CHAPTER 19

TREATMENT OF CRIMEAN-CONGO HEMORRHAGIC FEVER

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19.1. CURRENT THERAPY: RIBAVIRIN USE AND HEMATOLOGICAL SUPPORT

Onder Ergonul

19.1.1. Ribavirin

19.1.1.1. Introduction

Ribavirin is a synthetic purine nucleoside analog with a modified base and D-ribose sugar, also known as virazol, first synthesized by Sidwell and colleagues in 1972 [43, 49] (Fig. 19-1). It is of particular interest, because it was the first synthetic nucleoside to exhibit broad spectrum antiviral activity, and it is one of few antiviral drugs in clinical use effective against agents other than HIV and herpesviruses [43]. It inhibits the replication of a wide range of RNA and DNA viruses in vitro, including orthomyxo, paramyxo, arena, bunya, flavi, herpes, adeno, pox, and retroviruses [49]. In current clinical practice, ribavirin is commonly used for certain viral infections (Table 19-1). Most notably, it is used in combination with interferon-α for treatment of HCV infection [66]. Ribavirin aerosol is used for treatment of pediatric infection by respiratory syncytial virus [19]. It is the only antiviral drug that could be also used in viral hemorrhagic fever syndromes. Besides Crimean-Congo hemorrhagic fever (CCHF), it is used in Lassa fever [70]. Viruses in the

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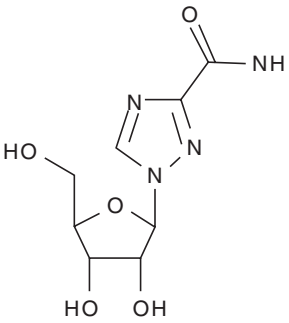


Fig. 19-1. The chemical structure of ribavirin.

Table 19-1. The activity spectrum of ribavirin

In vitro activity
Orthomyxo, paramyxo, arena, bunya, flavi, herpes, adeno, pox, and retroviruses
In vivo benefits
HCV
RSV
Influenza A and B
VHF: Lassa, hemorrhagic fever with renal syndrome, Rift Valley, CCHF
SARS

Bunyaviridae family are generally sensitive to ribavirin [92]. A prospective, randomized, double-blind, placebo-controlled trial of 242 patients with serologically confirmed Hantaan virus in the People's Republic of China found a sevenfold decrease in mortality among ribavirin-treated patients [54], other studies did not confirm these benefits. Ribavirin was found to be effective against CCHF virus (CCHFV) in vitro [99, 104].

19.1.1.2. Mechanisms of action

More than 30 years since its discovery, the mechanism of action of ribavirin still remains controversial. Ribavirin is clinically administered as the nucleoside. Adenosine kinase is the cellular enzyme responsible for conversion to ribavirin monophospate (RMP) (Box 19-1).

The antiviral mechanism of ribavirin is not fully defined but relates to alteration of cellular nucleotide pools and inhibition of viral messenger RNA synthesis [97]. Intracellular phosphorylation to the mono, di, and triphosphate derivatives is mediated by host cell enzymes. In both uninfected and respiratory syncytial virus (RSV)-infected cells the predominant derivative is the triphosphate, which has an intracellular elimination half life of less than 2 h [76]. Ribavirin triphosphate is generally the predominant metabolite [76].

The broad spectrum antiviral activity of ribavirin can potentially be attributed to its multiple mechanisms of action. As a purine analog, it can function in multiple cellular and viral processes. An important aspect of the antiviral activity of ribavirin may stem from the ability to act via multiple mechanisms simultaneously [43]. Five distinct mechanisms were suggested. Some of these actions potentiate others [49]. Ribavirin monophosphate competitively inhibits cellular inosine-5′- phosphate dehydrogenase and interferes with the synthesis of guanosine triphosphate (GTP) and thus nucleic acid synthesis in general. Ribavirin triphosphate also competitively inhibits the GTP dependent 5′-capping of viral messenger RNA. Ribavirin is an inhibitor of inosine monophosphate dehydrogenase, an inhibitor of RNA capping, a polymerase inhibitor, a lethal mutagen, and an immunomodulatory agent [43].

19.1.1.2.1. Ribavirin as an immunomodulatory agent

Ribavirin has also been postulated to act via another indirect antiviral mechanism, by enhancing the host T-cell response. This conclusion stems from observations in hepatitis C virus (HCV) infected patients that ribavirin can reduce serum alanine aminotransferase (ALT) levels without significantly reducing levels of circulating HCV RNA as determined via PCR [25]. The ribavirin is thought to induce a switch in T-helper cell phenotype from type 2 to type 1 [57]. The T-helper type 1 response is associated with cellular immunity and with expression of IL-2, γ-interferon, and tumor necrosis factor-α [71].

In vitro inhibitory concentrations of ribavirin may reversibly inhibit macromolecular synthesis and proliferation of uninfected cells, suppress lymphocyte responses [50], and alter cytokine profiles in vitro. However, this effect was not studied in vivo. The cytokines IL-6 and TNF-α were found to be higher among fatal CCHF patients, whereas there was no significant difference in the levels of IL-10 between the favorable and fatal cases [29]. However, the immunomodulatory effect of ribavirin was not studied on CCHFV yet.

19.1.1.3. In vitro activity of ribavirin against CCHFV

In an in vitro study, ribavirin was shown to inhibit the viral activity, and some CCHF viral strains appeared more sensitive than others [104]. In contrast, a dose of ribavirin at least nine times greater was required to induce a comparable inhibitory effect on the yields of Rift Valley fever virus, for which the drug has been shown to inhibit replication in monkeys and rodents [104]. After intraperitoneal infection of infant mice with CCHF virus, virus titers in liver remained significantly higher than in other organs except serum. Within the liver, virus antigen was first found by immunofluorescence assays (IFA) in Kupffer cells followed by more extensive hepatic spread. Later, virus was found in other organs including brain and heart. Ribavirin treatment significantly reduced infant mouse mortality and extended the geometric mean time to death. Ribavirin treatment reduced CCHF virus growth in liver and significantly decreased, but did not prevent, viremia [99].

