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Concentrations of human interferons α and β in rabbit body fluids

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Summary

Human interferons α and β were administered to rabbits and their concentrations in body fluids were determined with time. The routes of administration used were intravenous, subcutaneous, intramuscular, intraduodenal and intrarectal. Plasma, lymph and cerebrospinal fluid (CSF) were examined as body fluids. There was no relationship between human interferons α and β and the routes of administration or concentration in the body fluids. The distribution of α and β in the body fluids varied markedly with the routes of administration.

Introduction

The biological method which has conventionally been used for quantitative determination of human interferon requires much time and skill and, furthermore, is difficult to use for the quantitative determination of extremely small amounts of interferon. Therefore, there are only a few reports of concentrations of interferon in the blood. For example, the concentration of interferon in blood in man has been reported by Cantell and Phyhälä (1973), Skreko et al. (1973) and Cantell et al. (1974). It is said that the half-life after intramuscular injection is 4.8 h (Merigan et al., 1975) or 2.8 h (Jordan et al., 1974).

Although it is believed that interferon has species specificity, it is known that human interferon exerts an effect on rabbit cells. Therefore, concentrations of

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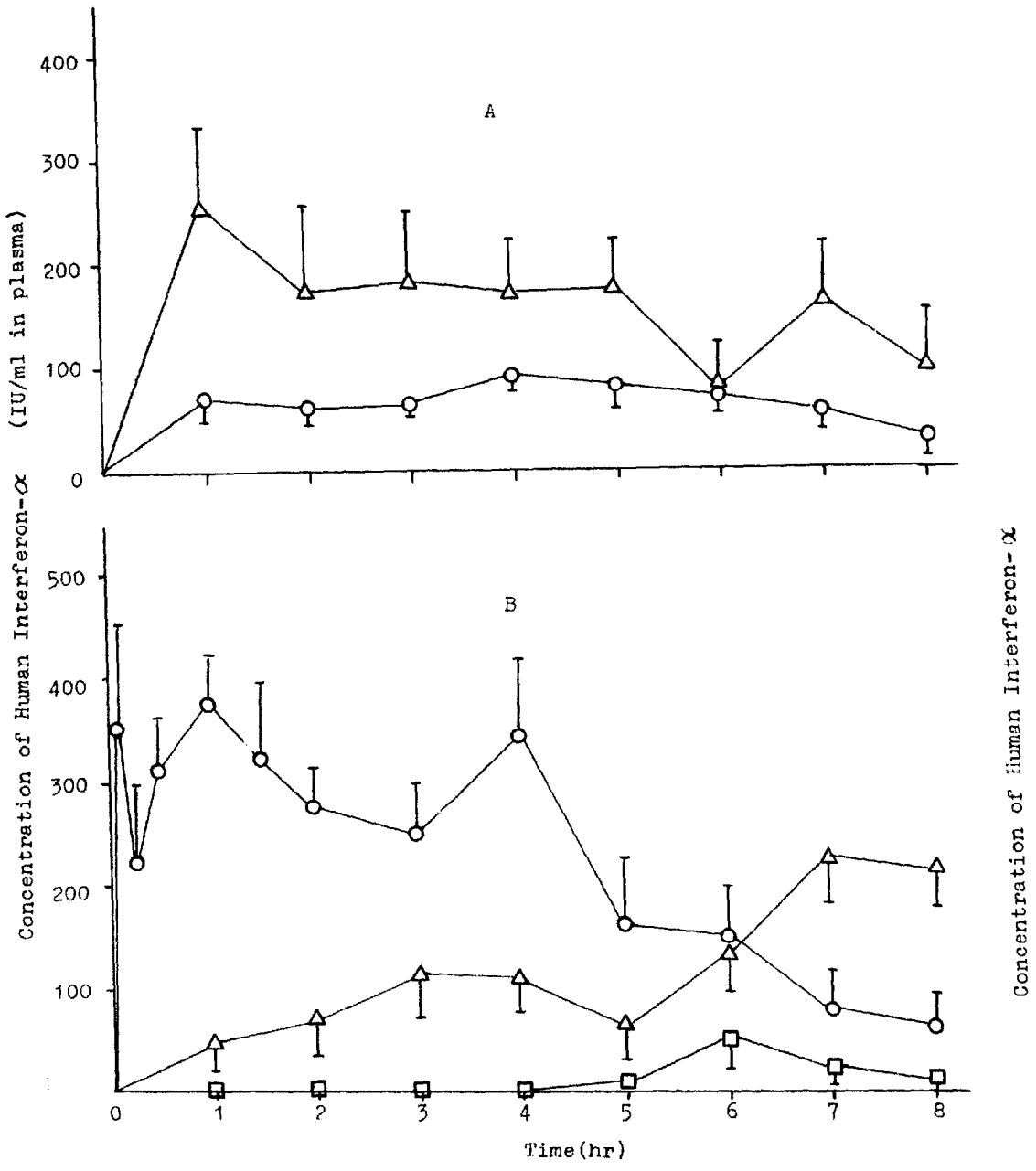

