PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 146 394-397 (1974)

Clearance of Human Interferon in Rabbits Induced to Make Rabbit Interferon (38112)

LIISA PYHALA AND K. CANTELL
(Introduced by Monto Ho)
Department of Virology, Central Public Health Laboratory, Helsinki, Finland

Human leukocyte interferon is rapidly cleared from the circulation of rabbits (1), gibbons (2), and man (3) after iv injection, but after im injection a stable serum level can be maintained for 12–24 hr in several species, including man (4). The im route of administration is used in current clinical studies with human leukocyte interferon (3). Very little is known about the clearance of exogenous interferon from the blood during and after the induction of endogenous interferon production. Such information is relevant to the combined clinical use of interferon inducers and exogenous interferon. Finter (5) reported that mouse interferon injected iv 18–24 hr after interferon induction by Newcastle disease virus (NDV) or poly rI:rC was cleared from the blood more slowly in these animals than in controls. We approached the problem by utilizing the "species specificity" of interferons. Since rabbit interferon has no antiviral activity in human cells (6), even small amounts of human interferon can be detected in the presence of large amounts of rabbit interferon. We injected concentrated human leukocyte interferon iv or im into rabbits at different times after interferon induction by NDV and followed the serum levels of rabbit interferon and human interferon. The results are presented below.

Materials and Methods. The production of interferon in human leukocyte suspensions and its concentration by precipitation with potassium thiocyanate have been described previously (1). Human leukocyte interferon was assayed by vesicular stomatitis virus (VSV) plaque reduction in a line of human amnion cells (U-cells), and the titers are expressed in reference units. The interferon preparation used contained 3.5×10^5 units per ml and about 50 mg protein per ml.

The Hertfordshire strain of NDV was used to induce rabbit interferon. It was grown in the allantoic cavity of 11-day-old chick embryos and the preparation used contained 256 HA units per ml. Five milliliters were injected into the marginal ear vein of conventional rabbits weighing between 3.3 and 3.6 kg. Blood samples were collected at intervals, and the sera were dialyzed against pH 2 for 6 days at 4° and then dialyzed back to neutrality. The rabbit serum interferon was assayed in primary rabbit embryo fibroblasts by VSV plaque reduction as described earlier (1), and the titers are expressed in reference units. The rabbit serum interferon used in the experiments below was collected at 4 hr after NDV injection and contained 200,000 units per ml. In rabbit embryo fibroblasts the human leukocyte interferon showed about one-third of its activity in U-cells. The rabbit serum interferon had no detectable activity in U-cells.

Five milliliters of human interferon were injected iv or im into rabbits at different times after iv injection of 5 ml NDV as well as into control rabbits not receiving NDV. Blood samples were collected at intervals, the sera were dialyzed against pH 2 for 6 days and back to neutrality, and assayed for interferon in U-cells and in rabbit embryo fibroblasts. Normal rabbit sera reduced the VSV plaque counts at low dilutions, but no such activity was seen when normal sera were diluted 1:20 or more.

Results. Figure 1 shows the kinetics of the appearance of rabbit serum interferon after induction with NDV. Interferon was already detectable in the sera at 1 hr, the peak was

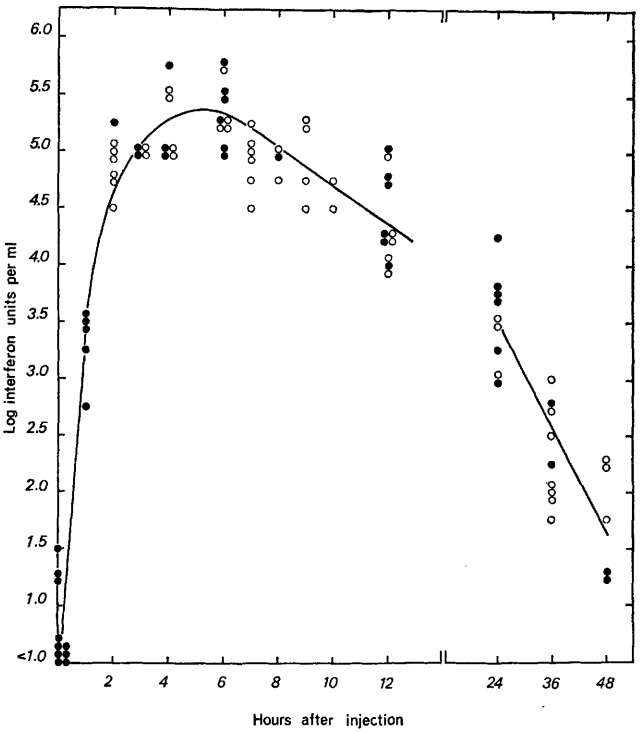

FIG. 1. Serum interferon production in rabbits induced by iv injection of NDV. All serum samples were assayed for interferon in rabbit embryo fibroblasts. ● no injection of human interferon. ○ iv or im injection of 1.75 million units of human leukocyte interferon 0, 1, 3, 6, or 24 hr after NDV, collection of blood samples at intervals up to 48 hr.

reached at 4–6 hr, and thereafter the titers declined slowly. The iv or im injections of 1.75 million units of human interferon at different times had no detectable effect on the kinetics.

Figure 2 shows the clearance of iv-administered human interferon from the blood of rabbits injected 1, 6, or 24 hr earlier with NDV. It can be seen that the clearance rate did not differ essentially from that of the control rabbits.

Similar experiments were done to study the fate of im-administered human interferon in rabbits injected with NDV. A dose of 1.75 million units of human interferon was injected simultaneously with NDV or at different times after it, and the level of circulating human interferon was followed. NDV induction had no demonstrable effect on the clearance pattern of the circulating human interferon after im injection (Fig. 3).