19.1.1.4. Clinical observational studies on ribavirin

The clinical data related to effectiveness of ribavirin stems from the studies on chronic infection with the HCV infection. The effect of ribavirin use in CCHFV infection was not evidenced by randomized clinical trials. The effectiveness of its use was described by observational studies [26, 33, 67, 74]. In the first clinical report in 1995, the observation was limited to three health-care workers (HCW), who had been infected with CCHFV infection [33]. All three patients were severely ill with low platelet and white blood cell counts, raised aspartate transaminase, and evidence of impaired haemostasis. According to severity criteria defined by Swanepoel [95], all had an estimated probability of death of 90% or more. The patients became afebrile, and their haematological and biochemical abnormalities returned to normal within 48 h of ribavirin treatment, and all made a complete recovery. This finding was found to be encouraging for the use of ribavirin in CCHFV infections, but did not constitute evidence of efficacy. After this study, ribavirin was introduced to be used in CCHFV infections.

Mardani et al, compared the fatality rate among patients suspected of having CCHF who received treatment with oral ribavirin and those who did not [67]. As many as 97 (69.8%) of 139 treated patients suspected of having CCHF survived, and 61 (88.9%) of 69 treated patients with confirmed CCHF survived. The efficacy of oral ribavirin was 80% among patients with confirmed CCHF and 34% among patients suspected of having CCHF. This study used historical control, and did not stratify the patients according to the severity of the patients.

Ergonul et al, described the role of ribavirin therapy for 35 confirmed CCHF patients. About 86% of the patients were considered to have severe cases of CCHF. Eight patients were given ribavirin, and all eight of them survived. The study results suggested the use of ribavirin especially for the severe cases [26] (Fig. 19-2).

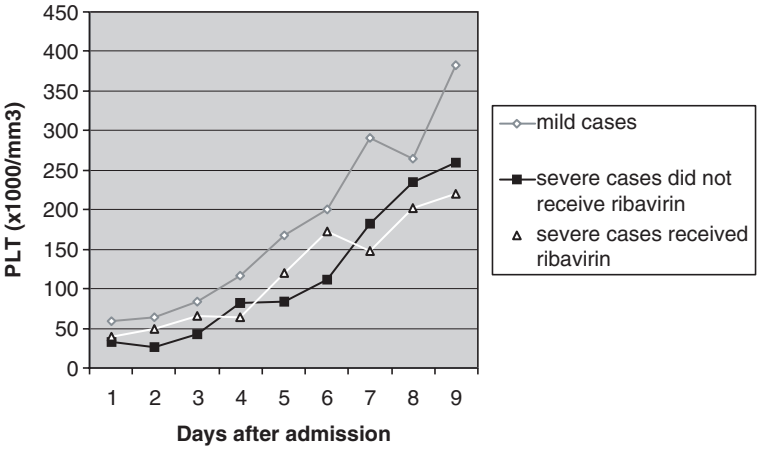


Fig. 19-2. The course of platelet count after admission.

The cases in this study were grouped according to the severity criteria defined by Swanepoel [95].

Ozkurt et al. described the efficacy of ribavirin therapy for CCHF among 60 confirmed CCHF patients in Eastern Turkey. Mean recovery time was shorter in the cases treated with ribavirin than those of control. But, the need for blood and blood product, mean hospitalization duration, fatality rates, and hospital expenditure values were not significantly different between two group of patients, who received ribavirin or not [74].

Almost all the authors claimed that, they could not perform a randomized clinical trial (RCT), because of ethical constraints. The lack of randomization is the main criticism for these observational studies. However, sometimes the observational studies could give qualified information, if they could be well designed [101]. But, the researchers should be aware of the potential confounders. While performing the clinical outcome studies on ribavirin use in CCHF, there are significant confounders;

1. Severity of the infection might differ. There are mild and severe forms of the disease, because of several reasons. (see also Chapter 16).
2. Number of days from onset might differ. Some patients could get the ribavirin at an earlier, prehemorrhagic phase of the infection, whereas some patients could get the ribavirin at a later phase, hemorrhagic phase of the infection.
3. The severity of the gastrointestinal symptoms might differ. Some patients cannot get drug via oral route, because of severe hematemesis. This parameter is important in pharmacological effectivity.

If the ribavirin was saved for the severe cases, an observational comparative study between the ribavirin given and not given group would have a misclassification bias in favor of not using ribavirin. The patients who would not receive ribavirin would be usually the mild cases. A well designed observational study should minimize the effect of these confounders to avoid the misclassification bias.

According to the clinical observation of the cases, the effective treatment for CCHF could be considered hypothetically in two phases (Fig. 19-3). The first phase, starts from the exposure to the virus, and characterized by the viremia, which usually lasts 5–15 days. In clinical terms, this period starts from the onset of prehemorrhagic symptoms (fever, myalgia, nausea, and vomiting), and ends up with the bleeding from various sites. The antiviral effect of the ribavirin most likely occurs in the first phase. The second phase is characterized by the decline of viremia, but bleeding from the various sites starts. In this phase, cytokines are released extensively, and the coagulation cascade is disrupted in some patients, and disseminated intravascular coagulation was noted [29]. The antiviral action of the ribavirin is less likely in this phase. The immunomodulatory effect is not known yet. Alternative drugs targeted to disseminated intravascular coagulation (DIC) or sepsis could be considered in the second phase of the disease course.