Fig. 1. Human interferon α concentration (mean $\pm$ S.E.) in rabbit plasma after administration of human interferon α by different routes. Key: (A) (○——○), injection into the duodenum (1.0×10^4 I.U. per animal); (△——△), injection into the rectum (1.0×10^4 I.U. per animal). (B) (○——○), intravenous injection (1.0×10^4 I.U. per animal); (△——△), subcutaneous injection (1.0×10^4 I.U. per animal); (□——□), intramuscular injection (1.0×10^4 I.U. per animal).

human interferons α and β in plasma, lymph and CSF in rabbits were determined by the trypsin method described previously by the authors (Naito et al., 1983).

Materials and Methods

Reagents

Interferon α (1.0×10^6 I.U., Kyoto Red Cross Blood Center); interferon β (1.0×10^6 I.U., Mochida Pharmaceuticals, Tokyo), kallikrein (1971 KU, Bayer AG, Leverkusen, F.R.G.); trypsin (Sigma Chemicals, St. Louis, MO, U.S.A.); trypsin inhibitor (Sigma); aprotinin (Trasyrol, Bayer AG).

Animals

The experimental animals were male albino rabbits (Keari, Japan) weighing 2.3–2.5 kg. They were given no food for 24 h and free access to water prior to the experiment. Each group consisted of 5 rabbits.

Collection of body fluids

Samples of plasma, lymph and CSF were collected according to the method of Naito et al. (1981).

