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The effect of enhancers on the buccal absorption of hybrid (BDBB) α-interferon

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Abstract

The pharmacokinetics of hybrid (BDBB) α-interferon was studied in rats following buccal and intravenous dosing. Analyses were carried out using a commercially available immunoassay. A bioavailability of 0.014% was obtained for buccal absorption with a T_{max} of 60 min. The intravenous data gave a volume of distribution of 119 ml, the clearance was 41.3 ml h^{-1} and the half-life for elimination was 120 min. A variety of absorption enhancers were also tried with α-interferon using the buccal dosing route. Sodium taurocholate gave the best results with a dose related response, the area under the curve showing a greater than 10-fold increase at 2% (w/v) concentration and almost 20-fold increase at 4% concentration. The order of efficacy of the enhancers was: 1% sodium dodecyl sulphate > 5% Tween 80 > 5% cyclodextrin > 5% ethanol. Both 5% PEG 400 and 5% salicylate had no statistically significant effects.

Key words: α-Interferon; Peptide; Pharmacokinetics; Buccal absorption; Absorption enhancer; Bioavailability

1. Introduction

Hybrid (BDBB) α-interferon is a novel recombinant form of α-interferon. It is a 166 amino acid residue peptide of 19.5 kDa that has a broad range of antiviral and antiproliferative activity (Meister et al., 1986; Gangemi et al., 1989; Hochkeppel et al., 1992). The α-interferons are currently being used clinically for the treatment of a number of different disease states which include hairy-cell leukaemia, Karposi's sarcoma in AIDS, non-A, non-B hepatitis and basal cell carcinoma (Baron et al., 1991). The preferred routes of administration for α-interferons are subcutaneous and intramuscular and, to a lesser extent, intravenous injection. Yoshikawa et al. (1985) examined gastrointestinal absorption in rats and described the use of fusogenic, lipid:surfactant (oleic acid/HCO60) mixed micelles as absorption enhancers for human α-interferon into the lymphatics from the large intestine. Interest has also been expressed in the buccal delivery of peptide drugs (Merkle et al., 1991; Harris and Robinson, 1992; Ho et al., 1992). Delivery via this route has the advantage of

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avoiding acid and enzyme mediated degradation and first pass metabolism by the liver. It also provides an opportunity for cytokines such as α-interferon to interact with the oropharyngeal associated lymphoid tissue as has been suggested by Bocci (1991). The plasma levels of peptides dosed via this route would not be expected to be good. Studies were therefore undertaken to examine the pharmacokinetics of buccally dosed hybrid α-interferon in the absence and presence of a variety of absorption enhancers.

2. Materials and methods

Hybrid (BDBB) α-interferon was supplied by Dr A. Meister, Department of Biotechnology, Ciba-Geigy, Basle. The enzyme-linked immunoassay (ELISA) kit was obtained from Anawa Laboratories, Zurich. Ethanol, polyoxyethylene sorbitan monooleate (Tween 80) and sodium dodecyl sulphate (SDS) were supplied by BDH Laboratories, Poole, Dorset. Polyethylene glycol 400 (PEG 400) taurocholic acid (sodium salt) and salicylic acid (sodium salt) were obtained from Sigma, Poole, Dorset. Hypnorm (fentanyl and fluanisone) came from Janssen Pharmaceuticals, Brussels and dimethyl-β-cyclodextrin from Janssen Chimica, Hyde, Cheshire. The animals used were male, Wistar rats from Bantin and Kingman, Hull.

Initial experiments with 1% Evans Blue helped to establish a protocol for studies to be carried out on buccal absorption in the rat. The dye was instilled into the buccal cavity, in the lower, outer margins of the gingiva. The animal's head had to be maintained at a height of 25 mm during continuous Hypnorm sedation and dye then remained in the buccal cavity and did not enter the trachea or oesophagus over 2 h, providing a volume of 50 μl was not exceeded.

