

# Interferons in the Therapy of Severe Coronavirus Infections: A Critical Analysis and Recollection of a Forgotten Therapeutic Regimen with Interferon Beta

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## ABSTRACT

The pharmacological and immunological properties of interferons, especially those of interferon beta, and the corresponding treatment strategies are described, and the results of studies with different interferons in coronavirus infections are analysed. Furthermore, the data obtained with high-dosed native interferon beta in life-threatening acute viral diseases as well as the results of clinical pilot studies with high-dosed recombinant interferon beta-1a are provided because they serve as the rationale for the proposed therapeutic regimen to be applied in acute viral infections. This regimen differs from those approved for treatment of multiple sclerosis and consists of interferon beta-1a administered as a 24 hour intravenous infusion at a daily dose of up to 90 µg for 3–5 consecutive days. Since under this regimen transient severe side effects can occur, it is analysed which patients are suitable for this kind of treatment in general and if patients with severe coronavirus infections could also be treated accordingly.

## Introduction

The outbreak of the recent respiratory syndrome COVID-19 caused by the novel coronavirus SARS-CoV-2 has spread from China to many countries in the world. On 11 March 2020 the World Health Organization (WHO) made the assessment that COVID-19 can be characterized as a pandemic [1]. Although most affected patients suffer from mild to moderate symptoms, the total number of fatal cases exceeds that of other coronavirus infections, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), [2–4].

Regardless of the fact that currently numerous therapeutic options are under review, so far no effective therapy could be identi-

fied [5–7]. Among others, interferons are considered as possible effective antiviral drugs against coronavirus infections.

## Properties of interferons and corresponding treatment strategies

Interferons are (glyco-)proteins with antiviral activity. They are members of the cytokine family. Since they are expressed rapidly during the process of a viral infection they form an essential part of a very early and virus-unspecific host defense mechanism against multiple viruses [8]. Some viruses including coronaviruses are weak interferon inducers and hence hardly activate this natural defense mechanism of the body. An indicator of the importance of this interferon evasion strategy is the finding that in cell culture and ani-

mal experiments interferons can strongly inhibit the replication of coronaviruses. There are still other defense mechanisms of the body so that most patients with a coronavirus infection recover after illness [9–12]. Thus, interferon therapy in coronavirus infections could be considered as a substitution of a compound which is not sufficiently produced by the body during these diseases. The clinical and pharmacological/immunological implications of the said interferon substitution in an acute coronavirus infection will be discussed below.

Beside their antiviral properties, interferons are characterized by antiproliferative activities relative to numerous malignant and non-malignant cells. Furthermore, they modulate cell differentiation and a variety of humoral and cellular immune functions. [8, 13–16]. The role interferons play in immunity and autoimmunity is rather complex, not only protective but also pathogenic effects are described or discussed, respectively [11].

Based on their protein structures and cell-surface receptors, interferons are divided into type I interferons (several interferon alpha subtypes, interferon beta, interferon epsilon, interferon kappa, interferon omega), type II interferons (interferon gamma) and type III interferons (several interferon lambda subtypes). For more than 35 years interferons have been produced in large quantities for clinical application via human diploid cell cultures (native interferons) and more frequently via non-human host cells such as *E. coli* and Chinese hamster ovary (CHO) cells using recombinant DNA technology (recombinant interferons). Various interferons are available as approved drugs [15–19]. Because interferons are to a large extent species-specific or have “defined host-ranges” [20], respectively, the pharmacological effects of human interferons can expediently and reliably be investigated only in man, in non-human primate models and in human cell cultures.

During decades of clinical experience with various interferon preparations in numerous diseases clinicians had to learn that very different dosing regimens and routes of administration are required in order to exploit a specific pharmacodynamic activity of a certain interferon preparation in the treatment of a particular disease [14–26]. High doses of interferons must be applied in order to create high serum levels which are probably essential for treatment focused on antiviral or antiproliferative interferon activities. In contrast, immunomodulation is often achieved with low doses whereby opposing effects (activation or inhibition) can be observed depending on a variety of conditions [13, 14, 27, 28]. Interferon beta, due to its stronger hydrophobicity, has a much higher tissue affinity compared to interferon alpha. Intramuscular (IM) or subcutaneous (SC) injections of interferon beta result in only low serum levels. Whenever high serum concentration of interferon beta is targeted, this cytokine requires the intravenous (IV) route of administration. High serum levels are, however, not required in order to exploit the immunomodulating potential of interferon beta since corresponding effects can be observed with IM or SC administration of even low doses [22, 27, 29–33].