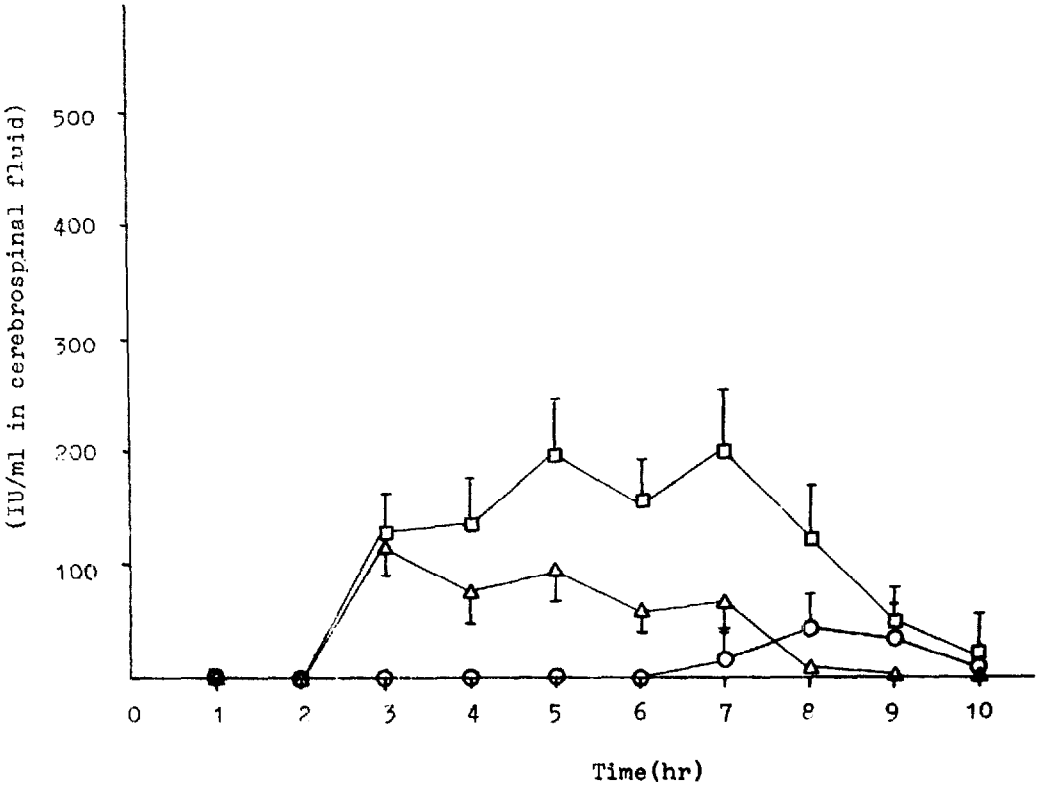

Fig. 2. Human interferon α concentration (mean ± S.E.) in cerebrospinal fluid of rabbits after administration of human interferon α by different routes. Key: (○——○), subcutaneous injection (1.0×10^4 I.U. per animal); (△——△), intramuscular injection (1.0×10^4 I.U. per animal); (□——□), injection into the duodenum (1.0×10^4 I.U. per animal).

Administration of interferons α and β

Intravenous administration. 1 ml (1.0×10^4 I.U.) of interferon α per animal was injected into rabbits. Blood samples were collected 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min after the injection. Lymph and CSF samples were collected every hour for 10 h after the injection. 1 ml (1.3×10^4 I.U.) of interferon β per animal was injected, and blood samples, lymph and CSF samples were collected in a way similar to that for interferon α.

Subcutaneous administration. 2 ml (1.0×10^4 I.U.) of interferon α per animal or 2 ml (2.0×10^4 I.U.) of interferon β per animal were injected. Blood samples were collected every hour for 8 h after the injection of interferon α or every hour for 10 h

