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Enteric absorption of human interferons α and β in the rat

Velio Bocci [1], Fausto Corradeschi [1], Antonella Naldini [1] and Enzo Lencioni [2]

[1] *Institute of General Physiology, Faculty of Pharmacy, University of Siena, (Italy)*
and [2] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Siena (Italy)*

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Summary

The absorption of both human lymphoblastoid interferon α and natural interferon β from the large intestine has been evaluated in the rat. We have shown that the addition of Labrafil R.M.1944 to the citrate buffer containing interferon α favours its absorption through the enteric mucosa in a significant way while it has practically no beneficial effect for interferon β. It appears worthwhile to pursue this line of investigation because the enteric route may enlarge the field of therapeutic application of interferon.

Introduction

Recently there has been a revival of interest on assessing the colorectal absorption of polypeptide drugs and Gardner et al. (1983), Ritschel and Ritschel (1983), Yoshikawa et al. (1984) and ourselves (Bocci et al., 1985) have reported that insulin, gastrin and interferon (IFN) could be, at least in part, absorbed by the rectal mucosa. By using an appropriate sorption promoters containing ursodeoxycholate (UDC) we could consistently obtain in the rat some absorption of human IFNα incorporated into suppositories. This work, however, raised a few important questions.

(1) Can IFN absorption be improved if IFN comes into contact with the far more extensive colonic mucosa rather than being limited to the rectal mucosa?
(2) Of the two types of IFNs, α and β, which are physicochemically and antigenically different, is any one preferentially absorbed?
(3) Is there any advantage in testing other promoters or trying to inhibit intestinal proteolysis.

The following results shed light on these questions.

Materials and Methods

Human lymphoblastoid IFNα (2.7×10^7 reference units/ml: spec. act. 6.6×10^7 reference units/ml) was obtained through the courtesy of Dr. N. Finter (Wellcome Biotechnology Ltd., Beckenham, U.K.). Human natural IFNβ was graciously donated by Dr. A. Valeri (Sclavo S.p.A., Siena, Italy). Doses of 1.7 (IFNα) and 1.9 (IFNβ) megaunits in a total volume (with adjuvants) of 0.2 ml and 0.3 ml were tested per rat, respectively.

The following formulations were prepared for both IFNs:

(1) IFNs diluted in saline.
(2) IFNs diluted in 0.1 M, pH 5.0 citrate buffer.

Correspondence: V. Bocci, Università degli Studi, Istituto di Fisiologia Generale, Via Laterina 8, 53100 Siena, Italy.

This was tested in order to reduce the micro-environmental pH and possibly proteolysis.

(3) IFNs diluted in citrate buffer additioned with Labrafil R.M.1944 (Gattefossè, B.P. 603, F-69 804 Saint-Priest Cedex, France). Labrafil is a preparation containing apricot kernel oil and PEG-6 transesters and is practically atoxic.

(4) IFNs diluted in saline containing 4 mg sodium ursodeoxycholate (UDC)

(5) IFNs diluted in saline containing 4 mg UDC to which was added Labrafil.

All formulations were prepared and used at once.

Forty-five male adult Wistar rats of an average body weight of 330 g were used throughout. In order to minimize the fecal content of their large bowels, the animals were fasted for 36 h before the experiment but water was allowed ad lib. During deep ether anaesthesia, a minimal laparatomy was performed to expose the ileal–coecal junction. IFN doses in 0.2–0.3 ml vols. as indicated above were injected into the caecum lumen. Penicillin (100,000 U) and streptomycin (0.1 g), as powders, were deposited on the site of injection and the peritoneal cavity was closed again in about 3 min. The animal was then kept for 9 h in a restraining cage until the end of the experiment. For comparative purposes some rats were injected subcutaneously at two sites of the abdomen with the same IFN doses. Blood (200 μl) was collected from the tail and was diluted into 200 μl of heparinized (10 U) saline and the diluted plasma was kept frozen at $-20°C$ until IFN measurements were performed. At autopsy, for the purpose of measuring IFN recovery, the content of the large intestine was recovered and diluted with saline containing 25% rat normal serum. The solution was immediately filtered through a Millipore filter (0.22 μm) and the filtrate was dialyzed at $+1°C$ against saline with 2 changes for 2 days. The dialysate was filtered again and frozen before IFN assay. This procedure was indispensable for preventing contamination and toxicity to the cell monolayer used in the assay.

IFN assay. Titres of IFN in plasma and in colorectal washings were assayed measuring the inhibition of the plaque-forming activity of vesicular stomatitis virus (VSV) on HEp2 (for IFNα) and Wish (for IFNβ) cells in microtiter plates as described by Langford et al. (1981). All samples were assayed at least twice in duplicate and the assays were always made employing the appropriate international standard preparations. All titres corrected for the initial dilution were reported in IU/ml. Values among controls and experimentals have been compared after evaluation of the respective area under curve (AUC, expressed as means $\pm$ S.D. of IFN IU/ml plasma/h) during the experimental period as reported in Fig. 1.

