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The Transmucosal Absorption of Recombinant Human Interferon-α B/D Hybrid in the Rat and Rabbit

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Abstract

For therapeutic proteins such as interferon-α B/D, a non-parenteral route of delivery is desirable. Possible sites of administration include the various regions of the gastrointestinal tract and airways, and this paper reports the bioavailability of interferon-α B/D via these routes in the rat and rabbit.

Apart from the stomach, detectable levels of interferon-α B/D in the serum were achieved via all routes. Bioavailabilities were less than 1%, except from the lung (6·8% in the rat) and nasal cavity (2·9% in the rabbit). Absorption from the gastrointestinal tract was similar for both species, but in the nasal cavity of the rabbit was sixfold that of the rat, and in the lung of the rat was tenfold that in the rabbit. Absorption from all routes, except the buccal cavity, resulted in detectable biochemical changes in the liver of the rabbit.

Comparison with reports from other groups show differences in the extent of absorption of interferon-α B/D and of natural or homologous recombinant interferon-α. The non-parenteral delivery of biochemically active amounts of interferon-α B/D is thus demonstrated.

With the availability of commercially viable supplies of therapeutic peptides and proteins has come the challenge of administering these agents to the patient without recourse to a needle. The buccal, oral, rectal, nasal, pulmonary, vaginal, ocular and transdermal routes have all received attention (Juliano et al 1992) and all have possibilities and limitations. The choice of route depends very much on the drug/disease combination under investigation. Interferon-α (molecular weight 19·5 kDa) is a large molecule to consider for transmucosal delivery. This disadvantage may be offset by the small amounts required for therapeutic activity; the recommended dose for the treatment of chronic active hepatitis B is $4{\cdot}5 \times 10^6$ int. units (Ryff 1993) which represents approximately 8 μg protein. Absorption of interferon-α has been demonstrated when given via the buccal cavity (Steward et al 1994), lung (Kinnula et al 1989; Patton et al 1994), large intestine (Bocci et al 1986) and, when absorption enhancers were included, the nose (Shim & Kim 1993). In the lung, a difference has been seen between natural and recombinant interferon-α, with the recombinant protein showing much reduced absorption compared with the natural molecule (Maasilta et al 1991). The purpose of this report is to examine the transmucosal absorption of the novel recombinant hybrid human interferon-α B/D hybrid (Meister et al 1986), a species that has been shown to have different properties from recombinant interferon-α (Hochkeppel et al 1992).

Materials and Methods

Materials

Recombinant human interferon-α B/D hybrid (sp. act. 250×10^6 int. units mg^{-1}) and monoclonal antibodies to Mx protein were, respectively, the gifts of Dr A. Meister and Dr H. Towbin, Ciba Pharmaceuticals, Basle, Switzerland. Biotinylated sheep anti-mouse antibody and biotin-streptavidin-horseradish peroxidase were from Amersham Life Science, Aylesbury, UK. HRP Colour Development Reagent was from Biorad, CA, USA. Hypnorm (fentanyl and fluanisone) was purchased from Janssen Pharmaceuticals, Brussels, Belgium, Sagatal (sodium pentobarbitone) from Rhone Merieux Ltd, Harlow, UK, and Halone (halothane) from RMB, Animal Health Ltd, Dagenham, UK. All other chemicals were of reagent grade quality. Male Wistar rats, 380 ± 50 g, were from Bantin and Kingman, Hull, UK, and female New Zealand White rabbits, 2 kg, were from Froxfield, UK.

Animal experiments

Animals were sedated with an intramuscular dose of Hypnorm (100 μL/rat, 400 μL/rabbit) with maintenance doses of 40 μL (rat) and 200 μL (rabbit) every 40 min. When dosing via the trachea, ileum or colon, the animals were anaesthetized with either an intravenous dose of 32 mg kg^{-1} Sagatal (rats) or by inhalation with Halone (rabbits) after which the wounds were closed and the animals maintained under sedation. Where indicated, animals dosed via the gastrointestinal tract were fasted overnight before the experiment.

Recombinant human interferon-α B/D hybrid was given as a solution in 200 mM mannitol, 30 mM phosphate (pH 7·6) at the volumes described below and at the doses shown in Table 1.

Intravenous: 100 μL into the dorsal tail vein (rat) or 200 μL into the marginal ear vein (rabbit).

Buccal cavity: 50 μL (rat) and 100 μL (rabbit) instilled into the lower, outer margins of the gingiva; the head was raised to a height of 25 mm throughout the experiment to retain the dose at the site (Steward et al 1994).

