Recombinant Human Interferon Alpha-2a: Delivery to Lymphoid Tissue by Selected Modes of Application

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The effect of different parenteral administration routes (i.d., s.c., i.v.), infusion rates, and albumin contents of the drug vehicle on the cumulative recovery of recombinant human interferon alpha-2a (rIFN alpha-2a) in lymph and on its concentration in blood and lymph was determined in sheep. Blood samples were withdrawn from a jugular vein catheter and lymph was collected from a cannulated efferent popliteal lymphatic duct. The concentration of rIFN alpha-2a in lymph and blood plasma samples was measured by an enzyme immunoassay. Following i.v. infusion of $2 \times 10^7$ U of rIFN alpha-2a, the peak concentrations measured in blood plasma and lymph, respectively, were 8250 and 14 U/ml. The concentration measured in lymph after i.d. or s.c. administration of the same dose was about 10^4 times higher (peak concentration, 3.1 \times 10^6 U/ml), while blood plasma levels remained low (peak concentration, 315 U/ml). The cumulative recovery of rIFN alpha-2a in lymph following i.d. or s.c. administration was 59.2 ± 7% (mean ± SD; N = 8) and was affected neither by the infusion rate nor by the coadministration of albumin. Our data indicate that following i.d. or s.c. administration, rIFN alpha-2a (MW 19,000) is absorbed mainly by the lymphatics. This results in high levels of rIFN alpha-2a in the lymph which drains from the application site to the regional lymph nodes. The knowledge gained in this investigation may help to improve the mode of administration and therapeutic efficacy of protein drugs whose targets are lymphoid cells.

KEY WORDS: recombinant human interferon alpha-2a (rIFN alpha-2a); parenteral administration; lymph concentration; blood plasma concentration; cumulative lymph recovery; sheep.

INTRODUCTION

Interferons are proteins which can be used as antiviral, cytostatic, or immunomodulatory agents. Based mainly on empirical approaches, several routes and schedules of administration have been used, but neither an optimal route nor an optimal dosage schedule is yet at hand. This is particularly true when interferons are used as immunomodulatory drugs (1).

Interferons can be considered as paracrine hormones acting physiologically in microenvironments, rather than as circulating proteins (2). Thus in order to reproduce the physiological distribution of interferon (3), Bocci has proposed that lymphatic absorption of interferons should be facilitated (4).

So far only a few studies have examined the lymphatic delivery of interferons. Yoshikawa et al. measured the concentration of interferon in the thoracic duct of rats following enteral administration. They demonstrated that the administration of interferon with mixed micelles resulted in a highly selective delivery into the lymphatics, but the total amount absorbed was very small (~0.05%) (5–8). Bocci et al. studied the lymphatic absorption of interferon following parenteral administration in rabbits. They showed that after subcutaneous injection, the total amount of interferon recovered in thoracic duct lymph was less than 0.04%. Recoveries increased from two- to eightfold when interferon was injected with albumin or with hyaluronidase, respectively (9).

To our knowledge no studies have examined the concentration of interferon in the peripheral lymphatic system following parenteral administration. In the present work we investigated the influence of different parenteral administration routes (i.d., s.c., i.v.), infusion rates, and albumin contents of the drug vehicle on the absorption of recombinant human interferon alpha-2a (rIFN alpha-2a) by lymphatic and blood vascular routes. The experiments were done in sheep after the efferent lymphatic duct of the popliteal lymph node had been cannulated. The results obtained indicate that following i.d. or s.c. administration, rIFN alpha-2a (MW 19,000) is absorbed mainly by the lymphatics. This results in high levels of rIFN alpha-2a in the lymph which drains to the regional lymph nodes.

MATERIALS AND METHODS

Interferon Preparation

Human serum albumin-free lyophilized rIFN alpha-2a
containing 18 × 10^6 U per vial (Ro 22-8181/658, Lot No. GFER 13 031, F. Hoffmann-La Roche & Co.) was used. The amount of rIFN alpha-2a is expressed in international units (U) of antiviral activity determined in the vesicular stomatitis virus MDBK cell assay. The specific activity of the pure unformulated substance was 2–4 × 10^8 U/mg protein (protein purity of >98%). Details of the products and purification procedures are described elsewhere (10).