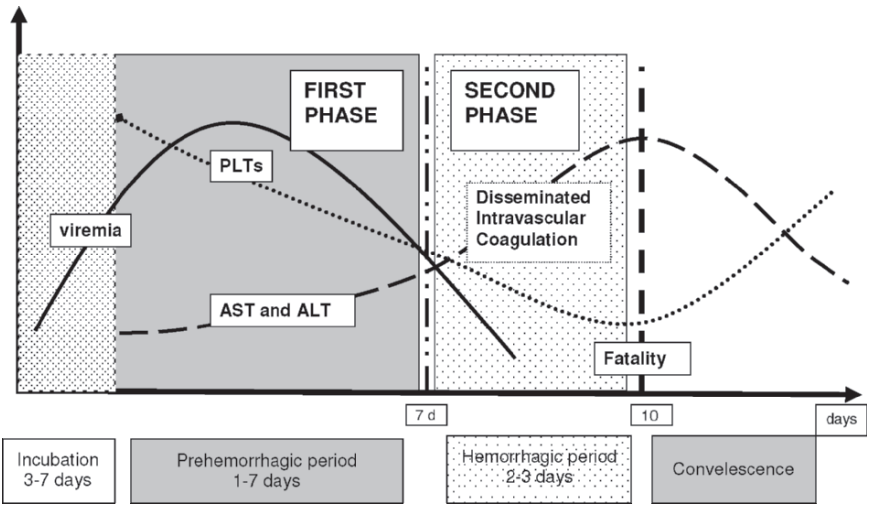


Fig. 19-3. The probable role of the ribavirin and alternatives in Crimean-Congo haemorrhagic fever (CCHF) infection course. The effective treatment for CCHF should be considered in two phases. The first phase, starts from the exposure to the virus, and characterized by the viremia, which usually lasts 5–15 days. In clinical terms, this period starts from the onset of prehemorrhagic symptoms (fever, myalgia, nausea, and vomiting), and ends up with the bleeding from various sites. Ribavirin is most likely to be effective in the first phase. The second phase is characterized by the decline of viremia, and clinically the onset of bleeding from the various sites. The immunologic mechanisms, such as cytokine storm, disrupt the coagulation cascade in some patients, and disseminated intravascular coagulation occurs. For these patients other treatment alternatives should be considered. Most likely, the ribavirin is less effective in this phase. Ribavirin could be effective in second phase, mainly because of its immunomodulatory effect. However, immunomodulation was not suggested as the main action for ribavirin.

19.1.1.5. Absorption, distribution, and elimination

Ribavirin is actively taken up by gastrointestinal nucleoside transporters located in the proximal small bowel, and oral bioavailability averages approximately 50% [49]. Extensive accumulation occurs in plasma, and steady state is reached by about 4 weeks. Food increases plasma levels substantially, so ingestion with food may be prudent [49]. Following single or multiple oral doses of 600 mg and 1,200 mg, peak plasma concentrations average 0.8 μg/mL and 3.7 μg/mL, respectively. After intravenous doses of 1,000 mg and 500 mg, plasma concentrations average approximately 24 μg/mL and 17 μg/mL, respectively.

The apparent volume of distribution is large (~10 L/kg) due to ribavirin's uptake into cells. Plasma protein binding is negligible. The elimination of ribavirin is complex. The plasma half life averages 30–40 h after a single dose but increases to approximately 200–300 h at steady state. Ribavirin triphosphate

concentrates in erythrocytes, and red blood cell levels gradually decrease with a $t_{1/2}$ of about 40 days. Hepatic metabolism and renal excretion of ribavirin and its metabolites are the principal routes of elimination. Hepatic metabolism involves deribosylation and hydrolysis to yield a triazole carboxamide. Ribavirin clearance decreases threefold in those with advanced renal insufficiency (CL_{cr} 10–30 mL/min); the drug should be used cautiously in patients with creatinin clearance of less than 50 mL/min [49].

Oral and intravenous forms are available in many countries, and the dosage was given in Box 19-2. The total duration of treatment was defined as 10 days [33]. If the laboratory results get better, then the ribavirin could be stopped. The effectivness of IV and oral forms were not compared yet.

Emergence of viral resistance to ribavirin has not been documented in most viruses but has been reported in Sindbis and HCV [107], although it has been possible to select cells that do not phosphorylate it to active forms.

19.1.1.6. Adverse events

Systemic ribavirin causes dose-related reversible anemia due to extravascular hemolysis and suppression of bone marrow [53]. Associated increases occur in reticulocyte counts and in serum bilirubin, iron, and uric acid concentrations. High ribavirin triphosphate levels may cause oxidative damage to membranes, leading to erythrophagocytosis by the reticuloendothelial system [49]. The half life of ribavirin metabolites is relatively short in cultured fibroblasts and lymphoblasts, although the nucleotides are much more stable in erythrocytes [76]. This accumulation of ribavirin in erythrocytes is responsible for the reversible hemolytic anemia that is a side effect of clinical ribavirin therapy [1].

Bolus intravenous infusion may cause rigors. About 20% of chronic hepatitis C patients receiving combination interferon–ribavirin therapy discontinue treatment

Box 19-1. The action mechanism of antiviral agents against RNA viruses

The replication strategy of the RNA viruses relies either on enzymes in the virion (the whole infective viral particle) to synthesize its mRNA or on the viral RNA serving as its own mRNA. The mRNA is translated into various viral proteins, including RNA polymerase, which directs the synthesis of more viral mRNA [97].

Box 19-2. The dosage of ribavirin in CCHF infections

About 30 mg/kg as an initial loading dose, then 15 mg/kg every 6 h (4 × 1 g) for 4 days, and 7.5 mg/kg every 8 h (4 × 0.5 g) for 6 days was recommended [27].

early because of side effects. In addition to interferon toxicity, oral ribavirin increases the risk of fatigue, cough, rash, pruritus, nausea, insomnia, dyspnea, depression, and particularly anemia. About 8% of patients require ribavirin dose reduction because of anemia. Hemolytic anemia, hypocalcemia, and hypomagnesemia were reported in patients, who received ribavirin because of severe acute respiratory syndrome (SARS) [17, 63].

However, no adverse event related to ribavirin therapy was noted among CCHF patients. Mainly because of two reasons, (1) acute course of the disease, that might not allow time to observe the side effects, and (2) overshadowing of the disease findings, which are the same with the potential adverse events, such as anemia.