In order to prolong the elimination half-life and thus to decrease the necessary administration frequency, some companies have developed pegylated forms of their respective interferon preparations. Such preparations are available for human use since the early 2000s [18, 34, 35].

Whenever feasible and appropriate, a local/topical application of the interferons should be considered since a relatively high local concentration of interferon can be achieved with a relatively low but therapeutically effective dose, and hence systemic adverse drug reactions can be reduced or completely avoided [14, 18, 36].

The therapeutic areas where interferons are used cover acute and chronic viral infections, malignancies and immune disorders [15–26]. Due to the variety of indications, it is not surprising that a regimen successfully used in one disease can be ineffective in another. There are approved therapeutic regimens for recombinant interferon alfa preparations concerning chronic viral hepatitis B and C and some malignant diseases. Recombinant interferon beta preparations are only approved for the treatment of multiple sclerosis. For the experimental treatment of other diseases including acute viral diseases, the use of other dose regimens of interferon beta-1a is suggested [22].

### Preclinical and clinical studies of interferons in coronavirus infections

Interferons can inhibit the replication of coronaviruses *in vitro* and show clinical effects in animal models [37–53]. In order to see if such promising results could also be achieved in a clinical setting, clinical studies using interferons, typically in combination with other antiviral drugs, were performed to treat SARS and MERS [54–59]. In these studies, only a minor or even no therapeutic benefit was observed, as outlined in review papers covering not only the aforementioned clinical studies but also several case reports [5, 60–64].

What could be the reason for the failure of interferons in SARS and MERS? In the studies performed so far, dosage and administration routes were chosen as they have been approved for interferons in the treatment of other diseases, namely chronic viral hepatitis (recombinant interferon alfa) or multiple sclerosis (recombinant interferon beta). Apparently, the investigators did not pay sufficient attention to the acute character of a coronavirus induced pneumonia demonstrating quite different pathological conditions compared to the approved indications, and thus potentially requiring a different treatment approach. To attain a direct antiviral effect with a systemic administration of interferons, high daily doses leading to high serum levels maintained for several days are required as shown, for example, in the treatment of herpes zoster with native interferon alpha or beta [65–67]. With the regimens used in the above mentioned studies as to SARS and MERS, however, only relatively low serum levels of interferons could be achieved.

Which interferon preparation of the already approved ones should be used and how should they be dosed and administered to achieve a therapeutic effect in acute viral infections in general and in coronavirus caused pneumonia in particular? In cell culture experiments, interferon beta is clearly superior to other interferons as to inhibiting replication of coronaviruses [5, 9, 37, 40, 41, 45, 48, 50, 68]. This superiority of interferon beta is also valid for other viruses such as herpes simplex [69]. Accordingly, interferon beta should be the interferon of choice in the treatment of acute viral infections. Regarding the dosage of this type of interferon in acute viral infections, data are available for native interferon beta.

## Native interferon beta in the treatment of acute viral diseases

In the early 1980s the German competent health authority approved a drug product containing a native interferon beta produced by human fibroblasts (tradename: Fiblaferon®) for treating acute life-threatening viral diseases such as viral encephalitis and disseminated herpes zoster [16, 67]. However, the documentation relative to this product is fairly unknown to the international scientific community because the vast majority of clinical data were published in books and/or in German language. Since Fiblaferon® is not marketed anymore, a recombinant interferon beta preparation would be the only available alternative in order to assess if such preparation could also demonstrate beneficial clinical effects in a scenario of an acute viral infection such as coronavirus induced pneumonia. Before suggesting an appropriate therapeutic regimen for a recombinant interferon beta, the data as to the native interferon beta are shortly reported here. Additionally, some unpublished data as to a recombinant CHO-derived interferon beta-1a given by the IV route are provided.

The therapeutically effective regimen for the treatment of acute systemic viral diseases with native interferon beta was developed by Heidemann et al. [70, 71] in immune-compromised patients suffering from herpes zoster. According to the “Heidemann scheme” the native interferon beta is administered in a daily dose of 0.5 million IU per kg body weight (max. 25 million IU per day) as a continuous 24-hour IV infusion for 3–5 consecutive days. The dose for the last day can be given in a ratio of 2:1 spread over two days. On the basis of this treatment schedule, other acute viral diseases including virus encephalitis and virus pneumonia were also successfully treated [16, 67, 72, 73]. Due to severe side effects that were frequently observed with this regimen, all patients had to be hospitalized under intensive care conditions. All patients showed high fever and/or other moderate to severe flu-like symptoms. Furthermore, rapid changes of laboratory parameters, especially leukocytopenia, thrombocytopenia and increase of transaminases, required daily laboratory monitoring, including determination of the partial thromboplastin time. All side effects disappeared shortly after termination of treatment. Careful attention had to be paid to fluid balance and fluid substitution. For the infusion, the native interferon beta was dissolved in a body weight dependent volume (up to 500 ml) of a physiological saline solution plus human albumin.

