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APROTININ CONCENTRATIONS IN BODY FLUIDS IN RABBITS

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SUMMARY

Aprotinin was administered to rabbits subcutaneously, intramuscularly, and by injection into the auricular vein, portal vien and duodenum to determine its concentration in the plasma. The concentration of aprotinin in the lymph and in the cerebrospinal fluid was also determined when it was injected into the auricular vein and duodenum in rabbits.

It was found that aprotinin is slightly absorbed from the digestive tract and transferred into the lymph and cerebrospinal fluid.

The concentration of aprotinin in the cerebrospinal fluid was also determined in 2 patients who received drip infusions of aprotinin.

INTRODUCTION

It is thought that aprotinin is a polypeptide containing 16 kinds of amino acids and that its molecular weight is approximately 6 500 (Anderer and Hornle, 1965). Aprotinin was first discovered as an inhibitor of kallikrein (Kraut et al., 1930). Furthermore, it seems that aprotinin acts as an inhibitor of trypsin, plasmin and other proteolytic enzymes. As far as the authors know, Beller et al. (1966) first reported on the distribution, half-life, and transplacental passage of aprotinin in humans. According to them, the half-life of aprotinin is 151 min and aprotinin does not pass through the placental barrier.

Keller (1969) found that aprotinin was absorbed into the blood and transferred into the kidney in mice. Also, he observed that when aprotinin was intravenously injected into rats, 70% of the total dose was present in the kidneys 12 h after injection and that the concentration in the kidneys decreased within several days.

Arndts et al. (1970) confirmed that aprotinin was concentrated in the kidneys when [^{131}I]-labelled aprotinin was intravenously injected into rats and mice, and it was marked in the cortex of the kidneys.

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Whether or not aprotinin passes through the blood–cerebrospinal fluid barrier is discussed in a report by Fanciullaci et al. (1969). According to them, aprotinin seems to enter the cerebrospinal fluid through the blood–cerebrospinal fluid barrier and reaches a high concentration in the fluid. The explanation given is that cerebrospinal fluid obtained from humans given aprotinin did not cause contraction of uterine muscles of rats. However, in that particular case, the concentration of aprotinin in the cerebrospinal fluid was not quantitatively measured; rather it was judged, on the basis of the determination of aprotinin-like activity, that aprotinin passes through the blood–cerebrospinal fluid barrier.

One of the authors have previously reported the plasma concentration of aprotinin when the agent was intravenously injected into humans (Naito et al., 1980). In the present work, aprotinin was administered to rabbits in various ways to determine its concentration in the plasma, lymph and cerebrospinal fluid. It was found that when aprotinin was injected into the duodenum, it was slightly absorbed into the blood; no such findings are in the literature. Furthermore, when aprotinin was administered to 2 patients by means of intravenous drip, it was found in the cerebrospinal fluid.

MATERIALS AND METHODS

Animals

The experimental animals were male albino rabbits (Keari, Japan) weighing 2.5–2.7 kg. They were given no food for 24 h prior to the experiment and were anesthesized with ether (Nakarai Chemicals, Kyoto, Japan).

Plasma sampling

A T-shaped catheter (Okasan Kagaku, Kyoto, Japan) treated with silicone was filled with heparin (Shimizu Seiyaku, Shimizu, Japan). The catheter was inserted into the carotoid artery, and a blood specimen (2.5 ml) was collected with a syringe which had been rinsed with a 10% solution of sodium citrate (Nakarai Chemicals, Kyoto, Japan). The sample was centrifuged at 3 000 rpm for 5 min to separate 1 ml of plasma for quantitative analysis.

Collection of lymph

Lymph specimens were collected from the thoracic duct by inserting a polyethylene tube (No. 3, 1 mm in external diameter) into the thoracic duct according to the method of Bollman et al. (1948).

Collection of cerebrospinal fluid

After the animals were anesthesized with 0.2–0.3 ml of 10% sterile amobarbital sodium solution (Nippon Shinyaku, Kyoto, Japan) injected into the auricular vein, an intravenous syringe (21-gauge X3/4 in T.W., Termo) was inserted into the cisterna magna beneath the medulla oblongata to collect 0.25 ml of cerebrospinal fluid.

Administration of aprotinin

A solution of aprotinin (Bayer AG, Leverkusen, G.F.R.) was administered to rabbits in various ways.