Stomach: 100 μL by gavage (rat only).

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Table 1. The serum pharmacokinetics of recombinant-α B/D hybrid after delivery via the gastrointestinal tract.

Route	Dose (10^6 int. units kg^{-1})	C_{max} (int. units mL^{-1})	t_{max} (min)	AUC (int. units mL^{-1})	Bioavailability (%)
Buccal cavity					
Rat	33	37 ± 12	15–240	83 ± 16	0·045
Rabbit	35	25 ± 10	180	53 ± 9	0·051
Stomach					
Rat	33	Not detected			
Ileum					
Rat*	47	115 ± 20	30–240	530 ± 74	0·21
Fasted	47	440 ± 110	30–120	1580 ± 360	0·61
Rabbit					
Fasted	12	110 ± 70	15	290 ± 175	0·82
Colon					
Rat*	33	215 ± 75	30–120	695 ± 230	0·38
Rectum					
Rat	33	145 ± 30	15–120	340 ± 170	0·18
Rabbit	17·5	85 ± 50	15	90 ± 35	0·09

*Animals were dosed into a ligated section of the gastrointestinal tract as described in Materials and Methods. The results are the mean ± s.e.m. for four to six rabbits.

Ileum/colon: 80 μL (rat) and 100 μL (rabbit) injected through the wall of the exposed ileum (10 cm above the stomach) or colon (10 cm below the stomach); where indicated, the injection was into a 1-cm ligated section.
Rectum: 80 μL (rat) and 100 μL (rabbit).
Nasal cavity: 20 μL (rat) and 10 μL (rabbit) dosed into the left nostril.
Trachea: 10 μL (rat) or 100 μL (rabbit) given by direct bolus injection through the wall of the exposed trachea 1 cm below the thyroid gland.

Blood samples (0.5 mL) were collected from the dorsal tail vein (rat) or the marginal ear vein (rabbit). Serum was prepared and stored at −18°C. The rabbits were killed 24 h after dosing and the livers removed and stored at −70°C for Mx protein determination.

Analytical methods

The initial dose and all serum samples were analysed by an enzyme immunoassay specific for interferon-α B/D hybrid (Anawa, Zurich, Germany). The limit of detection was 10 int. units mL^{-1} and the coefficient of variation was 6·3%.

Measurement of Mx protein in liver samples was by Western Blotting (Horisberger & De Staritzky 1989). Cell extracts containing 100 μg protein were separated on a 12% SDS polyacrylamide gel and transferred to a nitrocellulose membrane which was incubated in a 1:1000 dilution of antibody. Antibody-antigen complexes were detected using the biotin-streptavidin-horseradish peroxidase system. Blots were standardized against a WISH cell extract containing 25 ng Mx protein, as determined by an enzyme-linked immunoassay (Towbin et al 1992), and quantified by densitometry.

Calculation of bioavailability

The area under the plasma vs time curves (AUC) was measured using the trapezoid rule. AUC was calculated over the complete time course. The AUC for the test route was referenced to the AUC for the intravenous dose where bioavailability was defined as 100%.

Results and Discussion

Table 1 shows the serum pharmacokinetics of recombinant human interferon-α B/D hybrid after administration via different regions of the gastrointestinal tract. All sites, except the stomach, gave detectable serum levels of hybrid interferon-α B/D but with bioavailabilities of less than 1%. The buccal cavity was the least permeable site. The bioavailability via this route for hybrid interferon-α was one twentieth of the value reported for recombinant interferon-α2 (Paulescu et al 1988). While some of the discrepancy may be attributed to different dosing and assay techniques (Steward et al 1994), the data lend support to the conclusion of Maasilta et al (1991) that different species of interferon-α have different pharmacokinetics following transmucosal delivery. Both rat and rabbit buccal mucosae showed the same degree of permeability towards interferon-α B/D (0·045 and 0·051%, respectively). Rabbit buccal mucosa is thought to be a better model than rat for human buccal tissue because it is less keratinized (Dowty et al 1992). The results suggest that absorption of interferon-α via this route in man may also be poor.