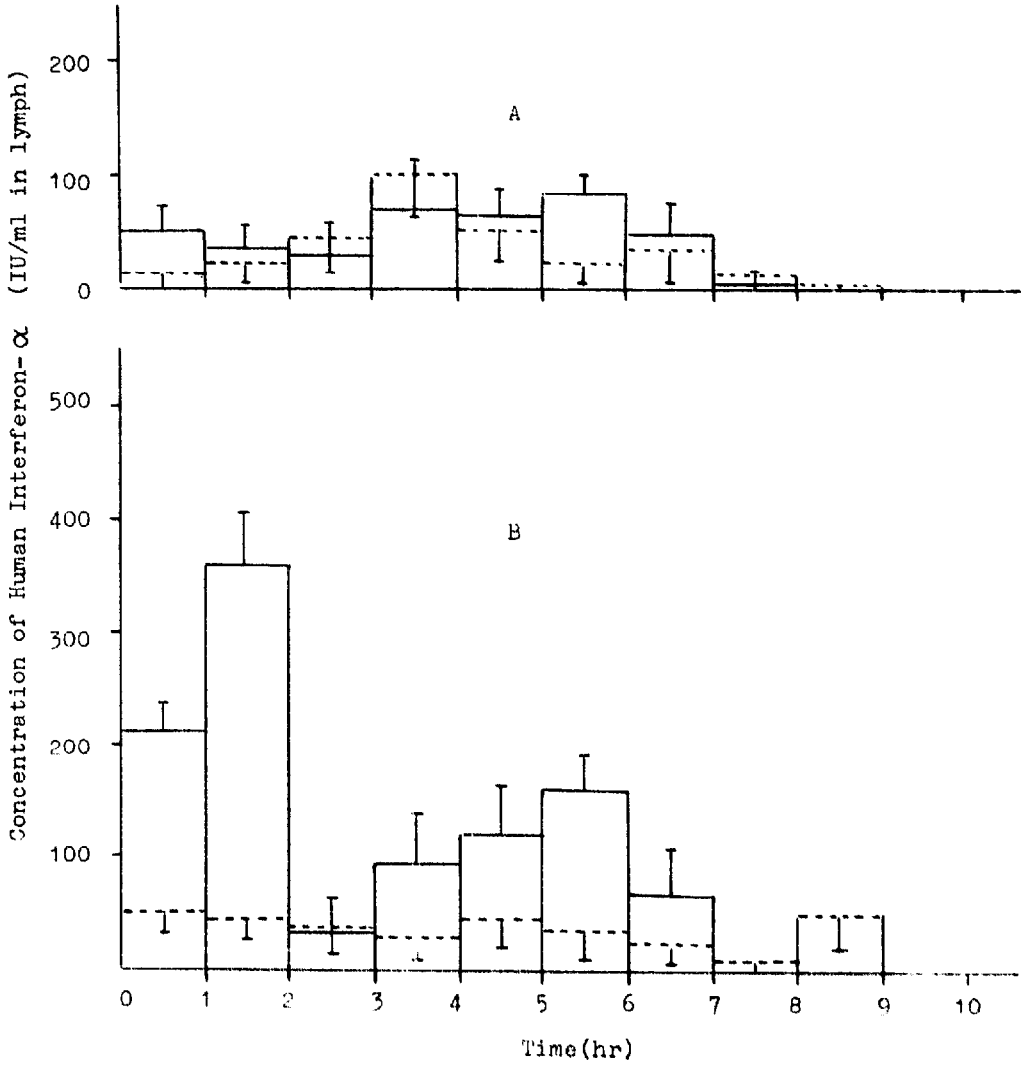

Fig. 3. Human interferon α concentration (mean ± S.E.) in lymph of rabbits after administration of human interferon α by different routes. Key: (A) ———, intramuscular injection (1.0×10^4 I.U. per animal); - - - - - -, injection into the rectum (1.0×10^4 I.U. per animal). (B) ———, intravenous injection (1.0×10^4 I.U. per animal); - - - - - -, subcutaneous injection (1.0×10^4 I.U. per animal).