When blood samples were collected, aprotinin was administered in 5 ways (subcutaneously, intramuscularly, and by injection into the duodenum, auricular vein and portal vein). Five ml (50 000 KIU)/head of aprotinin was injected into the auricular vein and portal vein, and blood samples were collected 15, 30, 45, 60, 90, 120, 180 and 240 min after injection. Two ml (100 000 KIU)/head of aprotinin was injected subcutaneously and intramuscularly, and blood samples were collected 15, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after injection. Six ml (300 000 KIU)/head of aprotinin was injected into the duodenum, and blood samples were collected 15, 30, 45, 60, 90, 120, 180, 240, 360, and 480 min after injection.

When lymph specimens were collected, 5 ml (50 000 KIU)/head of aprotinin was injected into the auricular vein, and 6 ml (300 000 KIU)/head of aprotinin was injected into the duodenum. Lymph samples were collected 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420 and 480 min after each injection.

When cerebrospinal fluid samples were collected, aprotinin was injected into the auricular vein and duodenum. When it was injected into the auricular vein, 5 ml (50 000 KIU)/head of aprotinin was used, and samples of cerebrospinal fluid were collected 15, 30, 45, 60, 90, 120, 150, 180 and 240 min after injection. When it was injected into duodenum, 6 ml (300 000 KIU)/head of aprotinin was used, and samples of cerebrospinal fluid were collected 15, 30, 45, 60, 90, 120, 150, 180, 240, 360 and 480 min after injection.

Determination of aprotinin

According to the method described in the previous paper (Naito et al., 1980), a definite amount of kallikrein (Bayer AG, Leverkusen, G.F.R.) was added to a definite amount of plasma, lymph, or cerebrospinal fluid and the mixture was coupled with aprotinin. An excessive amount of kallikrein was quantitatively determined by using *p*-tosyl-α-arginine methylester (TAME) (Nakarai Chemicals, Kyoto, Japan) as a substrate to obtain the content of aprotinin.

Patients

The concentration of aprotinin in the cerebrospinal fluid was measured in two patients.

The subjects were patient D (a 65-year-old man weighing 60 kg) and patient K (a 42-year-old man weighing 55 kg), both of whom had ventricular drainage due to head injuries. Aprotinin (500 000 KIU) was dissolved in 50 ml of physiological saline solution and was mixed with 150 ml of Solita T_2 solution (Shimizu Seiyaku, Shimizu, Japan). The mixture was administered to the patients for 1 h by means of intravenous drip. Samples of cerebrospinal fluid were collected just after drip infusion and 0.5, 1, 2, 3 and 6 h after drip infusion.

RESULTS AND DISCUSSION

Fig. 1 shows the aprotinin concentration in the plasma when it was intravenously injected into rabbits. According to this figure, pharmacokinetic parameters were obtained by using a conventional method (Table 1). The parameters were substituted for

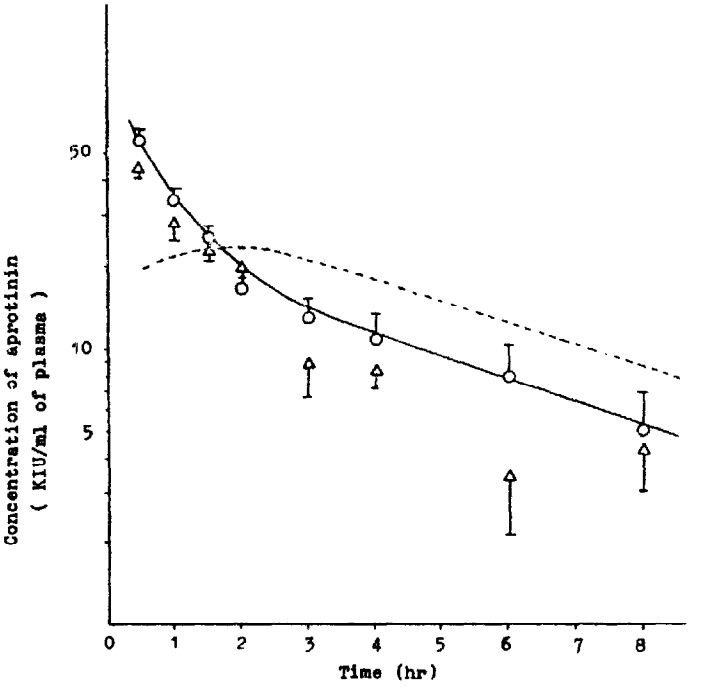


Fig. 1. Plasma level of aprotinin. ○, ear vein injection (50 000 KIU/head); △, portal vein injection (50 000 KIU/head); ——— and - - - - - -, calculated curves of aprotinin in a central and a tissue compartment, respectively, obtained from Eqns. 1 and 2 in Scheme 1.