Preclinical studies indicate that ribavirin is teratogenic, embryotoxic, oncogenic, and possibly gonadotoxic. In vivo genotoxicity of ribavirin among three patients with CCHF were studied [98]. The micronucleus and the sister chromatid exchange test were found to be higher among all three patients, during and right after the ribavirin therapy. A month later, the test results became normal. This finding revealed that ribavirin has a reversible in vivo genotoxic effect in humans [98]. To prevent possible teratogenic side effects, up to 6 months is required for washout following cessation of long-term treatment [49]. The use of ribavirin is contraindicated among pregnant women. Ribavirin is in Food and Drug Administration (FDA) pregnancy category X.

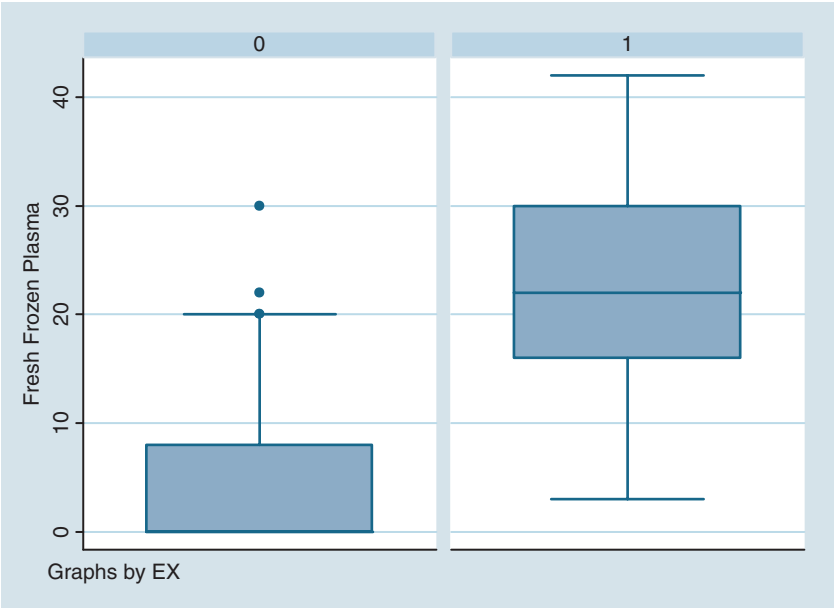
19.1.2. Supportive therapy

Potential bleeding foci of the patients should be considered and conservative measures should be taken, such as use of histamine receptor blockers for peptic ulcer patients, avoidance of intramuscular injections, and not using aspirin or other drugs with actions on the coagulation system. Non-steroidal anti-inflammatory drugs should be avoided. Fluid and electrolyte balance should also be monitored meticulously.

Supportive therapy is the essential part of the case management. It includes the administration of thrombocytes, fresh frozen plasma (FFP), and sometimes erythrocyte preparations. The replacement therapy with these blood products should be performed by checking the complete blood count, which should be done one or two times a day. In clinical practice, checking the thrombocyte level once a day would be sufficient [27].

Thrombocyte solution or FFP are the products to be replaced according to the deficit of the individual patient. Figure 19-3 depicts the tremendous amount of thrombocyte solution and FFP that were given to the patients in Turkey [28]. The fatal cases received significantly ($p < 0.001$) higher amount of thrombocyte suspensions and FFP (Fig. 19-4).

Sometimes, it is hard to decide where to start or stop the blood product replacement. Therefore, we reviewed the general rules of platelet and FFP use by adapting to CCHFV infection.



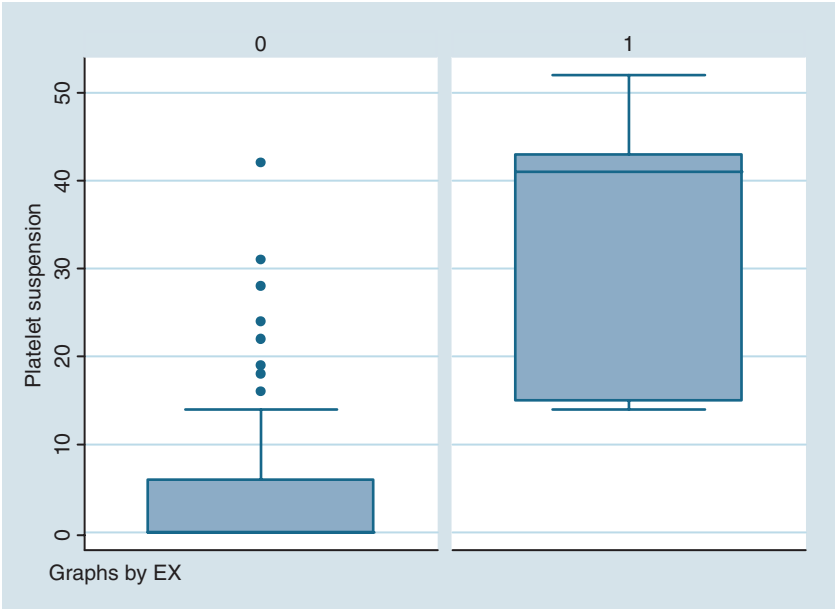


Fig. 19-4. The number of units of (A) fresh frozen plasma or (B) platelet suspensions given to CCHF patients. The box plots on the right represents total units given to patients who scummed to the disease and, on the left, those who survived.

19.1.2.1. Platelets

Random-donor platelets (RDPs): Platelet concentrates are prepared by separating platelets from a single unit of whole blood so that a minimum of 5.5×10^{10} platelets are suspended in approximately 50 mL of plasma (1 U). Platelets should be separated from whole blood within 8 h of collection. Depending on the container in which RDPs are stored, the shelf life varies from 3 to 5 days when stored at room temperature.