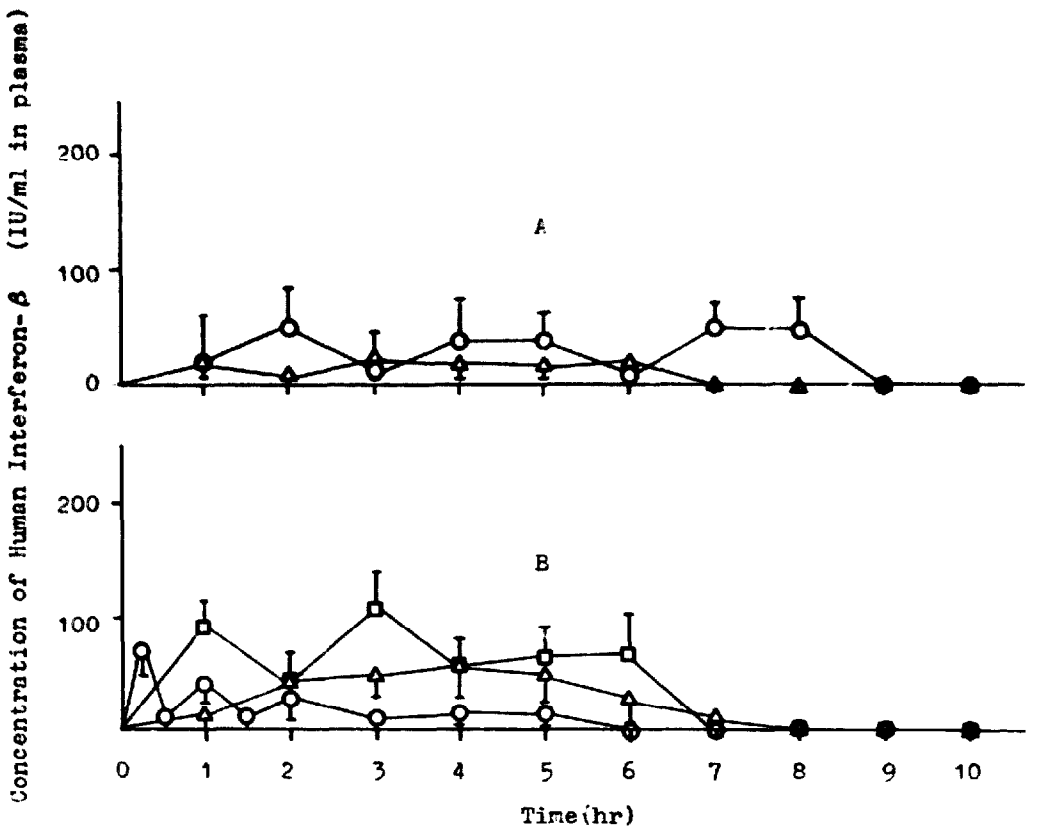

Fig. 4. Human interferon β concentration (mean ± S.E.) in rabbit plasma after administration of human interferon β by different routes. Key: (A) (○——○), injection into the duodenum (2.0×10^4 I.U. per animal); (△——△), injection into the rectum (2.0×10^4 I.U. per animal). (B) (○——○), intravenous injection (1.3×10^4 I.U. per animal); (△——△), subcutaneous injection (2.0×10^4 I.U. per animal); (□——□), intramuscular injection (6.0×10^4 I.U. per animal).

after injection of interferon β. Lymph and CSF samples were collected every hour for 10 h after the injection of both interferons.

Intramuscular administration. 2 ml (1.0×10^4 I.U.) of interferon α per animal or 2 ml (6.0×10^4 I.U.) of interferon β per animal were injected. Blood samples, lymph and CSF samples were collected in a way similar to that for subcutaneous administration.

Intraduodenal administration. 2 ml (1.0×10^4 I.U.) of interferon α per animal or 2 ml (2.0×10^4 I.U.) of interferon β per animal were injected. Blood samples, lymph and CSF samples were collected in a way similar to that for subcutaneous administration.

Intrarectal administration. 2 ml (1.0×10^4 I.U.) of interferon α per animal or 2 ml (2.0×10^4 I.U.) of interferon β per animal were injected. Blood samples, lymph and CSF samples were collected in a way similar to that for subcutaneous administration.

Both interferons α and β were dissolved in physiological saline solution before use.

Determination of interferons α and β

The trypsin method was used according to the method previously described (Naito et al., 1983).

Results and Discussion

Figs. 1–3 show the concentration curves of human interferon α in plasma, lymph and CSF after injection into rabbits by various routes. Figs. 4–6 show the results of determination of concentrations of human interferon β in those body fluids after injection into rabbits.

As shown in these figures, the concentration curve pattern in body fluids is considered to be inappropriate for pharmacokinetic treatments. Therefore, area under the concentration–time curves (AUC) of interferons in plasma, obtained by

