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# Oral administration of human recombinant interferon-$\alpha_2$ in rats

L. Paulesu, F. Corradeschi, C. Nicoletti and V. Bocci

*Institute of General Physiology, University of Siena, Siena (Italy)*

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## Summary

The absorption of human recombinant interferon-$\alpha_2$ (IFN$\alpha_2$) from the oropharynx has been investigated in the rat. We have shown that small amounts of interferon can be absorbed and measured in blood. The absorption of IFN$\alpha_2$ dissolved in saline was not improved by the addition of different promoters. The practical usefulness of a small absorption of interferon has been discussed.

## Introduction

The administration of interferon (IFN) by oral route has been found ineffective because of the gastrointestinal proteolysis (Wills et al., 1984). However, absorption of antibodies administered by oral route has been noted in newborns, suggesting that the gut mucosa can easily absorb immunoglobulins at least during the first days of life (Rogers Brambell, 1970).

Gardner et al. (1983), Ritschel and Ritschel (1983), Yoshykawa et al. (1984) and Bocci et al. (1985, 1986) have reported that insulin, gastrin and IFN can be, at least in part, absorbed by the rectal mucosa. Unexpectedly, Pacini et al. (1987) have detected some biological activity as well as protein-bound radioactivity in plasma following oral administration of tumor necrosis factor (TNF) in rabbits.

These results as well as the possibility that a small amount of proteins might be absorbed at the oropharynx level compelled us to investigate whether IFN in saline or with absorption promoter could be absorbed by oral administration.

## Materials and Methods

*IFN preparation*

Human recombinant (R-) IFN$\alpha_2$ obtained through the courtesy of Dr. I.I.A. Tabachnick and Dr. P.P. Trotta (Schering-Plough Research Division, Bloomfield, NJ), had a potency of $42.0 \times 10^8$ IU/ml; it was at least 98% pure and contained only a trace of human albumin.

Doses of 3.2 megaunits in a total volume (with adjuvants) of 0.1 ml were tested per rat in the following formulations:

(1) IFN diluted in saline;
(2) IFN diluted in saline with Labrafil R.M. 1944 added (Gattefossè, B.P. 603, F-69804 Saint-Priest Cedex, France). Labrafil is a preparation containing apricot kernel oil and PEG-6 transesters and is practically atoxic;
(3) IFN diluted in saline containing 3 mg sodium ursodeoxycholate (Na-UDC) In comparison

*Correspondence*: V. Bocci, Institute of General Physiology, University of Siena, Via Laterina 8, 53100 Siena, Italy.

with other sorption promoters, this adjuvant has the advantage of not being harmful in physiological dosages; and

(4) IFN diluted in saline containing Na-UDC to which Labrafil was added.

All formulations were prepared with or without 0.5% methylcellulose and used at once. Methylcellulose was added to increase the viscosity and stickiness of the solution in the hope of delaying a rapid swallowing of the IFN solution.

Twenty-seven male adult Wistar rats with an average body weight of 330 g were used throughout. The animals were allowed food and water ad libitum before the experiment. During light and transient ether anesthesia, 0.1 ml of R-IFN$\alpha_2$ preparation was dropped in the middle portion of the tongue. For comparative purposes some rats were injected subcutaneously at two sites of the abdomen with the same IFN doses. Blood, collected from the tail (150 $\mu$l) was diluted into 150 $\mu$l of heparinized (10 U/ml) saline and the diluted plasma was kept frozen at $-20\,^{\circ}C$ until IFN measurements were performed.

*IFN determination*

The IFN assay was carried out in microtiter plates (Costar, U.K.) as described by Langford et al. (1981) using human amniotic cells (Wish) and vesicular stomatitis (VSV, Indiana strain) as a challenge virus. All samples were assayed at least twice in triplicate. The assays were always made employing the international reference preparations for human IFN$\alpha$ (obtained from the National Institute for Biological Standards and Control, London). The standard of human IFN$\alpha$ (G-023-901-527) with a defined potency of 4.3 Log/vial, when reconstituted in 1.0 ml of sterile distilled water, yielded in our assay system a geometric mean titer of 4.8 $\log_{10}$ IU/ml (S.D. = 0.069; $n = 16$). All titres corrected for the initial dilution are reported in IU/ml.

