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Colorectal administration of human interferon-α

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Summary

The possibility that human interferon-α may be absorbed by the colorectal mucosa has been evaluated in the rat. Suppository bases per se do not allow interferon absorption as it was undetectable in peripheral plasma. Inclusion of physiological amounts of Na-ursodeoxycholate favours some interferon absorption although plasma levels remain at a much lower level after comparative subcutaneous administration. Although the results are encouraging, we have been unable to achieve a marked increase of interferon levels in the portal blood that may render rectal administration a selective route in the treatment of hepatitis.

Introduction

The rectal administration route of polypeptide drugs has been until now a rather neglected area of research interest. Recently Gardner et al. (1983), Ritschel and Ritschel (1983) and Yoshikawa et al. (1984) have reported that insulin, gastrin and interferon (IFN) in the presence of adjuvant could be, at least in part, absorbed by the colorectal mucosa. About two years ago we undertook a project aiming at clarifying whether IFN could be absorbed by the rectal mucosa; Vlatković et al. (1979) have claimed that as little as 1.2×10^6 IU IFN-α administered in the form of suppositories in acute virus B hepatitis was clinically effective. Unfortunately IFN was not titrated in the plasma of their patients so that it remained doubtful whether the clinical improvement was really due to IFN. However, this original observation was interesting and deserved an experimental study to ascertain which absorption

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agent, if any, could favour IFN bioavailability. IFN could be useful either as an antiviral agent and/or as an immunoadjuvant in the treatment of acute (Levin and Hahn, 1982) and chronic (Galasso, 1981) hepatitis particularly if hepatic bioavailability could be improved by facilitating IFN absorption via colorectal mucosa. The present results shed some light on this problem.

Materials and Methods

Human lymphoblastoid IFN-α (2.75×10^7 reference units/ml: specific activity 6.6×10^7 reference units/ml) was obtained through the courtesy of Dr. K. Fantes (Wellcome Biotechnology, Beckenham, U.K.). Each mini-suppository was prepared as follows. (a) An emulsion of saline solution of IFN-α (100 μl containing 2.75 megaunits) in 100 mg paraffin oil added with 40 mg Arlacel 481 (Atlas) was made and it was incorporated at 42°C into 700 mg of a hydrophobic suppository mass (Suppocire D.M., Gattefossé). The ratio eventually used between the emulsion and the mass was 1. (b) A saline solution of IFN (100 μl) was directly incorporated at 40°C into 700 mg of a hydrophilic suppository mass (Novata E, Henkel). This preparation was used either as such or after addition of 80 mg N-lauroylsarcosine-sodium salt. Moreover additions of either 40, 8 or 4 mg Na-ursodeoxycholate (Na UDC) were tested.

43 male adult Wistar rats of an average body weight of 300 g were used throughout. In order to minimize the fecal content of the large bowel the animals were fasted for 36 h before the experiment but water was allowed ad libitum. During deep ether anaesthesia, the suppository (800 mg $\pm$ 20 mg) was rapidly inserted into the rectum and, to prevent loss of material, the anal orifice was closed with a purse-string suture. The animal was then kept for 1–9 h in a restraining cage until the end of the experiment. For comparative purposes, some rats were injected with different IFN dosages in two sites in the subcutaneous tissue of the abdomen. Blood (200 μl) collected from the tail was diluted into 200 μl of heparinized (10 U/ml) 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% of rat normal plasma. The solution was then filtered through a Millipore filter (0.22 μm) and the filtrate was dialyzed at $+1$°C against saline with two changes for two 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 cells in microtitre plates as described by Langford et al. (1981). All samples were assayed at least twice in duplicate. The assays were always made employing the U.K. Medical Research Council (MRC) Research Standard B, 69/19, for human leukocyte interferon (obtained from National Institute for Biological Standards and Control, Holly Hill, Hampstead, London). The MRC sample of

human IFN with a defined potency of 3.69897 $\log_{10}$ IU/ml, when reconstituted in 1.0 ml of sterile distilled water had, in our assay system, a geometric mean titer of 3.98001 $\log_{10}$ IU/ml (S.D. = 0.044; n = 10). All titres corrected for the initial dilution were reported in IU/ml.