Pheresis–apheresis platelets or single-donor platelets (SDPs): Platelets collected by apheresis techniques from a single donor, using blood cell separators, contain a minimum of 3.0×10^{11} platelets suspended in 200 to 400 mL of plasma. Apheresis platelets have a shelf life ranging from 24 h to 5 days and are stored at room temperature. These products account for most platelet transfusions in the developed countries.

HLA-matched platelet concentrates: If the donor of an apheresis platelet concentrate is selected because the donor's human leukocyte antigen (HLA) type is matched to the recipient's HLA type (due to the development of alloimmunization to HLA), the apheresis platelet product is considered to be an HLA-matched platelet component.

Use of platelet transfusions is indicated to control active bleeding or to prevent hemorrhage associated with a deficiency in platelet number or function. Platelets are used prophylactically to prevent bleeding when the platelet count is less than 10,000–20,000/μL. There is a growing trend to reduce the prophylactic platelet transfusion "trigger" to counts as low as 5,000–10,000/μL in stable patients without significant hemorrhage [69].

Dose and infusion rate:

RDPs The average adult platelet concentrate dose is one unit of RDP per 12 kg of body weight. There is a growing trend in the United States to define a standard "dose" of platelet concentrates. This standard varies among different institutions from 4 U, 6 U, or 8 U of platelet concentrates to be infused per transfusion episode. Platelet units usually are pooled into one bag by the transfusion service prior to issue. They should be infused within 4 h at a rate that depends on the patient's ability to tolerate the volume. RDPs may be "volume reduced; that is, the pooled RDPs undergo centrifugation that allows separation of platelets from the platelet poor plasma. Excess platelet poor plasma is removed, and the therapeutic dose then is concentrated into approximately 100 mL. Volume-reduced platelets are infused more rapidly than nonvolume-reduced platelets and must be infused within 4 h of pooling [69].

Pheresis platelet-SDPs. One SDP concentrate is considered a therapeutic dose, equivalent to six units of RDP. SDPs should be infused as rapidly as possible, depending on the patient's ability to tolerate the infused volume.

HLA-matched platelets. The dose and infusion rate are similar to those of SDPs.

Expected outcome: Platelet transfusions should result in prevention or resolution of bleeding caused by thrombocytopenia or platelet dysfunction. As a general rule, platelet counts should be obtained 18–24 h post-infusion. The average-sized adult who receives 1 U of RDP per 12 kg of body weight or one SDP dose should have a posttransfusion platelet increment of 30,000–50,000 μL. Patients who have smaller or no increment at 18–24 h post-infusion should have platelet counts performed 10–60 min after the next platelet transfusion. If the 10 to 60 min posttransfusion increment is minimal or not increased at all, the possibility of refractoriness caused by alloimmunization should be entertained. Antibody-related platelet destruction is often related to the development of HLA-specific antibodies in response to foreign donor HLA antigens [79]. The patient then should be considered for HLA-matched platelet transfusions or crossmatched platelet concentrates. Poor posttransfusion platelet survival, in addition to being caused by alloimmunization, is often seen in conjunction with fever, sepsis, disseminated intravascular coagulation, and others. Patients in these settings are considered to be refractory on a nonimmunologic basis and are not expected to benefit from HLA-matched platelet transfusions. Patients who have life-threatening hemorrhage may require larger platelet doses to be therapeutic. Currently, controversy is growing as to whether patients requiring prophylactic platelet transfusions would benefit more from repetitive small-dose platelet transfusions or be better off using extra high-dose platelet transfusions. Randomized, well-controlled studies to answer this issue have not yet been done [69].

In CCHF, since disseminated intravascular coagulation occurs in the disease course, platelet destruction is expected, and the rapid increment of PLT level after transfusion may not be observed.

19.1.2.2. Fresh frozen plasma

FFP is plasma that is separated from whole blood and frozen within 8 h of collection and has normal levels of all clotting factors and anticoagulants. Three different types of FFP are available for transfusion: (i) Standard FFP is as just described; (ii) Donor-retested FFP is plasma that is donated, frozen, and stored for 112 days. The donor then returns, and if all infectious disease testing is still negative, the original unit is released into the inventory. Donor-retested plasma has the value of closing the window period for the infectious diseases that are currently tested for HIV, HVC; (iii) pooled solvent detergent-treated plasma is frozen, and plasma has been sent to a commercial company to be treated by a solvent detergent process that will prevent the transmission of envelope viruses (i.e., HIV, HCV). The value of this product is that it will prevent transmission of any envelope virus by blood transfusion. It is a pooled product, and recipients are exposed to 1,500–2,500 donors with each transfusion of a unit of frozen plasma [69].

FFP is indicated to replenish clotting factors in patients with demonstrated deficiencies, such as prothrombin time or partial thromboplastin time greater than 1.5 times normal, international normalized ratio (INR) > 1.6. FFP is most commonly used in the setting of acquired coagulopathy, such as in patients with liver disease, DIC, or excess warfarin effect [69].

The average adult dose is determined by the clinical situation and the underlying disease process. It is reasonable to administer plasma at a dose of 10–15 mL/kg of body weight (2–4 U of FFP) followed by laboratory evaluation to determine responsiveness and to decide the interval between doses. The infusion rate is determined on the basis of the patient's clinical need and hemodynamic status. Plasma does not contain red cells, and therefore, cross-matching is not required. The ABO type of the donor should be compatible with the recipient. FFP is thawed at 37°C and must be transfused with 24 h of thawing if used for coagulation factor replacement. Plasma can be thawed and maintained in the refrigerated state for up to 5 days, but there is some loss of coagulation factor V and a greater decrease in factor VIII [69].

Expected outcomes: Improvement of coagulation factor deficiency is expected as assessed by the prothrombin time (INR), partial thromboplastin time, or specific factor assays [69].

The other alternatives could be considered the possible pathogenetic mechanisms of the infection. The therapeutic agents, that were considered in hemophagocystosis and DIC could also be studied in CCHF infection. The agents considered in DIC are shown in Table 19-2. Corticosteroids accompanied with ribavirin were reported to be useful at the early stage of CCHF [60]. However, this experience was limited to six patients, only.