## Proposed dosage of recombinant interferon beta in acute viral infections

In the late 1980s clinical pilot studies with a recombinant CHO derived interferon beta-1a were performed in 15 adult male and female patients suffering from viral hepatitis, viral encephalitis or herpes zoster (data not published). The Heidemann scheme was applied and final daily doses of 50 to 150 µg (declared as 10–30 million IU) - corresponding to 0.6–2.7 µg per kg individual body weight - were administered. Daily doses of more than 1.3 µg interferon beta-1a per kg body weight could hardly be given for 5 consecutive days due to high fever, leukopenia and/or increase of transaminases. Therapy had to be discontinued prematurely in 2 out of 4 patients who were treated with doses between 1.3 and 1.7 µg per kg body weight, and in additional 2 out of 2 patients treated with higher dosage. Howev-

er, in all 9 patients treated with < 1.3 µg per kg body weight therapy could be performed as scheduled. Side effects were very similar to those observed under high dose native interferon beta and also disappeared shortly after termination of treatment.

According to these data, a daily dose of 1.2 µg per kg body weight (max. 90 µg per day) seems to be the maximum tolerated dose (MTD) of interferon beta-1a given as a 24-hour continuous IV infusion for 3–5 consecutive days. Thus, relative to the IU the MTD for the recombinant interferon beta-1a is apparently lower than the MTD for the native interferon beta. In juvenile herpes simplex virus encephalitis, a dose of 1.0 µg (declared as 0.2 million IU) interferon beta-1a per kg body weight (max. 60 µg per day) was used as a 24-hour continuous IV infusion and well tolerated but showed no additional therapeutic effect to aciclovir which was given as the basic antiviral treatment [74]. In viral encephalitis, higher dosage of interferon beta-1a than in other acute viral diseases might be required due to the repairing activities of interferon beta on the blood-brain barrier which is disturbed in viral encephalitis [75, 76]. Nevertheless, the aforementioned MTD should be widely exploited not only in viral encephalitis but also in other severe acute viral diseases in order to achieve a potential therapeutic effect.

The data about interferon beta composed and analysed here are intended to encourage clinicians to perform clinical studies using recombinant interferon beta-1a in severe viral infections with another but probably more appropriate regimen ( $\leq 90$  µg daily given as a 24-hour continuous IV administration for 3–5 consecutive days) than the approved ones in multiple sclerosis (30 µg IM once weekly for Avonex® or 44 µg SC three times a week for Rebif®, respectively). With the suggested regimen, however, more severe and still other adverse drug reactions can occur because of the high daily dose and the IV route of administration.

It has to be emphasized that the proposed dose and regimen is only appropriate for treating acute viral infections and only valid for the non-pegylated recombinant interferon beta-1a but not for other approved recombinant interferon beta preparations, i.e. pegylated interferon beta-1a or E. coli derived interferon beta-1b preparations, where the highest tolerated daily dose - if given as continuous 24-hour IV infusion for 3–5 days - has not yet been determined. A detailed discussion of the comparability of the different interferon beta preparations as well as of the determination of their biological and specific activities is given elsewhere [22, 77, 78].

## Feasibility of the proposed interferon beta-1a dosage in coronavirus infections

Is the regimen proposed for interferon beta-1a in acute viral infections also applicable for treating severe coronavirus infections?

Due to the expected side effects of the proposed regimen for interferon beta-1a in coronavirus infections, only patients with a life-threatening course of their disease will be suitable candidates for this kind of treatment. During severe coronavirus infections, fever, lymphocytopenia and increase of transaminases are often observed [2, 3, 9, 79]. These symptoms may prevent high dose IV administration of interferon beta-1a since they are also common side effects of the proposed therapeutic regimen. Therefore, suitable patients have to be determined at an early stage of their disease, and treatment has to be started when the patients are still in

a condition and willing to tolerate the side effects mentioned above. It has still to be decided if the MuLBSTA score [80] or another score predicting the risk of mortality in viral pneumonia may be useful to identify such patients.