In all the subsequent experiments, male Wistar rats weighing 380 ± 50 g were used and were sedated using an initial dose of 100 μl intramuscularly (i.m.) of Hypnorm and subsequent doses of 40 μl i.m. every 40 min. After taking a predose blood sample from the tail vein, the animal was maintained with its head supported at the set level of 25 mm and was dosed buccally with 50 μl of an α-interferon solution (25 μl into each cheek) either with or without absorption enhancers. Throughout all the experiments the buccal dose of interferon was 40×10^6 U kg^{-1} except the intravenous dose, which was 1×10^6 U kg^{-1}. After the predose sample, and following the buccal dosing other samples were taken at 0.5, 1, 2, 3 and 4 h and were centrifuged after clotting in order to obtain the serum. Analyses were carried out using the ELISA. This assay is a sandwich immunoassay using two monoclonal antibodies with one labelled using horseradish peroxidase and the other coated onto polystyrene beads. The samples were incubated at room temperature for 48 h with both antibodies before being washed thoroughly with water and undergoing a second incubation with the *o*-phenylenediamine reagent. The reaction was stopped using 0.1 M sulphuric acid and the samples were read in the spectrophotometer at 492 nm. Standards were analysed in duplicate but samples from the animals could only be analysed singly. The limit of detection with this assay was 10 mU/ml and the coefficient of variation was 6.3%.

3. Results

The serum profile after buccally dosing hybrid α-interferon alone is given in Fig. 1 and can be compared with the serum profile for intravenous (i.v.) dose shown in Fig. 2. These data provided a means for deriving area under the curve (AUC) values and from these a bioavailability of 0.014% was obtained for the buccal route with a T_{max} of 60 min. The i.v. data gave a volume of distribution of 119 ml, with a clearance of 41.3 ml h^{-1} and an elimination half-life of 2 h.

The effects of different classes of surface-active agents were investigated. Sodium taurocholate (Fig. 3) was the only agent to give a dose-dependent increase in α-interferon absorption and able to improve the bioavailability by greater than 10-fold. A summary of pharmacokinetic data for these and other agents is presented in Table 1. The results show that only salicylate and PEG 400 had no statistically significant ef-

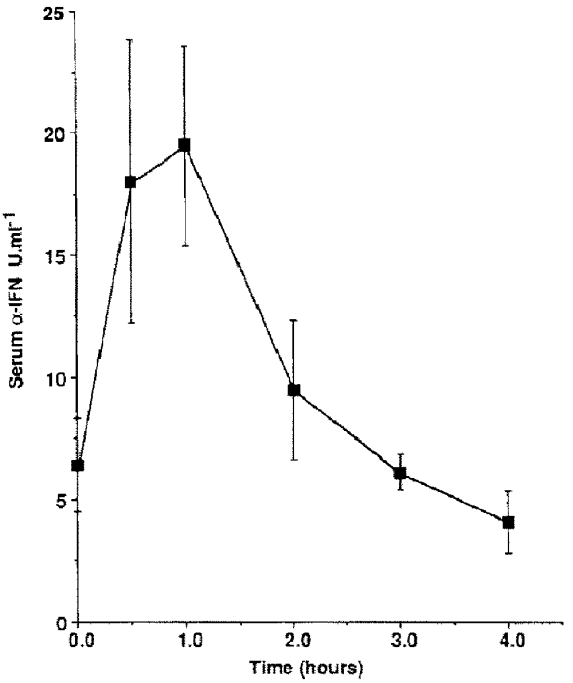

Fig. 1. Serum levels of hybrid (BDBB) α-interferon following a buccal dose of 40×10^6 U kg^{-1} to rats (means $\pm$ SE).

