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*Report*

# Development of Interferon Suppositories. I. Enhanced Rectal Absorption of Human Fibroblast Interferon by Fusogenic Lipid via Lymphotropic Delivery in Rats

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We attempted to enhance the rectal absorption of human fibroblast interferon (HuIFN-β) in rats by the administration of suppositories containing fusogenic lipid and a nontoxic surfactant. Suppositories containing either the lipid (linoleic acid) or the surfactant [HCO60; polyoxyethylated (60 mol) hydrogenated castor oil] alone failed to enhance the absorption of HuIFN-β. However, suppositories containing both linoleic acid and HCO60 facilitated the rectal absorption of HuIFN-β. The absorbed HuIFN-β was selectively delivered via the lymph.

**KEY WORDS:** interferon suppository; enhanced rectal absorption; fusogenic lipid; lymphotropic delivery.

## INTRODUCTION

Owing to its large molecular size, it is generally not expected that absorption of interferon (IFN) from the alimentary canal will occur (1). We have found that fusogenic lipid–surfactant mixed micelles (MM) safely facilitated the colorectal absorption of various poorly absorbable drugs (2–7) as well as human fibroblast IFN (HuIFN-β) (8,9) and human leukocyte IFN (HuIFN-α) (10). We report here the potentiation of HuIFN-β absorption from the rectal suppository containing MM components and its lymphotropic delivery in rats.

## MATERIALS AND METHODS

Purified HuIFN-β (approximate MW, 22,000; sp act, $> 1 \times 10^7$ IU/mg protein; Toray Industries Inc., Tokyo) was used. Linoleic acid (LA) of 99.0% high-purity grade (Nippon Oil & Fats Co., Ltd., Tokyo) and HCO60 [polyoxyethylated (60 mol) hydrogenated castor oil from Nikko Chemicals Co., Ltd., Tokyo] were chosen as lipid and surfactant, respectively. Each minisuppository was prepared as follows. HuIFN-β ($3 \times 10^6$ IU) was homogeneously incorporated at 37°C into 600 μl of a melted hydrophobic suppository mass (Witepsol W 35, Dynamit Nobel Chemicals, Troisdorf-Oberlar, West Germany) containing (i) no adjuvants, (ii) linoleic acid (30%, w/w), (iii) HCO60 (10%, w/w), and (iv) both linoleic acid (30%, w/w) and HCO60 (10%, w/w).

Male Fisher 344 rats (Charles River Japan, Inc., Kanagawa) weighing 300 to 350 g (not fasted) were anesthetized by the intraperitoneal injection of pentobarbital. During the anesthesia the suppository, containing $3 \times 10^6$ IU HuIFN-β, was inserted into the rectum, and the anal canal was closed with a tissue cement. The blood from the carotid artery and the lymph from the thoracic duct were collected. To minimize the thermal inactivation of IFN, all samples were kept frozen at −100°C immediately after collection until IFN measurements. The antiviral activity of HuIFN-β was determined by measuring the quantity required for a 50% reduction of the cytopathic effect (11) on human amniotic cells (FL cells) challenged with vesicular stomatitis virus (New Jersey serotype). Minimum detectable levels by this method were 10 IU/ml for both serum and lymph. The titer of HuIFN-β was calibrated and expressed in terms of the HuIFN-β standard (Catalog No. G-023-902-527) of the National Institutes of Health, USA, for reference.

## RESULTS AND DISCUSSION

In our previous report, HuIFN-β dissolved in MM (LA + HCO60) solution was administered to the lumen of the rat colorectum, and selective lymphatic transfer (lymph to serum level ratio = 25 to 40) was observed (8). In the present experiment, HuIFN-β incorporated in a suppository mass containing no adjuvants and either linoleic acid or HCO60 did not yield detectable HuIFN-β levels in either the serum or the lymph. However, the administration of suppositories with both LA and HCO60 as MM components resulted in high concentrations (50–300 IU/ml, peak level, 1.5 hr after administration) in the lymph for 5 hr, while little HuIFN-β was detected in the serum (Fig. 1). Therefore, the lymphotropic property of HuIFN-β from the colorectum was retained with the administration of the suppository containing MM components.

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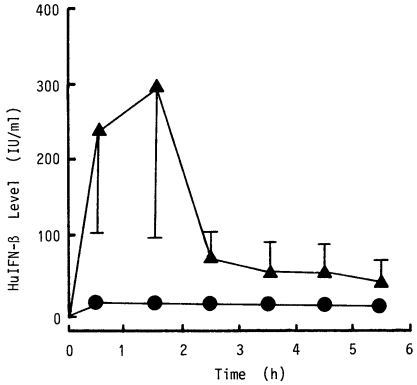


**Fig. 1.** Serum and lymph level of HuIFN-β in rats after the administration of rectal suppositories containing linoleic acid and HCO60. (●) Serum; (▲) lymph. Each points represents the mean ± SE of four experiments. The SE is indicated unless smaller than the point as plotted.

The peak level and the area under the curve of HuIFN-β in the lymph produced by rectal suppository administration (Fig. 1) were approximately one-fourth of those resulting from the administration of a MM solution (8). Generally, with the administration of suppositories, the dissolution rates of drug and adjuvants are considered to limit the drug absorption in comparison with their administration in solution. Further, the subsequent diffusion rates of HuIFN-β and MM components in the molten suppository are also expected to be lower than those in the MM solution, owing to the higher viscosity in the molten state. Muranushi *et al.* have suggested that the diffusion rates of MM components in the intestinal lumen affect the degree of the enhanced intestinal mucosal permeability (12). These factors may have contributed to the lower absorption of HuIFN-β from the suppository than from the previously administered solution (8). We have already reported that the effect of MM (LA + HCO60) as an absorption promoter for HuIFN-α was a temporary action with a reversible change of mucosal permeability (10). In addition, light and electron microscopy of the colorectal mucosal surface exposed to the suppository used in Fig. 1 showed that the epithelial cells remained intact (not shown). These results suggest that the suppository containing linoleic acid and HCO60 does not damage the colorectal mucosa.

Recently, Bocci *et al.* have measured the absorption of human lymphoblastoid IFN-α in the rat from a rectal suppository containing sodium ursodeoxycholate as an adjuvant (13). Although the observed blood levels were rather low, these authors speculated on considerable lymphatic participation in the colorectal absorption route. We have also reported that absorbed HuIFN-α (approximate MW, 18,000) as well as HuIFN-β was selectively transferred into the lymphatics with the aid of MM (10). We now conclude that HuIFN-α is also lymphotropically absorbed after the rectal administration of a suppository containing MM components.

This is the first report in which fusogenic lipid and surfactant as combined absorption promoters were applied in rectal suppositories for enhancing the absorption of HuIFN. We expect that further enhancement of HuIFN absorption can be achieved by regulating the components and the doses of lipid and surfactant in suppositories for clinical application.

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