Furthermore, severe cases of coronavirus infections are characterized by a hyper-inflammatory lung pathology induced by an excessive accumulation of inflammatory cells and high serum levels of pro-inflammatory cytokines (“cytokine storm”) [9, 12, 79, 81–84]. In a mouse model as to MERS mouse interferon beta administered via the intranasal route showed opposing effects on this inflammatory response depending on the time of administration. Early treatment with interferon beta on 6 and 24 hours post infection (p.i.), i.e. before peak virus replication occurred, protected mice from fatal outcome, while late treatment on day 2 and 4 p.i., i.e. after peak virus replication, resulted in fatal pneumonia through increased inflammatory cell infiltration in the lungs and enhanced pro-inflammatory cytokine expression [52]. In another mouse model as to MERS, the effects of early and late administered mouse interferon beta given by the SC route were not equally distinct or not found at all [53]. Regarding SARS, experiments were performed with cynomolgus macaques treated with pegylated human interferon alfa-2b administered by the IM route in two different settings: In the prophylactic group the animals received the drug on days -3, -1, 1, and 3, in the post exposure group on days 1 and 3 p.i. [39]. Interferon treatment reduced viral replication and pulmonary damage in both groups, even though these effects were more distinct in the prophylactic than in the post exposure group. Protective effects, i.e. prevention of severe inflammation and reduction of mortality, were also observed in other non-human primate models relative to MERS. In these experiments the monkeys (rhesus macaques or common marmosets, respectively) received human interferon alfa-2b or beta-1b, respectively, by the SC route. The initial dose was administered 8 hours p.i. followed by further one to three doses until 56 hours p.i., i.e. in these models all doses were given prior to the peak of clinical signs and viral loads [47, 49]. Taken together, the results obtained in animal models also indicate that interferon treatment of coronavirus infected patients should be started at an early stage of their disease in order to achieve a potential protective effect because a (too) late initiation of interferon treatment is possibly not only therapeutically ineffective but can even lead to an exacerbation of the disease.

As to humans, there are no data available how high doses of interferon beta-1a given by the IV route act on an exaggerated inflammation. In the treatment of multiple sclerosis with interferon beta, it is assumed that an anti-inflammatory effect of this cytokine is obtained by the approved IM or SC administration of medium doses [85]. In contrast, during local, i.e. intralesional, treatment of basal cell carcinoma with low doses of interferon beta-1a, often an inflammation of the lesion is induced before subsequent healing [86]. Similar observations were made after intralesional treatment of melanoma metastases with low doses of interferon beta-1a [32]. Accordingly, interferon beta can have anti-inflammatory and pro-inflammatory effects depending on the disease (stage), dosage and administration route. A dual mode of action in inflammation is also known for interferon gamma [13, 14, 28].

Relative to the infections with the novel coronavirus SARS-CoV-2, it will be interesting to learn the results of interferon studies planned or just started in China (ClinicalTrials.gov, search terms: “coronavirus” and “interferon”). In some of these studies, the patients receive a recombinant interferon alfa or beta preparation via oral spray or inhalation, respectively, possibly leading to a much higher drug concentration in the affected organ than with the “standard” SC or IM administration of different interferons previously used in SARS and MERS (see above). In a patient with COVID-19, however, the inhalation of interferon alfa-2b given together with lopinavir and ritonavir tablets did not prevent his lethal outcome [84].

## Concluding remarks

The therapeutic role that interferons can play in the treatment of coronavirus infections has still to be determined. Interferons are potent inhibitors of virus replication as shown in several cell culture and animal experiments (see above). Thus, interferons have been used and should also further be considered in the treatment of coronavirus infections. However, due to their pleiotropic effects and possible different actions depending to a large extent on the immune status, the outcome of any clinical application of interferons is often hard to predict [18, 21].

The relevance of results elaborated in cell culture or animal models needs to be scrutinized relative to various factors with impact on the specific clinical scenario. For example, the window for a supposed successful therapeutic administration of interferon after onset of symptoms but prior to the peak of virus replication is very different in humans and experimentally infected mice or monkeys. Furthermore, the peak for virus replication is generally unknown in patients. Also the comparison of the results obtained in one animal model with those found in another one is difficult due to differences in animal species, delivery route, dose, start, frequency and duration of administration, interferon subtype and/or active viral antagonism of innate immunity [52, 53]. In addition, there are still further aspects to be considered if findings in the different animal models studying antivirals for coronavirus infections are assessed [87].

A therapeutic regimen successfully used with an interferon in one human disease might not be effective or tolerated in another one or another stage of the disease. That could be the reason for the reported failure of interferon treatment in SARS and MERS where low-dose and medium-dose regimens were applied as approved for virus hepatitis and multiple sclerosis. In the past, high-dosed native interferon beta given by the IV route was successfully used for the treatment of various acute viral diseases. As to recombinant interferon beta-1a the MTD for a high dose IV administration has already been determined. It remains an open question if this dosage is therapeutically effective in severe coronavirus infections and if it can safely be applied in patients with a poor survival prognosis at an early stage of their disease.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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