Results and Discussion

We first undertook a screening of the rectal bioavailability of IFN from various suppository bases without the addition of sorption promoters. Although each suppository incorporated 2.75×10^6 IU IFN-α, no significant plasma IFN level (above 20 IU/ml) could be demonstrated in a series of rats 1.5, 3, 4.5, 6, 7.5 and 9 h after rectal administration. IFN colorectal recovery after autopsy was negligible and the conclusion was drawn that absorption was minimal or nil and, owing to the IFN sensitivity to intestinal proteinases (Bocci et al., 1968), massive inactivation had most probably taken place in the intestinal lumen. In fact, after subcutaneous injection of IFN-α, its plasma level rose rapidly reaching a maximum 2–3 h after administration and fell progressively thereafter (Fig. 1). There was a clear dependence between IFN plasma levels and injected doses.

In the second series of experiments, we investigated whether addition of well known sorption promoters such as either the Na-salt of N-lauroylsarcosine (80 mg), or Na-UDC (40 mg) to a hydrophilic mass (Novata E) would facilitate IFN

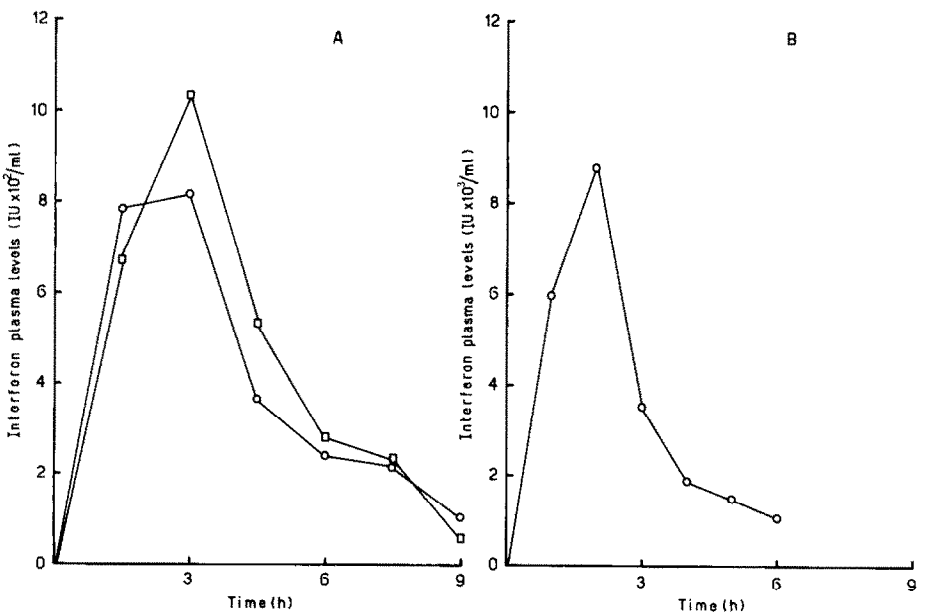

Fig. 1. Interferon plasma levels after subcutaneous injection of three different dosages of interferon-α to rats. Key: (A) O——O, 3×10^5 IU; □——□, 6×10^5 IU. (B) 2.75×10^6 IU.

absorption. However, in spite of the addition of promoters, no IFN plasma levels could be detected and the fact that plasma samples were slightly haemolyzed suggested that amounts of promoters used were excessive, might have been toxic and probably hindered absorption.

Thus, in the third series of experiments we decided to evaluate only doses of Na-UDC in physiological dosages (Fig. 2). This drug, in comparison with other adjuvants, has the additional advantage of not being harmful to the intestinal mucosa. It can be seen that definite IFN plasma levels are demonstrable: 8 mg Na-UDC sped up IFN absorption (C_{max} 1.5 h after administration) whereas 4 mg Na-UDC had a delayed C_{max} (3–4 h) but yielded more constant plasma levels. Therefore, the latter Na-UDC concentration (12 mg/kg body weight) may be preferable also because it is within the concentration range (600–900 mg body weight) of the drug used in humans for treatment of gall stones.