In the course of these studies the toxicity of the combined administration of NDV and interferon became clearly apparent. During the past 2 yr more than 150 rabbits have been injected iv or im with a few million units of human leukocyte interferon in our laboratories, and none has died. Likewise, of nine rabbits injected with NDV alone all survived. On the other hand, three of 10 rabbits receiving human interferon iv at 1–24 hr after NDV died within 2 days. Similarly, three of 20 rabbits which received human interferon im after NDV died within 2 days. A grayish and swollen liver was the only pathological macroscopic feature in the autopsy. Furthermore, most of the surviving rabbits receiving both NDV and interferon showed general weakness and lack of appetite.

Attempts were made to study the clearance of exogenous rabbit interferon from the blood of rabbits injected with NDV. One million units of the rabbit serum interferon were injected iv or im into seven rabbits and they all survived. However, of three rabbits administered the rabbit interferon iv 40–48 hr after

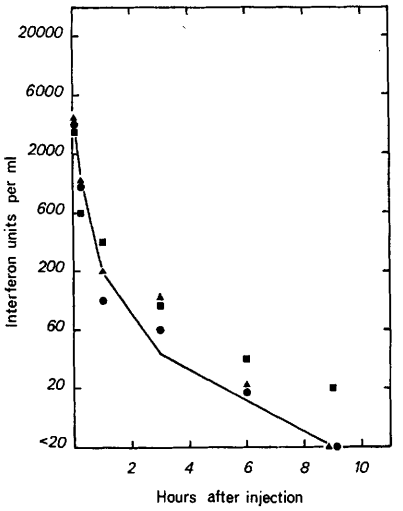

FIG. 2. Clearance of human interferon from the blood of rabbits induced with NDV. 1.75 million units of human leukocyte interferon was injected iv into three rabbits 1 (●), 6 (▲), or 24 (■) hr after NDV and into three control rabbits not receiving NDV. All sera were assayed for interferon in human U cells. The line represents the geometric mean of the interferon titers in the control rabbits.

NDV injection all died within 3 hr.

Discussion. From the titers in Fig. 1 and the published clearance rates for rabbit interferon (7) it can be estimated that the total amount of circulating interferon produced by a rabbit in response to NDV injection is of the order of hundreds of millions of units. Thus, the exogenous interferon injected in the experiments above amounts to only a small fraction of the endogenously produced interferon. It is not surprising that the injections of human interferon did not measurably influence the production or clearance of the endogenous rabbit interferon. The important point is that the production and clearance of the vast amounts of endogenous interferon had no detectable effect on the pharmacokinetics of the exogenously administered interferon. The mechanism by which interferon is cleared from the blood is still completely obscure. The present findings suggest that the clearance mechanism is not readily affected even by large amounts of endogenous interferon. Neither enhanced clearance nor a "saturation effect" was observed.

The stable level of circulating interferon after im injection is probably maintained by continual entrance and clearance of interferon into and from the blood. The present results suggest that the entrance of interferon from the site of injection into the blood is not influenced by the amount of interferon present in the blood.

It would have been ideal to use labeled exogenous rabbit interferon in the present study, but, unfortunately, such preparations are not yet available. Since a heterologous interferon was used, the results obtained do not completely rule out the possibility that the clearance of exogenously administered interferon may be affected by induction of endogenous interferon. This seems rather unlikely, however, because the clearance rates of homologous interferon from the blood in sev-

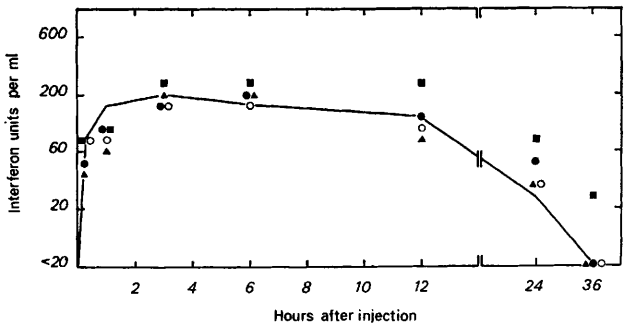

FIG. 3. Circulating human interferon after im injection into rabbits induced with NDV. Human leukocyte interferon (1.75 million units) was injected im into rabbits 0 (●), 1–3 (▲), 6 (■), or 24 (○) hr after NDV. Each symbol represents the geometric mean of the interferon titers in the sera of two to five rabbits. The line is drawn through the geometric mean titers in five control rabbits not receiving NDV.

eral species were found to be closely similar (8) and the pattern of circulating interferon after im injection of human leukocyte interferon is similar in different species (4). In other words, no "species specificity" has been demonstrated for the pharmacokinetics of interferons.

If potent and safe inducers become available, their clinical use may be limited by the fact that induction is followed by a refractory period during which the body cannot be reinduced to make more endogenous interferon. The present findings suggest that it will be possible to maintain a steady serum level even during the refractory period by im administration of exogenous interferon. Earlier work has shown that the exposure of cells to interferon *in vitro* can render them more susceptible to the cytotoxic action of certain interferon inducers (9). The present observations suggest that exogenous interferon can cause a similar increase in the toxicity even *in vivo*.

Summary. The rate of clearance of iv-injected human leukocyte interferon from the blood of rabbits was not affected by induction of endogenous interferon with NDV injected 1, 6, or 24 hr earlier. Likewise, im injection of the human interferon into rabbits together with, or at different times after NDV induction, gave a clearance pattern of circulating interferon similar to that in control rabbits.

Administration of exogenous human or rabbit interferon to rabbits injected previously with NDV resulted in increased toxicity.

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Received Jan. 31, 1974. P.S.E.B.M., 1974, Vol. 146.