An algorithm for the management of the cases was suggested in Table 19-3.

Table 19-2. Treatment modalities for disseminated intravascular coagulation [35] (Box 19-3)

1. Replacement therapy	Fresh-frozen plasma
2. Anticoagulants	Unfractionated and low molecular weight heparin
	Danaparoid sodium
	Recombinant hirudin
	Recombinant tissue factor pathway inhibitor
	Recombinant nematode anticoagulant protein c2
3. Restoration of anticoagulant pathways	Antithrombin
	Recombinant human activated protein C
4. Other agents	Recombinant activated factor VII
	Antifibrinolytic agents
	Antiselectin antibodies
	Recombinant interleukin-10
	Monoclonal antibodies against TNF and CD14

Table 19-3. An algorithm for the case management

Evaluation of the suspected case
Clinical symptoms (fever, myalgia, bleeding from various sites)
Patient history i. Referral from endemic area ii. Outdoor activities (picnic, tracking, etc.) in endemic area iii. History of tick exposure iv. Exposure to potentially viremic domestic animal blood
Laboratory tests (low platelet and high white blood cell count, elevated AST, ALT, LDH, CPK)
Preventive measures a. Isolate the patient b. Inform and educate colleagues and staff c. Use the barrier precautions
Investigations for confirmation Serum for PCR (early in disease) and ELISA (late in disease or convalescence) a. IgM positivity or PCR positive confirms diagnosis, IgG positivity cannot b. Sera for differential diagnosis
Decision making for therapy 1. Ribavirin 2. Do not neglect other causes of clinical picture. Starting doxycycline or equivalent should be considered 3. Hematological support a. Fresh frozen plasma to improve hemostasis b. Thrombocyte solutions 4. Respiratory support
Follow-up 1. No relapse occurs after the disease. Therefore there is no need for the follow up of the cases 2. HCWs exposed to the virus should be followed up with complete blood counts and biochemical tests for 14 days

Box 19-3. Disseminated intravascular coagulation and CCHF

Disseminated intravascular coagulation (DIC) results from activation of coagulation in the vascular tree. Accelerated platelet consumption is almost always seen. DIC can be distinguished from immune thrombocytopenia by finding prolongation of the prothrombin and partial th romboplastin times, decreased plasma fibrinogen, and elevated plasma fibrin–fibrinogen split products. DIC can be seen with infections (e.g., viral, Rickettsial, bacterial, malarial infections); obstetric catastrophes (abruptio placentae and the retained dead fetus syndrome); malignancies; trauma; and vascular abnormalities such as giant hemangiomas and aortic aneurysms. Generally, the treatment should be directed toward correcting the underlying cause. Support with plasma and platelet transfusions may be required for bleeding complications until the cause has been corrected. The thrombocytopenia gradually resolves as the infection is controlled [35, 108].

19.2. OLD AND NEW TREATMENT STRATEGIES

Ali Mirazimi

19.2.1. Introduction

Historically, treatment for Crimean-Congo hemorrhagic fever (CCHF) is limited and very poorly studied. However hemorrhagic fever virus infections can be approached by the following different therapeutic strategies [6]: (i) administration of high-titered neutralizing antibodies and/or (ii) treatment with antiviral drugs.

In more recent times, an immunotherapy approach has been described that uses passive transfer of CCHF convalescence sera from recovered patients [102]. The specific use of immunotherapy as a therapeutic approach will be discussed in Section 19.1.

Depending on the site of interactions and their molecular target, anti-viral drugs may be classified as follows: orotidine monophosphate decarboxylase inhibitors (i.e. pryrazofurin) [5, 96], inosine monophosphate dehydogenease inhibitors (i.e. ribavirin and its derivates) [11, 26, 89, 92], cytidine triphosphate Synthase inhibitors (i.e. cyclopentenylcytosine) [5, 68], S-adenosylhomocysteine hydrolase inhibitors (i.e. neplanocin A) [5], polyanionic substances (i.e. sulfated polymers) [8–10] and interferons and immunomodulaterors [90, 91].

Currently, there is no specific antiviral drug against CCHF, however, reports exist which describe a potential antiviral effect of ribavirin [26, 99]. It should be mentioned that no randomized, controlled studies exist, which confirms the potency of ribavirin against CCHF.

Another interesting area of treatment is the antiviral activity of interferons and immunomodulators against viral infections. Almost all hemorrhagic fever viruses have been shown to be sensitive to interferons [4, 7, 20, 23, 48]. However, in order to develop an agent, whose function exploits the antiviral activities of interferon, more knowledge of the antiviral mechanism of interferon against CCHFV is required. The role of interferon and the interferon-induced antiviral proteins will be discussed in Section 19.2.2.

During past years, several groups have become interested in studying CCHFV as it has a potential for being abused as a bioterrorism weapon; such research has increased our knowledge of the basic biology of the CCHFV [3, 4, 13, 34, 40, 46, 62, 85, 103]. This may lead to an improvement in therapy; an example could be the finding of inhibitors of the viral protein processing. During the last decade, the potential of RNA interference as a therapeutic agent against virus infection has been discussed. Recently many studies have displayed the efficacy of chemically synthesized short interference RNA as a tool to combat a diverse group of viruses. However it should be mentioned that before RNA interference can be used as therapeutic agents several questions need to be addressed, such

as: (1) the transient effect of short interference RNA, (2) delivery methods, and (3) cell uptake [15].

19.2.2. Interferons and interferon-stimulated antiviral proteins

Host responses to infection comprise two general categories: (1) Innate immunity, a rapid and phylogenetically nonspecific response to infection [61], and (2) adaptive immunity, long lived, and highly-specific immune response to infection [39, 75].

Innate immunity has been described as an essential immune response against emerging viruses. Central to the innate immune response is a large heterogeneous group of peptide mediators (interferons, chemokines, interleukins, and growth factors), these molecules are part of a complex network that regulates the immune and inflammatory responses [14, 61, 82, 100].