Eqns. 1 and 2 in Scheme 1 to obtain the concentration of aprotinin in central and tissue compartments (Fig. 1).

As Fig. 1 shows, the observed values were lower than the calculated values in the case

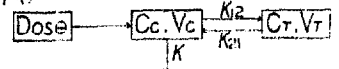
TABLE 1

EXPERIMENTAL AND PHARMACOKINETIC PARAMETERS * FOR PLASMA LEVEL OF APROTININ AFTER EAR VEIN INJECTION OF APROTININ (KIU/HEAD)

Experimental parameter		Pharmacokinetic parameter	
α	3.01 h	C_0	99 KIU/ml
β	0.35 h	K	1.12 h
A	77.0 KIU/ml	k_{12}	1.30 h
B	22.0 KIU/ml	k_{21}	0.94 h
		V_C	505 ml
		V_T	698 ml
		Area	88.4 KIU h/ml

* All parameters show values in the two-compartment model. α, slope of α-phase; β, slope of β-phase; A, intercept of α-phase, B, intercept of β-phase; C_0, plasma level of aprotinin at zero time; K, elimination rate constant; k_{12}, rate constant of transfer from the central to tissue compartment; k_{21}, rate constant of transfer from the tissue to central compartment; V_C, distribution volume of the central compartment; V_T, distribution volume of the tissue compartment; Area, area under the plasman concentration curve.

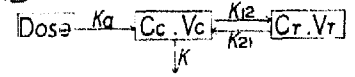
(A)



$$C_C = \frac{D}{V_C}\left\{\frac{(\alpha - K_{21})\cdot e^{\alpha t}}{\alpha - \beta} + \frac{(K_{21} - \beta)\cdot e^{\beta t}}{\alpha - \beta}\right\} \quad (1)$$

$$C_T = \frac{D}{V_T}\left\{\frac{K_{12}\cdot e^{\alpha t}}{\beta - \alpha} + \frac{K_{12}\, e^{\beta t}}{\alpha - \beta}\right\} \quad (2)$$

(B)



$$C_C = \frac{Ka\cdot F\cdot D}{V_C}\left\{\frac{(K_{21} - \alpha)\cdot e^{\alpha t}}{(Ka - \alpha)\cdot(\beta - \alpha)} + \frac{(K_{21} - \beta)\cdot e^{\beta t}}{(Ka - \beta)(\alpha - \beta)} + \frac{(K_{21} - Ka)\cdot e^{Ka\cdot t}}{(\alpha - Ka)\cdot(\beta - Ka)}\right\} \quad (3)$$

$$C_T = \frac{K_{12}\cdot Ka\cdot F\cdot D}{V_T}\left\{\frac{e^{\alpha t}}{(Ka - \alpha)\cdot(\beta - \alpha)} + \frac{e^{\beta t}}{(Ka - \beta)\cdot(\alpha - \beta)} + \frac{e^{Ka\cdot t}}{(\alpha - Ka)\cdot(\beta - Ka)}\right\} \quad (4)$$

Scheme 1

Equations for calculated curves of aprotinin in a central and a tissue compartment after ear vein injection (A) and subcutaneous or intramuscular injection (B).

of injection of aprotinin into the portal vein. This seemed to be because aprotinin is somewhat inactivated in the liver.

