, 42). When considering high-dose ribavirin as a treatment option, costs must also be addressed. In this study, additional costs include blood transfusions, hospitalisation, erythropoietin and additional doses of ribavirin. The lack of control group makes it impossible to estimate costs related to reduced working capacity. On the other hand, if this treatment is found to improve the response rate, the increased cost must be balanced against the insufficient treatment outcome of today.

16.3 FUTURE ASPECTS

The uniform dosing schedules of ribavirin today are contradictory to the variation in ribavirin elimination mainly based on renal function. Higher doses resulting in higher ribavirin plasma concentrations may be used to the benefit of genotype 1 infected patients in the future.

The probability of saturable ribavirin absorption must be acknowledged when higher doses are tested. A population pharmacokinetic analysis including higher plasma concentrations could result in a dose-predicting model of better accuracy for patients with normal renal function.

The problem of ribavirin induced anaemia needs to be further investigated if higher ribavirin doses are tested. We need to know more about erythropoesis and erythropoietin, with regards to doses and timing during antiviral treatment in hepatitis C infected patients. Costs related to higher ribavirin doses need to be assessed. A ribavirin prodrug less prone to result in anaemia seems promising. Phase III trials with viramidin are ongoing. Concentration-effect analysis of viramidine is essential to investigate.

In the future a controlled study investigating high-dose ribavirin would be of great interest. An obvious population would be non-responders on combination therapy with Peginterferon and ribavirin. This is a population to which no effective treatment is available, today. More knowledge concerning viral kinetics during high-dose ribavirin would also be of interest.
17 CONCLUSIONS

- In a population pharmacokinetic analysis, ribavirin clearance was strongly dependent on renal function. Ribavirin dosing should not be based on body weight alone. There was an important inter individual variability in ribavirin clearance, which suggests that therapeutic drug monitoring could be useful. A formula is presented, which can be used to estimate a ribavirin dose to reach a desired target concentration of ribavirin.

- The most important side effect of ribavirin, anaemia, depends on ribavirin plasma concentration, not on dose per body weight.

- A tendency towards a relation between ribavirin plasma concentration in genotype-1 infected hepatitis C patients and treatment outcome, however not significant, was found.

- It is feasible to treat patients with chronic hepatitis C with higher doses of ribavirin but serious side effects necessitates strict attention. The pharmacokinetic formula for ribavirin dose prediction needs to be revised for patients without renal impairment.

- Bioavailability declines with increasing ribavirin doses in a steady-state setting. This suggests that ribavirin absorption is saturable, which may have clinical implications for dosing. Long-term storage of ribavirin samples in whole blood at room temperature is not a stable storing condition. The best practice is to separate plasma and store samples frozen within 24 hours.
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and

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19 REFERENCES


