Potentiation of Enteral Absorption of Human Interferon Alpha and Selective Transfer into Lymphatics in Rats

Hiroshi Yoshikawa[1,3], Kanji Takada[1], Yu-ichiro Satoh[2], Norio Naruse[2], and Shozo Muranishi[1]

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Abstract: Enhancement of human leucocyte interferon (HuIFN-α) absorption from rat large intestine was studied with the aid of lipid, surfactant and lipid-surfactant mixed micelles. Neither the emulsified lipid (linoleic acid) nor the surfactant (HCO60, polyoxyethylated [60 moles] hydrogenated castor oil) alone were able to enhance the absorption of HuIFN-α. However, linoleic acid-HCO60 mixed micelles enhanced the absorption of HuIFN-α from the large intestine. Highly selective delivery of HuIFN-α into the lymphatics compared to the blood was also observed.

Cantell et al. have proposed that an efficient gastrointestinal absorption of interferon (IFN) will be difficult to achieve owing to its large molecular weight (1). We have found that with the aid of fusogenic lipid-surfactant mixed micelles (MM), the mucosal permeability of the alimentary tract of animals, especially in the colo-rectal region, was harmlessly and temporarily enhanced for poorly absorbable drugs (2–7) as well as human fibroblast IFN (HuIFN-β) (8). In this paper, we describe the absorption enhancement by MM of human leucocyte IFN (HuIFN-α) from the rat large intestine and selective delivery into the lymphatics compared to the serum.

Materials and Methods

Purified HuIFN-α (approximate molecular weight 18 k Dalton) with a specific activity of 3.6×10^6 international units (IU)/mg protein was kindly provided by Dr. K. Cantell, Central Public Health Laboratory, Helsinki, Finland. Linoleic acid (LA) of 99.0% purity grade (Nippon Oil & Fats Co., Ltd., Tokyo, Japan) as a lipid and polyoxyethylated (60 moles) hydrogenated castor oil (HCO60, Nikko Chemicals, Co., Ltd., Tokyo, Japan) as a surfactant were used. MM solution was prepared by dispersing LA (0.56% w/v) and HCO60 (0.4% w/v) in distilled water followed by sonication. The surfactant solution was prepared by dissolving HCO60 (0.4% w/v) in distilled water under vigorous shaking. The emulsion was prepared by dispersing LA (0.56% w/v) in distilled water under sonication. Test solutions for administration were prepared by dissolving HuIFN-α (1.5×10^6 IU/ml) in saline, HCO60 and MM solution, and LA emulsion, respectively.

Male Fisher 344 rats (Charles River Japan, Inc., Kanagawa, Japan) weighing 250–300 g (not fasted) were anesthetized by intraperitoneal injection of pentobarbital. *In situ* absorption experiments using rat large intestine were performed according to the method previously reported (7). Following a midline laparotomy, a closed loop of the entire large intestine (colon and rectum) was prepared by the ligation with a cannulated silicone tube into the proximal end of the colon and distal end of the rectum. The intestinal contents were removed by slow infusion of saline solution into the loop. Once the intestinal content was cleared, a gentle stream of air was applied to aid in the removal of residual fluids. Two ml of 37 °C test solution (saline, HCO60 and MM) or LA emulsion containing HuIFN-α (dose, 3×10^6 IU/rat) was introduced into the loop of the large intestine. Pretreatment experiments with MM solution were performed according to following procedure. The MM solution (0.56% w/v LA + 0.4% w/v HCO60) not containing HuIFN-α, was first introduced into the loop of the large intestine. After 2 h pretreatment, the MM solution in the intestine was entirely removed with the aid of air supplied by a syringe and washed out by saline solution. Immediately after washing, 2 ml of saline solution containing HuIFN-α (1.5×10^6 IU/ml) was infused in the loop. The blood from the carotid artery and the lymph from the thoracic duct were collected.

The antiviral activity of HuIFN-α was determined by measuring the 50% reduction of cytopathic effect on FL cells by vesicular stomatitis virus (9). Minimum detectable levels by this method were 30–40 IU/ml for the serum and 30–50 IU/ml for the lymph. The titer of HuIFN-α was calibrated and expressed in terms of the standard HuIFN-α (Catalogue No. G-023-901-527) of the National Institute of Health, U.S.A. for reference.

Results and Discussion

HuIFN-α dissolved in saline and HCO60 solution, or in LA emulsion and administered to the lumen of the large intestine, did not yield any HuIFN-α levels above the detection limit of the assay for serum and lymph. However, the administration with MM composed of LA-HCO60 produced high levels of HuIFN-α (160–1350 IU/ml) in the lymph for 5 h, while little HuIFN-α was detected in the serum (Fig. 1). The average ratios of HuIFN-α level in the lymph over that in the serum ranged from 11 to 20. This finding suggests that HuIFN-α

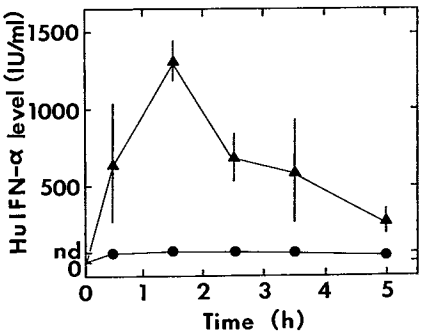

Fig. 1 Serum and lymph level of HuIFN-α after administration with mixed micelles (LA-HCO60) into the lumen of rat large intestine.
●, serum; ▲, lymph; nd, not detectable. Each point represents the mean ±S.E. of 3 experiments. S.E. is indicated unless smaller than the point as plotted.

[1] Department of Biopharmaceutics, Kyoto Pharmaceutical University, Kyoto, Japan
[2] Basic Research Laboratories, Toray Industries, Inc., Kamakura, Japan
[3] Address correspondence to: Hiroshi Yoshikawa Ph.D., Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto, 607, Japan

was absorbed through the intestinal mucosa with the aid of MM and was delivered into the lymphatics with high selectivity. Pretreatment with MM of the large intestinal mucosa did not stimulate the absorption of HuIFN-α, and therefore, the effect of MM as an absorption promoter for HuIFN-α is considered to be a temporary action without irreversible changes of mucosal permeability as previously reported for other poorly absorbable drugs (4, 5).

We found previously (8) that HuIFN-β (approximate molecular weight of 23 k Dalton) absorbed with the aid of MM from the rat large intestine was transferred into the lymphatics with lymph level/serum level ratios of 25–40. Therefore, the lymphotropic property of HuIFN absorbed from the large intestine by MM is considered to be independent of the type of HuIFN. Further systemic injections of HuIFN-β showed no selective lymphatic delivery (10). According to pharmacokinetic studies of HuIFN in various species including man, species differences were not observed (11, 12), and consequently, we may assume that in man lymphatic delivery of HuIFN would also occur as a result of the administration method used in this paper. The enteral route of HuIFN administration with lipid-surfactant mixed micelles may offer interesting clinical applications.

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