Animals

Nonpregnant, nonlactating white Alpine or black Jura ewes or wethers (50–70 kg) were obtained from Versuchsbetrieb Sennweid, Olsberg, Switzerland. Prior to experiments animals were housed in pens inside a barn with access to pasture in spring and summer. During experiments sheep were maintained in individual pens and fed on commercial pellets.

Surgical Procedures

All surgical procedures were done under aseptic conditions in a properly equipped operating theater. Surgical anesthesia was induced by intravenous injection of sodium thiopentone and maintained with halothane (Hoechst, Frankfurt am Main, FRG). The efferent duct of the popliteal lymph node was cannulated using the procedure described by Hall and Morris (11). Catheters were inserted into the jugular vein through the bore of a 14-gauge needle and anchored in position with stay sutures.

rIFN Alpha-2a Absorption Studies

The rIFN alpha-2a absorption studies were started 2–3 days after surgery. The general design of our absorption studies is shown in Fig. 1. After collecting blank blood and lymph samples, 2 ml of a rIFN alpha-2a solution (1 × 10^7 U/ml) was injected either i.d. or s.c. into the lower part of the hind leg or i.v. into the jugular vein. In some experiments human serum albumin was coadministered.

The s.c. and i.d. administrations were done with an injection device attached to the shaved leg. This device allows accurate injection of drug solutions into a defined place within the skin. The depth of injection was 2.25 mm for the s.c. administration and 1.25 mm for the i.d. administration. The infusion rate was controlled by an infusion pump (Micropump MP-20, Micrel, Greece) and was varied from 0.3 to 17 ml/hr. Lymph was collected continuously in heparinized tubes. The collection tubes were changed at 10-min intervals for the first hour after injection, at hourly intervals for the next 5–6 hr, and then at 4- to 12-hr intervals. The volume of each lymph sample was determined gravimetrically using a specific gravity of 1.0. Blood samples were withdrawn from a jugular vein catheter and transferred to heparinized tubes. The blood samples were taken at 15-min intervals for the first hour after injection and then hourly for the next 4 hr. Between periods of sampling the cannula was kept patent with a heparin lock (50 U/ml in saline). Care was taken to obtain a circulating blood sample by discarding the first 3 ml of aspirate. After centrifugation (2000 rpm, 20 min, 4°C) all lymph and blood plasma samples were kept frozen at −20°C until assayed.

rIFN Alpha-2a Assay

The concentration of rIFN alpha-2a was measured by an enzyme immunoassay method, utilizing a solid-phase sandwich principle (12). Briefly, rIFN alpha-2a was incubated with (mouse) monoclonal rIFN alpha-2a antibodies (LI-9) that were coated on a polystyrene bead and with (mouse) monoclonal rIFN alpha-2a antibody (LI-1) that was conjugated to horseradish peroxidase. Following this incubation step, the unbound material was removed by washing and the activity of peroxidase bound to the bead was measured using O-phenylenediamine as substrate. The resulting color intensity, which is measured photometrically, is directly proportional to the rIFN alpha-2a concentration in the sample. The reference standard had a specific activity of 1.7 × 10^8 U/mg of protein as determined against the NIH interferon standard. The assay sensitivity in lymph and blood plasma was 1 U/ml of rIFN alpha-2a. The concentration of rIFN alpha-2a measured with the enzyme immunoassay has been correlated with antiviral activity in the vesicular stomatitis virus MDBK cell assay (13).

RESULTS

Cumulative Recovery of rIFN Alpha-2a in Lymph

The effect of the administration route, infusion rate, and albumin content of the drug vehicle on the cumulative recovery of rIFN alpha-2a in the efferent popliteal lymph was determined. The recovery of rIFN alpha-2a in lymph was calculated as the product of the concentration in lymph and the volume of lymph and was expressed as a percentage of the administered dose.

The results of 12 experiments performed in six separate sheep are summarized in Table I. The cumulative recovery of rIFN alpha-2a in lymph following i.d. or s.c. administration into the lymph cannulated leg was 59.2 ± 7% (mean ± SD; N = 8) and was affected neither by the infusion rate nor by the coadministration of albumin. In contrast, following i.v. infusion or i.d. administration into the noncannulated leg, only a small amount of the administered dose was recovered in the lymph. The cumulative recoveries determined were 0.0015 ± 0.0007 and 0.006 ± 0.002%, respectively (N = 2).