The permeability of the remainder of the gastrointestinal tract towards interferon-α B/D was colon > ileum > rectum (Table 1). The poor performance of the more permeable ileal epithelium compared with that of the colon was in part due to the higher levels of protease activity in the ileum. When protease levels were reduced by fasting the animals overnight, absorption of interferon-α B/D from the ileum was increased threefold (Fig.1). In the rabbit, no attempt was made to restrict the dose to the site of administration by ligation. This did not adversely affect the bioavailability. The data presented here for recombinant hybrid interferon-α show a greater degree of absorption from the colon than that reported for natural interferon-α from the rat large intestine (Bocci et al 1986). The rat and rabbit showed very similar levels of absorption from the ileum (0·61 and 0·82%, respectively), but the rectal route was only half as efficient in the rabbit compared with the rat (0·09 and 0·18%). The poor permeability of rabbit rectum has been noted for

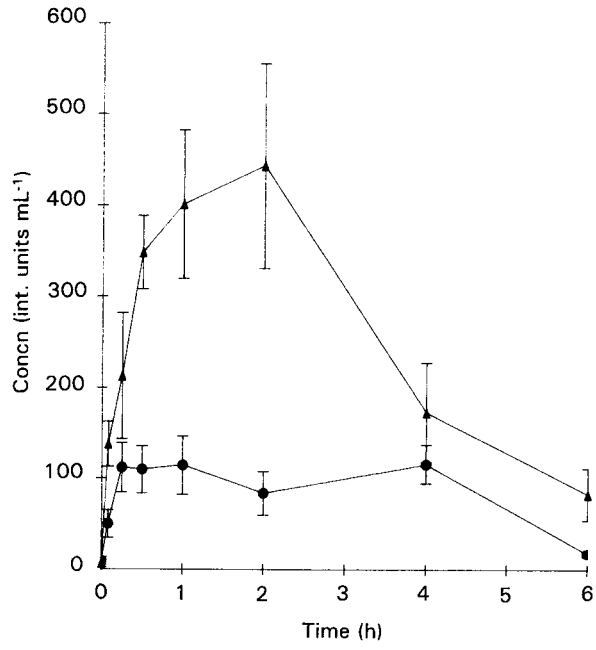

FIG. 1. The effect of fasting on the absorption of human recombinant interferon-α B/D hybrid from the rat ileum. Rats were given 47×10^6 int. units kg^{-1} interferon-α B/D into a ligated section of the ileum, as described in Materials and Methods. One group of rats (▲) was fasted overnight before dosing, the other group (●) had free access to food. The results are the mean ± s.e.m. of six animals.

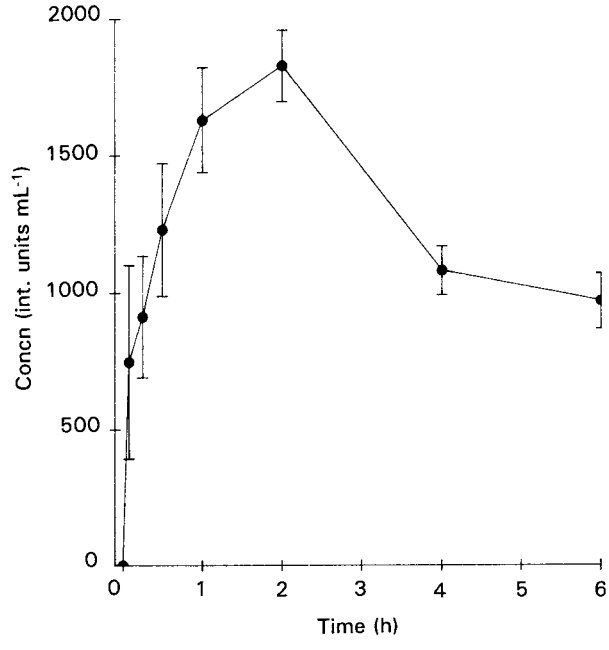

FIG. 2. The serum profile of recombinant interferon-α B/D after dosing via the trachea. Rats were given 13×10^6 int. units kg^{-1} interferon-α B/D by direct injection into the trachea as described in Materials and Methods. The results are the mean ± s.e.m. of six animals.

insulin and can be overcome to a certain extent by the use of absorption enhancers (Yamamoto et al 1992).

Table 2 shows the serum pharmacokinetics of interferon-α B/D after nasal and pulmonary (intratracheal) administration. In the rat, absorption via the nasal cavity was similar to that via the gastrointestinal tract. The bioavailability of interferon-α B/D from the nasal cavity of the rabbit (2·9%) was sixfold that in the rat (0·54%). The rabbit nasal cavity is also more permeable than the rat towards insulin (Deurloo et al 1989). Nasal absorption has been reported for recombinant interferon-α, but absorption enhancers were required to produce detectable serum levels (Shim & Kim 1993). The increased bioavailability of hybrid recombinant interferon-α vs a single species recombinant interferon-α is the reverse of the situation in the buccal cavity.