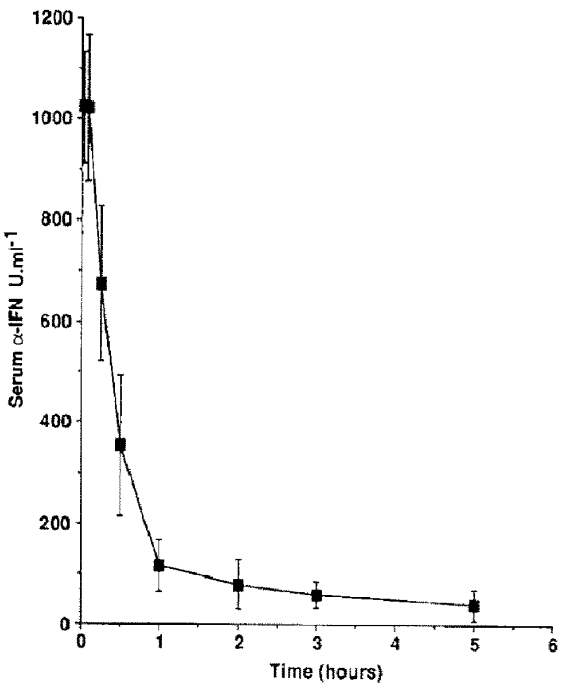

Fig. 2. Serum levels of hybrid (BDBB) α-interferon following an i.v. dose of 1×10^6 U kg^{-1} to rats (means $\pm$ SE).

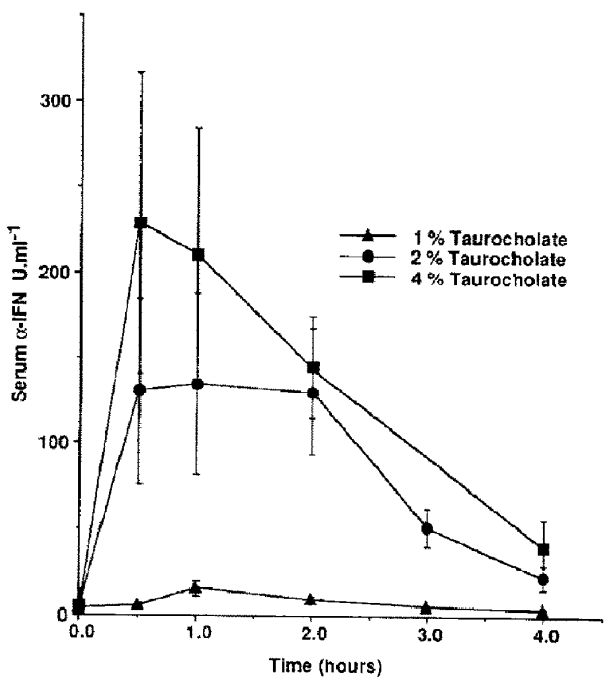

Fig. 3. The effects of sodium tautocholate on absorption of hybrid (BDBB) α-interferon dosed buccally at 40×10^6 U kg^{-1} to rats (means $\pm$ SE).

Table 1
Comparison of effects of enhancers with hybrid α-interferon alone

Enhancer (w/v)	AUC ± SE	% bioavailability	P
Control	42 ± 9.4	0.014	
Bile salt			
Taurocholate			
(20 mM, 1%)	33 ± 5.8	0.016	N.S.
(40 mM, 2%)	341 ± 99	0.17	< 1%
(80 mM, 4%)	624 ± 136	0.25	< 0.1%
Surfactant			
Tween 80 (5%)	184 ± 23	0.087	< 0.01%
SDS (1%)	252 ± 98	0.12	< 5%
PEG 400 (5%)	72 ± 16	0.026	N.S.
Others			
Ethanol (5%)	79 ± 8.5	0.043	< 5%
Salicylate (5%)	32 ± 2.0	0.016	N.S.
Cyclodextrin (5%)	101 ± 31	0.045	< 5%

fects while the others, were less than 10-fold in their difference from hybrid α-interferon alone.

4. Discussion

There was a difference between the doses given when comparing the buccal and intravenous routes. Data in the literature however, have suggested that α-interferon has dose-independent pharmacokinetics (Jang et al., 1992). The bioavailability value for hybrid (BDBB) α-interferon alone was different from the 1% absorption reported by Paulesu et al. (1988) for recombinant interferon-α_2 in rats. They were measuring plasma levels by using an antiviral assay for detection of the α-interferon and calculated their percentage bioavailability based on subcutaneous, rather than i.v. data. In their studies they also examined the effect of a bile salt, ursodeoxycholate, in the presence and absence of Labrafil. They observed that at 3% (w/v) concentration of the bile salt there was no improvement over the controls in the amount of interferon-α_2 absorbed, whether Labrafil was present or not, which again contrasts with the present observations.