Results and Discussion

First of all we tested the stability of both IFNs in the specified formulations. We did not know whether the addition of Labrafil and the formation of an oil-in-water emulsion could inactivate IFNα and/or IFNβ. Both IFNs were quite stable in all formulations.

Both IFNs were then injected either subcutaneously, or into the lumen of the large intestine distal to the ileal–coecal valve. Results obtained with IFNα and IFNβ are reported on the left and right hand side of Fig. 1, respectively. After s.c. injection of 1.67 megaunits IFNα the peak level in the plasma was reached within an hour and C_{max} was equivalent to 990 IU. IFN levels declined thereafter and were negligible after 9 h. Intestinal administration of the same dose of IFNα with either saline, or citrate buffer, or UDC in saline yielded a very low (between 10 and 15 IU) but fairly stable IFN plasma level throughout the experiment indicating a minimal absorption of IFNα from the intestinal lumen. The addition of 100 μl of Labrafil enhanced significantly ($P < 0.001$) IFN absorption so that a peak level of IFN (43 IU) was reached in the plasma 3 h after administration. Although inter-animal variability was considerable, an average of 30 IU were still present in the plasma after 9 h. Addition of UDC to the IFN in saline–Labrafil suspension did not improve the absorption of IFNα that, although significantly ($P < 0.001$) greater than the controls, was less marked than after addition of Labrafil alone.

After s.c. injection of 1.87 megaunits of IFNβ

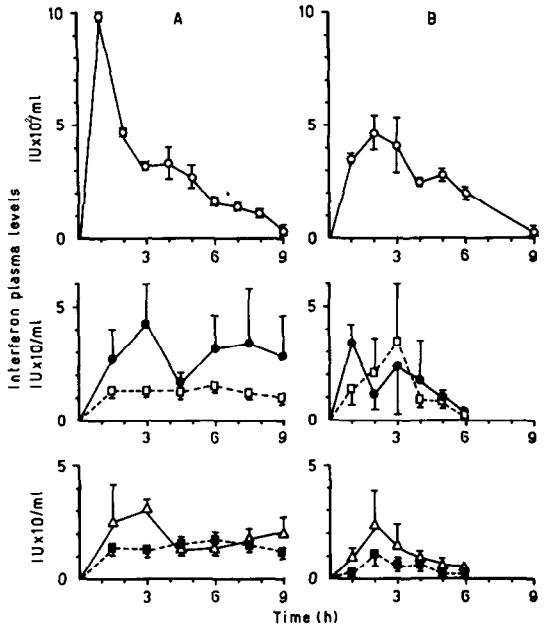

Fig. 1. Interferon plasma levels obtained in rats after administration of interferon α (panel A) and interferon β (panel B). Key: ○———○, subcutaneous administration. The following symbols are for situations after administration of interferons into the coecal lumen: □———□, IFN dissolved in citrate buffer; ●———●, IFN dissolved in citrate buffer and Labrafil; ■———■, IFN dissolved in saline containing UDC; △———△, IFN dissolved in saline additioned with Labrafil and UDC. Values are reported as mean ± S.D.

the peak level in the plasma was reached after 2 h and C_{max} was equivalent to only 465 IU. Although the dose of IFNβ was higher than that of IFNα, IFNβ attained in plasma far lower levels, confirming the notion that IFNβ diffuses poorly from the injection site to the plasma pool (Billiau et al., 1979; Vilcek et al., 1980). Intestinal administration of the same dose of IFNβ with UDC in saline yielded almost negligible IFN plasma levels. Absorption of IFNβ in citrate was slightly better (AUC: 7.3 ± 0.6 IU/ml plasma/h) probably because inhibition of proteolysis may have reduced local IFN inactivation (Cesario et al., 1973). The addition of Labrafil anticipated the peak plasma level at 1 h and the AUC was increased (10.3 ± 6.1) but the increase was not statistically significant. As observed for IFNα, further addition of UDC did not appear beneficial. Recoveries of both IFNs from the luminal content at the end of the experiment were negligible indicating that unabsorbed IFNs undergo complete inactivation.

These results confirm and extend previous observations made by Yoshikawa et al. (1984, 1985). They found that lipid–surfactant mixed micelles promoted the absorption via intestinal lymphatics of IFNβ from the large intestine and they detected high IFNβ levels in lymph. Obviously, plasma levels cannot overlap lymph levels because IFN present in lymph once it reaches the plasma pool is subjected to high dilution and rapid turnover (Bocci, 1985) and therefore, as we have observed, drops to levels below 50 IU. It appears noteworthy that IFNα gives more consistent and stable plasma levels than IFNβ and this could be due to the fact that IFNα in citrate buffer is more stable in the gut lumen and/or Labrafil enhances its absorption. These results are interesting in that they show that particularly IFNα can be absorbed at least in part via the large intestine and rectum. Moreover, absorption can be enhanced by simply mixing IFN dissolved in citrate buffer with Labrafil and therefore one can envisage administration of IFN per os by encapsulating the promoter with IFN in gastric-resistant capsule. If this pharmaceutical preparation is feasible and if IFN remains stable for months it could be tested in patients in order to evaluate therapeutic efficacy and tolerability.

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