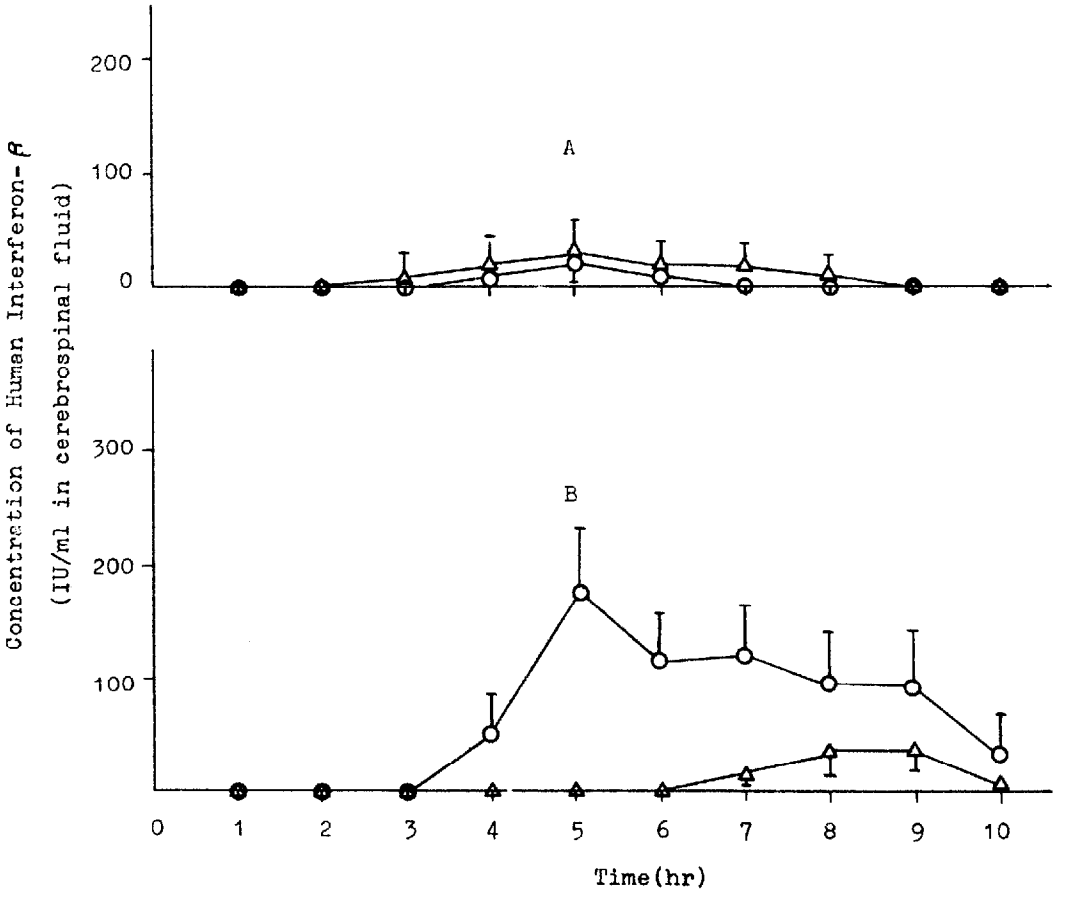

Fig. 5. Human interferon-β concentration (mean ± S.E.) in cerebrospinal fluid of rabbits after administration of human interferon by different routes. Key: (A) (○——○), injection into the duodenum (2.0×10^4 I.U. per animal); (△——△), injection into the rectum (2.0×10^4 I.U. per animal). (B) (○——○), subcutaneous injection (2.0×10^4 I.U. per animal); (△——△), intramuscular injection (6.0×10^4 I.U. per animal).

various administration routes, were determined by the trapezoidal rule, and the ratios of the areas under the concentration–time curves in lymph and CSF, when the plasma AUC obtained by each route was 1.00, were determined (Table 1). This method allows the comparison of the amounts of human interferon distributed in body fluids among the various routes of administration. As shown in Table 1, human interferon α could barely be detected in the CSF after intravenous, subcutaneous or intrarectal injection. On the other hand, a large amount of interferon α was determined in the CSF after intramuscular injection, and an extremely large amount of it was determined after the intraduodenal injection. The amount of interferon α transferred to the lymph was significantly larger than that transferred to the plasma after intramuscular injection, and it was slightly decreased after intravenous injection. After injection by other routes little or no interferon α was transferred to the lymph.