Aungst and Rogers (1989) used similar concentrations of enhancers in their experiments on buccal absorption in rats. They, like the present authors, found bile salts to be superior to other types of absorption promoters. Nakada et al. (1988) examined the effects of additives on the buccal absorption of human calcitonin, also in rats. They demonstrated large enhancement effects with sodium lauryl sulphate and bile salts although α- and γ-cyclodextrins showed no significant difference from the controls whereas our observations with β-dimethyl cyclodextrin were statistically significant.

When considering the toxicological potential of the formulations used in the present study, the distinction between a pharmacological enhancement and an enhancement due to a toxic effect is difficult to define. However, buccal tissue is considered to be robust (Merkle and Wolany, 1992) possibly due to its high cell turnover, and the toxicological issue with respect to absorption enhancers may not be as serious as with other mucosal surfaces such as the intestines.

In these experiments, a variety of absorption enhancers have been tried and although there were a number that were statistically significant, with the possible exception of the bile salt, they are unlikely to have any biological significance. Absorption via this route is relatively poor as would be expected from a stratified epithelium. There is much interest, however, in buccal delivery of α-interferons as pharmacological results appear to be promising in both animals (Schafer et al., 1972; Cummins et al., 1988; Paulesu et al., 1988; Georgiades et al., 1989; Young et al., 1990; Fleischmann et al., 1991, 1992) and man (Hutchinson et al., 1990; Obel and Koech, 1990). Antiviral resistance was transferred by spleen and blood cells from one group of mice receiving low oral doses of α-interferon to another group of recipient mice (Georgiades et al., 1989). Interferon dosed to lactating mice conferred protection on the neonatal weanlings that had received an oral dose of the lethal vesicular stomatitis virus. There was a 35% reduction in deaths and interferon was identified as being present in the milk (Schafer et al., 1972). Similarly, Cummins et al. (1988), using cats, found low, oral doses of human α-interferon prevented development of feline leukaemia virus which can be fatal. Low

dose oral administration of human α-interferon in cattle was found to be able to control the development of *Theileria parva* infection (Young et al., 1990). Clinical research with low dose oral α-interferon has also demonstrated beneficial effects in HIV seropositive patients (Obel and Koech, 1990) and in the treatment of chronic major aphthous stomatitis (Hutchinson et al., 1990). These studies point to a range of species of animals coupled with different pathophysiological conditions that can be affected by oral α-interferon.

Further studies are needed to determine if there is a relationship between pharmacological effects and blood levels of α-interferon. These studies have demonstrated that absorption of hybrid (BDBB) α-interferon does occur via the buccal route and that this absorption can be enhanced by the use of suitable additives.

5. References

Aungst, B.J. and Rogers, N.J., Comparison of the effects of various transmucosal absorption promoters on buccal insulin delivery. *Int. J. Pharm.*, 53 (1989) 227–235.

Baron, S., Tyring, S.K., Fleischmann, W.R., Coppenhaver, D.H., Niesel, D.W., Kimpel, G.R., Stanton, G.J. and Hughes, T.K., The interferons: mechanisms of action and clinical applications. *J. Am. Med. Assoc.*, 266 (1991) 1375–1383.

Bocci, V., Is interferon effective after oral administration? The state of the art. *J. Biol. Reg. Homeost. Agents*, 4 (1990) 81–83.

Cummins J.M., Tompkins, M.B., Olsen, R.G., Tompkins, W.A. and Lewis, M.G., Oral use of human alpha interferon in cats. *J. Biol. Response Modifiers*, 7 (1988) 513–523.

Fleischmann, W.R., Fields, E.E., Wang, J.-L., Hughes, T.K. and Stanton, G.J., Modulation of peripheral leukocyte counts in mice by oral administration of interferons. *Proc. Soc. Exp. Biol. Med.*, 197 (1991) 424–430.