Interferons are produced by leukocytes and fibroblasts in response to viral infection [44, 45] and results in the induction of antiviral pathways within a period of hours postinfection. The interferons have a long history of clinical application against some viral diseases [18]. As early as 1986, interferon-α was demonstrated to have a potent antiviral effect in patients with chronic hepatitis C [86].

In recent times, several groups have studied the antiviral activities of interferons against hemorrhagic fever viruses. These studies clearly demonstrate that interferons have significant antiviral activities against most of all hemorrhagic fever viruses in vitro and animal models. However, to date, no clinical data has addressed the effect of interferons against viral hemorrhagic fever and in particular CCHFV. Moreover, the use of interferons as a therapeutic agent against hemorrhagic fever diseases poses significant clinical challenges. A better understanding of interferons action is essential if current therapy is to be optimized and also if new strategies therapy are to be approached.

Isaacs and Lindenmann have discovered the antiviral activity of interferon class of molecules almost 50 years ago [58, 59]. Today interferons are divided into two groups, interferon types I and II. The type I interferons are major players in antiviral defense against almost all known viruses [48]. The type I interferons includes interferon-α, -β, and -λ [12, 87]. There are at least 14 subtypes of interferon-α genes, but only one β and λ gene. All type I interferons have antiviral activity as well as antiproliferative and immunomodulatory activities. Virus-infected or stimulated cells upregulate and secretes interferon type I. The secreted interferons induce other cells to express potent antiviral proteins and to activate additional antiviral mechanisms that will limit the viral spread. The antiviral activity of interferon-α and -β is not directly coupled to the interferon molecule per se. Rather, these molecules act indirectly, by inducing interferon-stimulated genes (ISGs), which in turn establish an antiviral phase in the target cells.

Type I interferons trigger the expression of a large number of ISGs. These ISGs have antiviral, antiproliferative, and immunomodulatory functions [21, 22]. Interferon-induced proteins include transcription factors, enzymes, cytokines, chemokines, and glycoproteins, although a large number of molecules need further characterization. Until now, a few antiviral proteins have been studied in detail. The best-characterized interferon-induced antiviral proteins are the Mx GTPase [64], the 2′-5′ oligoadenylate synthetases (2–5 OAS)/RNaseL [93], and the protein kinase R (PKR) [105].

MxA has been found to be one of the major antiviral proteins against a large group of viruses, including viruses of the Ortomyxo, Paramyxo, Togoviridae, Rhabdoviridae, Picrnaviridae, and Hepdnaviridae families [36–38, 47, 52, 65]. MxA belongs to the dynamin super family of large GTPases, whose members function in a variety of intracellular transport process. Recent studies have clearly demonstrated that MxA has a significant antiviral activity against CCHFV [3, 4]. Andersson and coworkers demonstrated that MxA colocalizes and interacts with CCHFV nucleocapsid protein in the perinuclear region of infected cells; it was therefore suggested that this interaction inhibits the virus replication process. Similar results have been observed for other members of Bunyaviridae [37, 64, 81]. These results together imply that all animal bunyaviridae may be restricted in their intracellular growth by MxA, and probably by the same mechanism.

Another recent report by Andersson and co-workers showed that ISGs, induced by interferon-α in human endothelial and hepatoma cells was sufficient to inhibit CCHFV growth significantly [4]. Yet, despite this effect, why does CCHFV still run its devastating course? It has been estimated that CCHFV causes mortality at rates belying tween 30–50% in infected patients. The most likely explanation is that interferon is induced at insufficient levels or too late in the course of infection to put MxA and other antiviral proteins in place to combat the CCHFV when it is urgently needed.

Additional proteins with potentially important antiviral activities are ISG20 [30–32], P56 [55, 56], RNA-specific adenosine deaminase 1 (ADAR 1) [83], promyelocytic leukemia protein (PML) [80] and guanylate-binding protein 1 (GBP-1) [2]. ISG20, an interferon-induced exonuclease, specifically degrades ssRNA [31]. Expression of ISG20 inhibits viral replication of vesicular stomatitis virus and human immunodeficiency virus [30, 32] in cell culture. Recent studies by Weber and Mirazimi have clearly shown that ISG20 has an antiviral activity against CCHFV by so far unknown mechanism (unpublished).

There is a critical need to identify new effective treatments for viral hemorrhagic fever and in particular CCHFV. One of the interesting issues is the interferons and other immunomodulators. Our present knowledge of the antiviral activity of interferon system is still limited. However, a better understanding of the complicated interaction between viruses and innate immune response will help to design new antiviral treatments and therapy.

19.3. ANTIBODIES TO CCHFV FOR PROPHYLAXIS AND TREATMENT

Dimiter S. Dimitrov

Infections with CCHFV elicit antibodies to the virus that are not present several (about 5–9) days after onset of illness [78, 88]. Patients who have died of CCHF do not usually develop a measurable antibody response [78]. It is likely that even if such patients did have low levels of CCHFV-specific IgG they still could be reinfected. For example, it was shown for West African sheep, which play a central role in the maintenance cycle of CCHF virus in disease-endemic areas because they serve as host for both the virus and the tick vector, that even sheep that were infected previously and had anti-CCHF virus IgG can be reinfected and transmit the virus [42, 106]. CCHFV-specific IgM remains elevated for 40 days after infection [42] but there are no reports whether such elevated levels could protect from infection or reinfection. However, such antibodies could play a protective role in secondary transmissions. The virulence of the virus is likely diminished in such transmissions possibly due to existence of subpopulations of virus adapted to a host that are selected after passage through another vertebrate host; such subpopulations seem to be less virulent and might have an altered capacity of transmission [41]. This is consistent with the observation that infectivity from secondary cases is unusual [94] unless heavy exposure to virus contaminated tissue has occurred [1, 73, 77]. However, the efficacy of treatment in such secondary transmissions is difficult to evaluate because of lack of controls and because the risk for fatal outcome of the secondary case is not so high. There is currently no specific antiviral therapy for CCHF approved for use in humans by the FDA.