## Results and Discussion

In order to examine whether IFN is absorbed via the oral route, we have used human IFN$\alpha_2$ because homologous and pure IFN$\alpha$ is not readily available and since no antibodies are formed during acute experiments, all mammalian IFNs have a similar pharmacokinetic behaviour (Cantell and Pyhälä, 1973).

To facilitate the absorption from the oropharyngeal mucosa, IFN$\alpha_2$ was administered in several formulations and in all of them the resulting IFN activity was quite stable.

Bioavailability of IFN$\alpha$ is practically quantitative after subcutaneous (s.c.) injection and the area under the curve (IFN concentration in plasma versus time) reflects well the phases of absorption, equilibrium and catabolism of the drug. Fig. 1 indicates that after s.c. administration the peak ($C_{max}$) is reached 2 h after administration; subsequently IFN levels declined rapidly and were negligible within 7 h. In these experiments $C_{max}$ was equivalent to $1150 \pm 120$. On the other hand oropharyngeal administration of the same dose of IFN$\alpha_2$ yielded very low (between 4 and 30 IU/ml) IFN plasma levels (Fig. 2) indicating in all cases a minimal absorption. However, even though the addition of either Labrafil or Na-UDC to the IFN dissolved in saline solutions did not significantly improve the absorption, it modified the pattern of the areas under curve. As can be seen in Fig. 2, the IFN plasma peak was delayed when Labrafil was

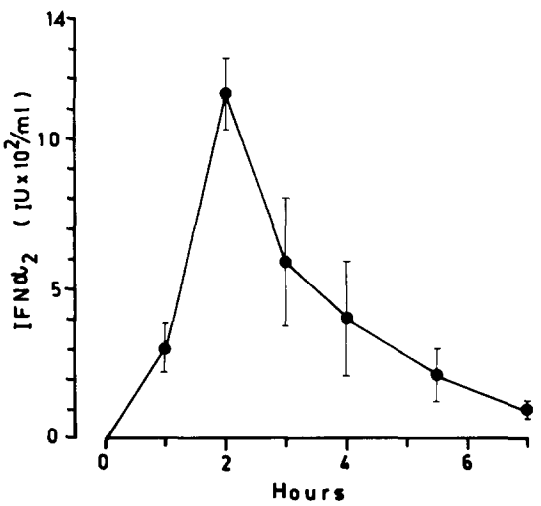


Fig. 1. Interferon plasma levels obtained in rats after subcutaneous injection of human recombinant interferon-$\alpha_2$. Values are reported as mean $\pm$ S.D.

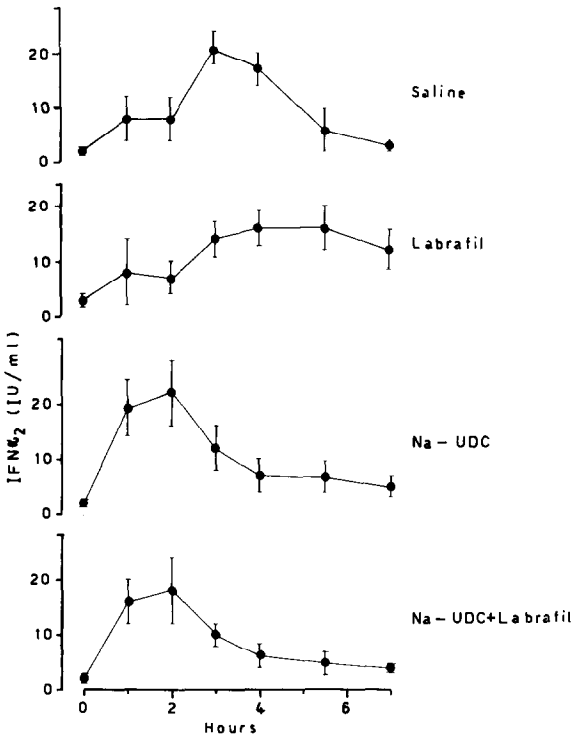


Fig. 2. Interferon plasma levels obtained in rats after oropharyngeal administration of interferon-$\alpha_2$ dissolved in saline or with addition of different promoters, without methylcellulose. Values are reported as mean ± S.D.

present and remained fairly constant until the end of the experiment.