At this stage it was considered worthwhile to evaluate whether IFN levels varied or were similar between peripheral and regional circulations. Although rectal administration yielded far lower IFN levels in the peripheral circulation than subcutaneous injection, there was the possibility of a portal-peripheral vein IFN gradient that would result in higher IFN concentrations in liver blood and possibly in improving therapeutic efficacy in hepatitis (Bocci, 1984). Because suppositories melt rapidly and spread all over the colorectal mucosa it is difficult to define how much IFN is absorbed by the upper, medial and lower hemorrhoidal plexus: if, at least in part, IFN reaches the inferior mesenteric vein, it enters the portal vein and hence the liver, otherwise it reaches the inferior vena cava and the general circulation. Table 1 shows that, as expected, rectal venous blood always contained far higher IFN levels than

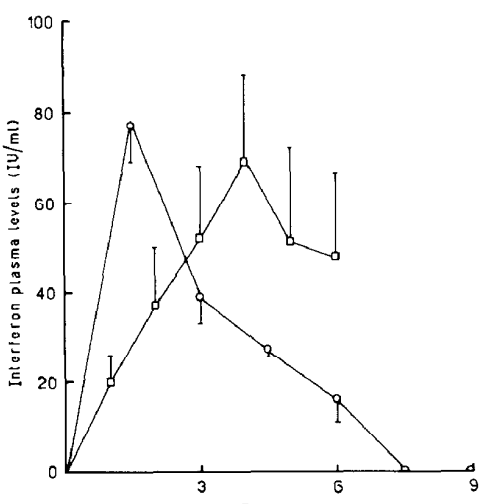

Fig. 2. Interferon levels (mean ± S.E.) in rat plasma after rectal application of suppositories containing 2.75 megaunits interferon-α added with Na-ursodeoxycholate as follows. Key: ○———○, 8 mg; □———□, 4 mg.

TABLE 1

IFN LEVELS (MEAN ± S.E. OF 4 RATS) IN PORTAL, CAVAL, RECTAL AND PERIPHERAL BLOOD AFTER RECTAL ADMINISTRATION OF IFN-α (2.75 megaunits) INCORPORATED INTO SUPPOSITORIES OF NOVATA E CONTAINING Na URSODEOXYCHOLATE (4 mg/SUPPOSITORY)

Time	IFN levels (IU/ml) (mean ± S.E.)			
	Portal blood	Rectal blood	Caval blood	Peripheral blood
1 h	76 ± 29	285 ± 54	88 ± 24	48 ± 8
2 h	26 ± 6	76 ± 13	37 ± 6	22 ± 0

portal, caval and peripheral (tail) blood. On the other hand, portal, caval (ascending) and peripheral blood levels do not differ significantly; the latter has, however, the lowest concentration owing to the fact that IFN is rapidly eliminated from the plasma pool (Bocci et al., 1982). This pattern is somewhat surprising but it may be due to considerable lymphatic absorption and to the fact that lymph drains into the general circulation.

In conclusion, this study has shown that with appropriate sorption promoters it is possible to induce some absorption of a large polypeptide such as IFN-α by the colorectal mucosa. Comparison of the IFN plasma levels obtained after subcutaneous or rectal administration indicates that IFN bioavailability is only about 2% with the latter route. On the basis of other studies (Bocci, 1985), it appears unlikely that the difference is due to the heterologousness of the system (i.e. human IFN in the rat). It may be possible to improve it by testing other promoters or selecting their optimal concentration and it seems worthwhile to investigate whether microenema rather than a suppository may increase distribution of IFN in the portal system. Finally, it may be rewarding to evaluate whether addition of proteinase inhibitors may reduce IFN inactivation in the colorectal lumen thus making this novel route of administration useful for clinical trials.

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