A formalin-inactivated suckling mouse brain-based vaccine has been used in Bulgaria and other parts of Eastern Europe and the former Soviet Union for protection from CCHFV infections. In the Rostov region of the former Soviet Union, 1,500 persons received the vaccine and showed a high frequency of detectable antibodies. Likewise, vaccine was given to several hundred human volunteers in Bulgaria, with resulting high antibody induction. With the relatively small target population of persons at risk for contracting CCHFV, the large-scale development and production of a CCHF vaccine by modern standards seems unlikely.

There was an early recognition of the possible benefits of treatments using serum prepared from the blood of recovered CCHF patients or gammaglobulin obtained from immunization of horses [51]. In more recent times, Bulgarian investigators suggested that immunotherapy treatment of seven patients with severe CCHF via passive simultaneous transfer of two different specific immunoglobulin preparations, CCHF-bulin (for intramuscular use) and CCHF-venin (for intravenous use), prepared from the plasma of CCHF survivor donors boosted with one dose of CCHF vaccine, resulted in quick recovery of all patients [102]. No side effects were observed and the patients were discharged

O. Ergonul and C. A. Whitehouse (eds.), Crimean-Congo Hemorrhagic Fever, 261–269.

in good health. Although there was no control group of cases of the same severity (an average of 25 cases per year are reported in Bulgaria), and firm evidence of its value is lacking, previous experience suggested that in such cases the prognosis is unfavorable even when the patients are treated with the intramuscular formulation (CCHF-bulin) – for 4 years (1985–1988) 15 such patients have died. The investigators attributed the success of this approach to the quick distribution of the antibodies through the circulation that is important for acute infections combined with a sustained and even increasing antibody concentration from the muscle depot. They suggested that the intravenous preparation be used for treatment of all cases of CCHF.

Advances in development of monoclonal antibodies and antibody engineering have raised hopes for new candidate drugs for prevention and treatment of CCHFV infections. Immunotherapy of CCHF has been based on polyclonlal antibodies obtained from serum of immunized animals or humans. Although such preparations could be effective, the use of monoclonal antibodies could allow better control of the composition of the therapeutic preparations and in some cases could be more effective. For example, the humanized monoclonal antibody Synagis, which is the first and only monoclonal antibody yet licensed for an infectious disease, is the preferred of the two available licensed products in most situations (the second one is the RSV-IGIV, RespiGam, which is a solution of IgG enriched in neutralizing antibodies to RSV). However, the mAbs recognize a single epitope that may limit their usefulness against pathogens that exhibit genetic variations [16]. This problem could be solved by generating mAbs against conserved epitopes and/or use of mAb cocktails [16, 24].

Recently, mouse monoclonal antibodies (mmAbs) specific for the two CCHFV envelope glycoproteins (Envs), G_N and G_C, were developed [13]. In neutralization assays on SW-13 cells, mmAbs to G_C, but not to G_N, prevented CCHFV infection. However, only a subset of G_C mmAbs protected mice in passive-immunization experiments, while some non-neutralizing G_N mmAbs efficiently protected animals from a lethal CCHFV challenge. Thus, neutralization of CCHFV likely depends not only on the properties of the antibody, but on host cell factors as well. In addition, non-neutralizing antibody-dependent mechanisms, such as antibody-dependent cell-mediated cytotoxicity, may be involved in the in vivo protection seen with some of the mmAbs.

Mouse antibodies could induce anti-mouse antigen immune responses and should by humanized if used in humans. Humanization decreases but does not eliminate the possibility for immunogenic effects and in some cases is difficult to do. We have initiated an experimental program aimed at the development of fully human mAbs (hmAbs) that recognize G_N and G_C and neutralize the virus by using our phage display libraries. G_N and G_C mediate the virus entry into susceptible cells, and have been recently expressed and characterized as membrane-associated proteins [13, 84, 103]. Currently there are no available human monoclonal antibodies (hmAbs) directed to epitopes on G_N and G_C.

We plan to use two different procedures for Env expression from non-infectious material and presentation in native conformations: (i) mammalian cell surface associated functional Envs, and (ii) recombinant expressed soluble Envs. Production of Envs in close to native conformation is of critical importance, for in many instances the ability to elicit potent neutralizing activity to oligomeric Envs depends on the retention of their native three-dimensional structure. As our second method for presentation of Env antigens, we use recombinant soluble Envs we have been developing. The ability to produce soluble forms of such glycoproteins using recombinant mammalian expression systems often allows for production of the protein in a form that is unperturbed in tertiary and quaternary structure. For example, viral Envs are invariably oligomeric, composed of homo or heterotrimeric or tetrameric forms, and the systems using high expression vectors and tissue culture often allows for rapid production of such native oligomeric versions of these types of proteins. Such soluble constructs can be more easily purified and they are quite useful in analyzing the structure and oligomeric nature of the proteins. Thus significant amounts of purified soluble Envs from all these viruses will be available for screening of PDLs.

The panning of PDLs with the soluble Envs and screening of the highest affinity binders will be performed by using standard protocols. We and others have successfully used these protocols for identification of hmAbs directed against epitopes on the HIV Env [72, 110–112]. Recently, we developed a novel methodology, sequential antigen panning (SAP), based on alternating various Envs during panning of PDLs, which was used to identify four new potent broadly HIV-neutralizing human monoclonal antibody Fabs (m12,14,16,18) [109]. We plan to use the SAP methodology for the Envs. If there are conserved neutralization epitopes on these viruses and the SAP approach is successful, we could obtain a hmAb(s) that is effective against several or all isolates in addition to nhmAbs specific to individual isolates. In preliminary experiments we identified three hmAbs that bind specifically to the Envs expressed by recombinant vaccinia virus and are being characterized for neutralizing activity by our collaborator R. Flick and epitope mapping (Zhu et al., in preparation).

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