There was no correlation between human interferon α and β administered by the

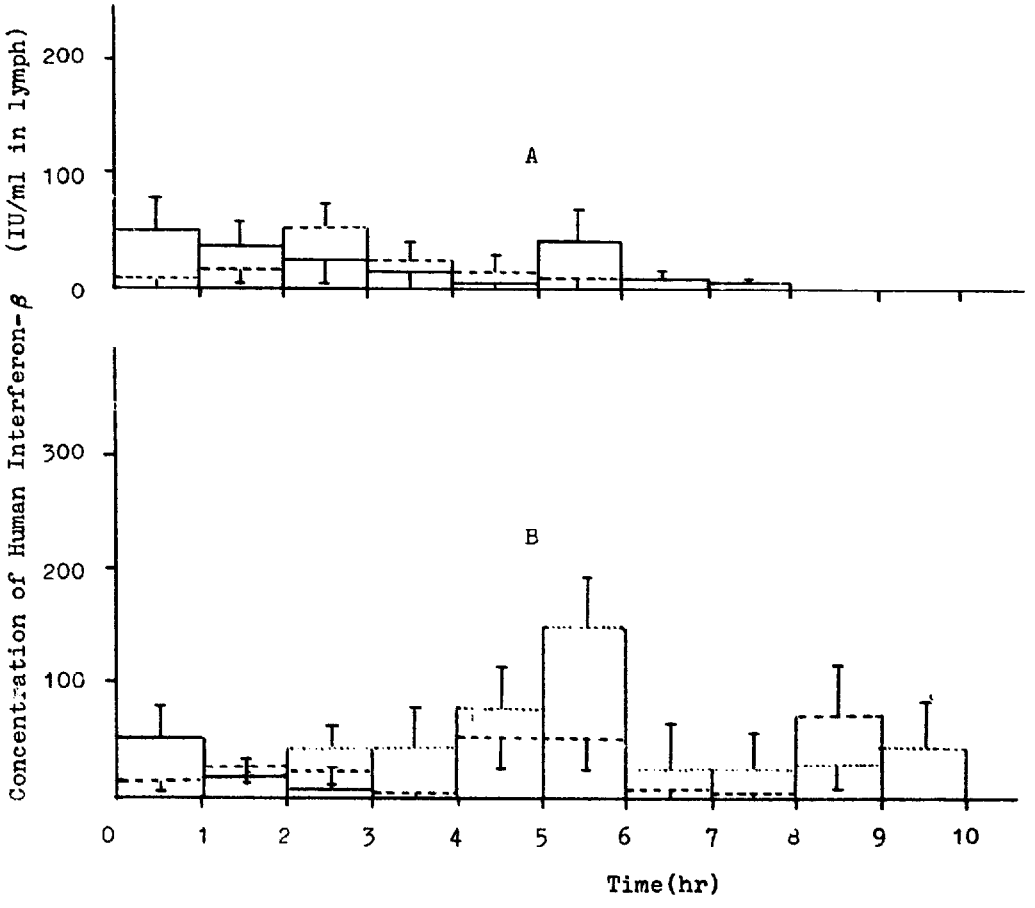

Fig. 6. Human interferon β concentration (mean ± S.E.) in lymph of rabbits after administration of human interferon β by different routes. Key: (A) ———, injection into the duodenum (2.0×10^4 I.U. per animal); - - - - - -, injection into the rectum (2.0×10^4 I.U. per animal). (B) ———, intravenous injection (1.3×10^4 I.U. per animal); - - - - - -, subcutaneous injection (2.0×10^4 I.U. per animal); · · · · · ·, intramuscular injection (6.0×10^4 I.U. per animal).

TABLE 1

AREA UNDER THE CONCENTRATION–TIME CURVE OF INTERFERON α OR β IN RABBIT BODY FLUIDS AFTER ADMINISTRATION OF INTERFERON α OR β

Administration route	Plasma		Lymph	Cerebrospinal fluid
	AUC	Ratio		
Interferon α				
i.v.	1919.8	1.00	0.81	0.00
s.c.	912.5	1.00	0.33	0.11
i.m.	80.0	1.00	4.88	4.71
duodenum	570.0	1.00	0.00	1.53
rectum	1155.0	1.00	0.27	0.00
Interferon β				
i.v.	113.8	1.00	0.79	0.00
s.c.	255.0	1.00	1.98	2.59
i.m.	402.5	1.00	1.11	0.25
duodenum	275.0	1.00	0.69	0.06
rectum	120.0	1.00	1.08	0.92

i.v. = intravenous injection; s.c. = subcutaneous injection; i.m. = intramuscular injection; duodenum = injection into the duodenum; rectum = injection into the rectum. AUC = area under the concentration–time curve (I.U.·ml^{-1}·h). Ratio was expressed when area under the concentration–time curve of interferon α or β in corresponding plasma to each administration route is 1.00. Each group consisted of 5 rabbits.

various routes. Because the chemical structure of α is different from that of β, this was to be expected.

The amount of human interferon β transferred to the lymph was greater than that transferred to the plasma after subcutaneous injection, and the amount decreased in the order of intramuscular injection then intrarectal injection. After subcutaneous injection, the amount transferred to the CSF was about 2.6 times as large as that transferred to the plasma. When it was intrarectally administered, the amount transferred to the CSF was almost the same as that transferred to the plasma. After administration by other routes it was not transferred at all, or only in an extremely small amount, to the CSF.

In conclusion, the amount of human interferon transferred to the various body fluids varies markedly with the route of administration.

Acknowledgements

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