The presence of Na-UDC anticipated the IFN plasma peak at 2 h and yielded a curve with a pattern resembling the one obtained after s.c. injection. The association of both Labrafil and Na-UDC does not appear advantageous because only the effect of Na-UDC can be observed. All the experiments described in Fig. 2 were repeated with the same formulations after addition of methylcellulose but the increase of viscosity did not improve the absorption of IFN.

In conclusion, our data indicate that a small amount of IFN$\alpha_2$, as detected by measuring its antiviral activity, can be absorbed and measured in blood after oral administration. Thus the classical concept that proteins cannot be absorbed at all after oral administration does not hold true anymore and it is likely that absorption occurs in the oropharyngeal mucosa. Unfortunately the periodic swallowing of saliva washes the IFN solution into the oesophagus and limits the absorption considerably. With the pharmaceutical preparations used here we have speeded up absorption only with Na-UDC but unfortunately this agent does not increase the rate of IFN absorption so that the total amount absorbed does not differ from the saline control. Comparison of the bioavailability between s.c. and oral administration indicates that the latter is minimal and casts doubts concerning its practical usefulness. However, it remains uncertain whether even a small absorption of IFN that certainly does not procure toxicity may significantly improve immunological parameters thus making this route more attractive. Further work with homologous IFN should clarify this aspect.

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## References

Bocci, V., Naldini, A., Corradeschi, F. and Lencioni, E., Colorectal administration of human interferon α. *Int. J. Pharm.*, 24 (1985) 109–114.

Bocci, V., Corradeschi, F., Naldini, A. and Lencioni, E., Enteric absorption of human interferons α and β in the rat. *Int. J. Pharm.*, 34 (1986) 111–114.

Cantell, K. and Pyhälä, L., Circulating interferon in rabbits after administration of human interferon by different routes. *J. Gen. Virol.*, 20 (1973) 97–104.

Gardner, C.R., Nishihata, T., Caldwell, L., Selk, J., Fix, S. and Higuchi, T., Absorption promoting adjuvants: animal studies on their effects on rectal drug absorption. *Bull. Tech. Gattefossè Rep.*, 78 (1983) 16.

Langford, M.P., Weigent, D.A., Stanton, G.J. and Baron, S., Virus plaque-reduction assay for interferon: microplaque and regular macroplaque reduction assay. In Pestka, S. (Ed.), *Methods in Enzymology, Vol. 78, Part A*, Academic, New York, 1981, pp. 339–346.

Pacini, A., Maioli, E., Bocci, V. and Pessina, G.P., Studies on Tumor Necrosis Factor (TNF). III. Plasma disappearance curves after intramuscular, subcutaneous, intraperitoneal and oral administration of human recombinant TNF. *Cancer Drug Delivery*, 4 (1987) 17–23.

Ritschel, W.A. and Ritschel, G.B., Rectal administration of insulin. *Bull. Tech. Gattefossè Rep.*, 78 (1983) 18.

Rogers Brambell, F.W., In Neuberger, A. and Tatum, E.L. (Eds.), *The Transmission of Passive Immunity from Mother to Young, Frontiers in Biology, Vol. 18*, Elsevier, Amsterdam, 1970.

Yoshikawa, H., Takada, K., Muranishi, S., Satoh, Y.I. and Naruse, N., A method to potentiate enteral absorption of interferon and selective delivery into lymphocytes. *J. Pharmacobiol. Dyn.*, 7 (1984) 59–62.

Wills, R.J., Spiegel, H.E. and Soike, K.F., Pharmacokinetics of recombinant alpha A interferon following IV infusion and Bolus IM, and PO administration to African green monkeys. *J. Interferon Res.*, 4 (1984) 399–409.