Fleischmann, W.R., Koren, S. and Fleischmann, C.M., Orally administered interferons exert their white blood cell suppressive effects via a novel mechanism. *Proc. Soc. Exp. Biol. Med.*, 201 (1992) 200–207.

Gangemi, J.D., Lazdins, J., Dietrich, F.M., Matter, A., Poncioni, B. and Hochkeppel H.-K., Antiviral activity of a novel recombinant human interferon-αB/D hybrid. *J. Interferon Res.*, 9 (1989) 227–237.

Georgiades, J.A., Kruzel, M.L. and Seman, G., Transfer of antiviral resistance by spleen and blood cells of mice receiving low oral doses of interferon alpha or gamma. *J. Interferon Res.*, 9 (1989) s213.

Harris, D. and Robinson, J.R., Drug delivery via the mucous membranes of the oral cavity. *J. Pharm. Sci.*, 81 (1992) 1–10.

Ho, N.F.H., Barsuhn, C.L., Burton, P.S. and Merkle, H.P., Routes of delivery: Case studies. Mechanistic insights to buccal delivery of proteinaceous substances. *Adv. Drug Del. Rev.*, 8 (1992) 197–235.

Hochkeppel, H.K., Grütter, M., Horisberger, M.A. and Lazdins, J.K., Human IFN-alpha hybrids. *Drugs Future*, 17 (1992) 899–914.

Hutchison, V.A., Lee-Ling Mok, W., Augendend, J.L., Cummins, J.M. and Richards A.B., Chronic major aphthous stomatitus: Oral treatment with low-dose α-interferon. *Mol. Biother.*, 2 (1990) 217–220.

Jang, S.H., Lee, S.H., Ryoo, S.H., Kim, S.H. and Lee M.G., Dose-independent pharmacokinetics of recombinant human interferon-alpha in rabbits. *Int. J. Pharm.*, 84 (1992) 273–278.

Meister, A., Uzé, G., Morgensen, K., Gresser,I., Tovey, M.G., Grütter, M. and Meyer, F., Biological activities and receptor binding of two human recombinant interferons and their hybrids. *J. Gen. Virol.*, 67 (1986) 1633–1643.

Merkle, H., Anders, R., Wermerskirchen, A., Rachs, S., and Wolany, G., Buccal routes of peptide and protein delivery. In Lee, V.H.L. (Ed.), *Peptide and Protein Drug Delivery*, Dekker, New York, Ch. 11, 1991, pp. 545–578.

Merkle, H.P. and Wolany, G., Buccal delivery for peptide drugs. *J. Controlled Release*, 21 (1992) 155–164.

Nakada, Y. Awata, N. Nakamichi, C. and Sugimoto, I., The effects of additives on the oral mucosal absorption of human calcitonin. *J. Pharmacobio-Dyn.*, 11 (1988) 395–401.

Obel, A.O. and Koech, D.K., Outcome of intervention with or without low dose interferon alpha in 32 HIV-1 seropositive patients in a referral hospital. *East Afr. Med. J.*, 67 (1990) ss71–ss76.

Paulesu, L., Corradeschi, F., Nicoletti, C. and Bocci, V., Oral administration of human recombinant interferon-2α in rats. *Int. J. Pharm.*, 46 (1988) 199–202.

Schafer, T.W., Lieberman, M., Cohen, M. and Came, P.E., Interferon administered orally: Protection of neonatal mice from lethal virus challenge. *Science*, 176 (1972) 1326–1327.

Yoshikawa, H., Takada, K., Satoh, Y., Naruse, N. and Muranishi, S., Potentiation of enteral absorption of human interferon alpha and selective transfer into lymphatics in rats. *Pharm. Res.*, 5 (1985) 249–250.

Young, A.S., Martin, A.C., Kariuki, D.P., Stagg, D.A., Wafula, J.M., Mutugi, J.J., Cummins, J.M., Richards, A.B. and Burns, C., Low-dose interferon alpha can control the development of Theileria parva infection in cattle. *Parasitology*, 101 (1990) 201–209.