Intratracheal administration was used to assess the pulmonary absorption of hybrid interferon-α. In the rat, a bioavailability of 6·8% was achieved by this route (Table 2). This is one-eighth the bioavailability reported for natural interferon-α (Patton et al 1994) and is consistent with the data of Maasilta et al (1991) who found a reduced absorption of recombinant interferon-α compared with natural interferon-α in man. Interestingly, the serum profile of interferon-α B/D (Fig. 2) was different from that of natural interferon-α. The absorption of natural interferon-α showed a lag of 2 h before reaching a plateau between 3–9 h (Patton et al 1994) whereas interferon-α B/D reached a peak at 2 h followed by a biphasic decline. The intratracheal route showed the most pronounced species difference with the bioavailability in the rabbit being 10% of that measured in the rat and of the same order of magnitude as found via the gastrointestinal tract. The low permeability of the rabbit trachea compared with other species has also been demonstrated by Wangensteen et al (1993) for inulin and dextran. The data from Kinnula et al (1989) suggest that the human lung has a low pulmonary permeability towards interferon-α.

In this study, serum concentrations of hybrid interferon-α were measured by immunoassay. To confirm that the absorbed material retained activity, levels of the interferon-inducible Mx protein were measured in the liver, the target organ in the treatment of hepatitis. The cross-species

Table 2. The serum pharmacokinetics of recombinant hybrid interferon-α after delivery via the airways.

Route	Dose (10^6 int. units kg^{-1})	C_{max} (int. units mL^{-1})	t_{max} (min)	AUC (int. units mL^{-1})	Bioavailability (%)
Nasal cavity					
Rat	33	215 ± 20	60–240	995 ± 120	0·54
Rabbit	1·75	150 ± 45	15	305 ± 40	2·90
Trachea					
Rat	13	1463 ± 105	120	6275 ± 495	6·8
Rabbit	15	120 ± 27	60–360	36223 ± 3686	0·7

The results are the mean ± s.e.m. for four to six rabbits.

Table 3. The induction of Mx protein in the liver after administration of recombinant interferon-α B/D hybrid via various routes. The level of Mx protein was measured in the liver 24 h after dosing groups of four rabbits via different routes with the doses described in Tables 1 and 2.

Site of administration	Mx protein in liver (ng (mg protein)$^{-1}$)
Control	4·87 ± 4·30
Buccal cavity	0·45 ± 0·45
Ileum	5·62 ± 1·12
Rectum	38·74 ± 19·36
Nasal cavity	20·27 ± 9·5
Trachea	11·75 ± 4·5

The results are the mean ± s.e.m.

specificity of interferon-α B/D is such that these measurements could only be made in rabbits (Horisberger & De Staritzky 1987). Table 3 shows the detection of increased levels of liver Mx protein in the rabbit after dosing via all routes, except the buccal cavity, demonstrating that interferon-α B/D was still active after transmucosal delivery and that sufficient material can be absorbed to induce biochemical changes in tissues. In all cases the increase in liver Mx protein was proportional to the serum levels of interferon-α B/D measured either as C_{max} or AUC. The changes in Mx protein levels were small compared with the 12·9 units achieved with an intravenous dose of $3·5 \times 10^6$ int. units, reflecting the low level of transmucosal absorption of interferon-α B/D. Mx protein is a clinical marker for interferon activity (Jakschies et al 1990) but there is, at present, no information on the correlation, if any, between the Mx response and the successful treatment of conditions for which interferon-α is a possible therapy. The induction of Mx protein does not imply that therapeutic levels of interferon-α can be achieved via transmucosal routes.

This paper demonstrates the transmucosal absorption of recombinant hybrid interferon-α B/D from the gastrointestinal tract and airways of the rat and rabbit. Bioavailabilities were low, being less than 1% via all routes except the lung (rat only) and nasal cavity (rabbit only). Absorption from all routes in the rabbit, except the buccal cavity, resulted in detectable biochemical changes in the liver, but this does not imply that sufficient material was absorbed for therapeutic activity. Only the airways showed species differences between the rat and rabbit.

Comparison with reports from other groups show some differences in the level of absorption of interferon-α B/D and natural or homologous recombinant interferon-α, but the data are not comprehensive and no clear pattern emerged. Direct comparisons are needed before firm conclusions can be drawn. None of the three types of interferon-α appear to be excluded from transmucosal absorption or are preferential candidates for the process. Dose-response and formulation studies are now needed to establish whether any of the routes can be exploited for therapeutic purposes.

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