Figs. 2 and 3 show aprotinin concentrations in the lymph and in the cerebrospinal fluid after injection of the agent into the auricular vein. Because the concentration curves of aprotinin were not smooth, it was impossible to calculate pharmacokinetic parameters. Nevertheless, it was found that an extremely large amount of aprotinin is transferred

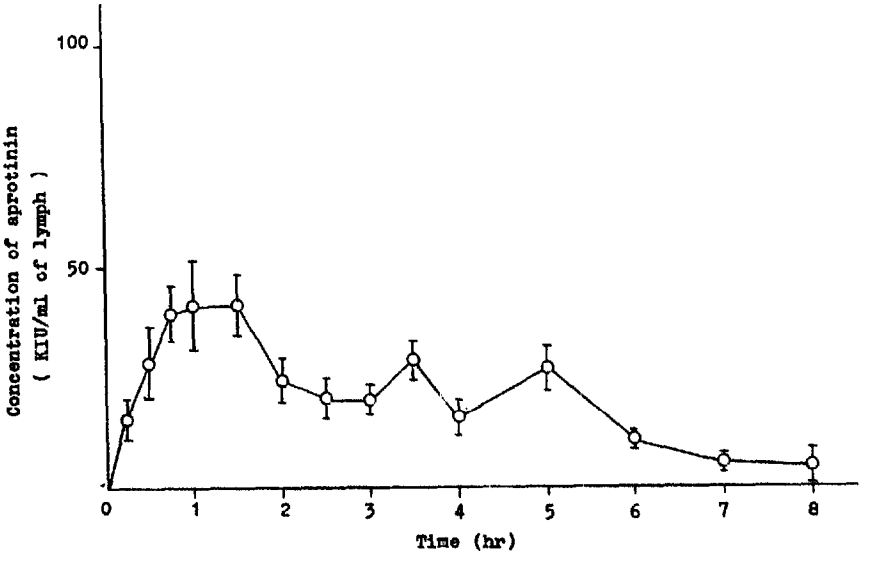


Fig. 2. Aprotinin in lymph after ear vein injection (50 000 KIU/head).

TABLE 2

CUMULATIVE AMOUNT OF APROTININ (KIU) IN LYMPH

Time (min)	Ear vein injection *			Duodenal admininstration **			
	Rabbit			Rabbit			
	A	B	C	D	E	F	G
15			83.2	0.0	0.0	7.8	1.6
30	41.7	50.5	238.5		4.9		8.3
45	83.7			10.1	4.9	7.8	
60	146.1	104.3	349.5			14.1	18.1
90	210.3		447.9	17.5	4.9	23.6	32.2
105	250.6						
120	285.6				4.9	23.6	50.4
150	330.9	164.8	496.3	27.4	8.8	25.9	58.5
180	357.8				17.8	25.9	60.6
210	380.8				29.1	25.9	72.4
240		202.3	554.6			25.9	72.4
300		217.8				25.9	
360			654.7	37.5		25.9	
420						25.9	
480		298.5		39.7		25.9	

* Aprotinin 50 000 KIU/head.
** Aprotinin 300 000 KIU/head.

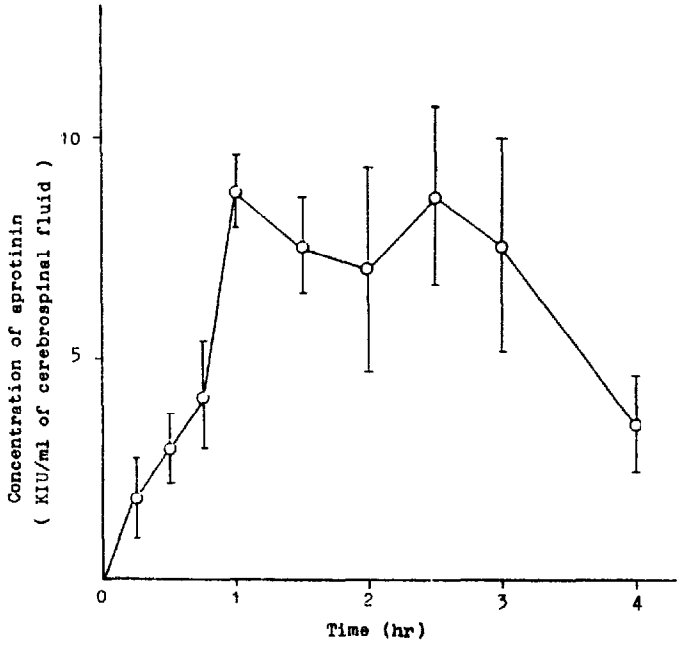


Fig. 3. Aprotinin in cerebrospinal fluid after ear vein injection (50 000 KIU/head).

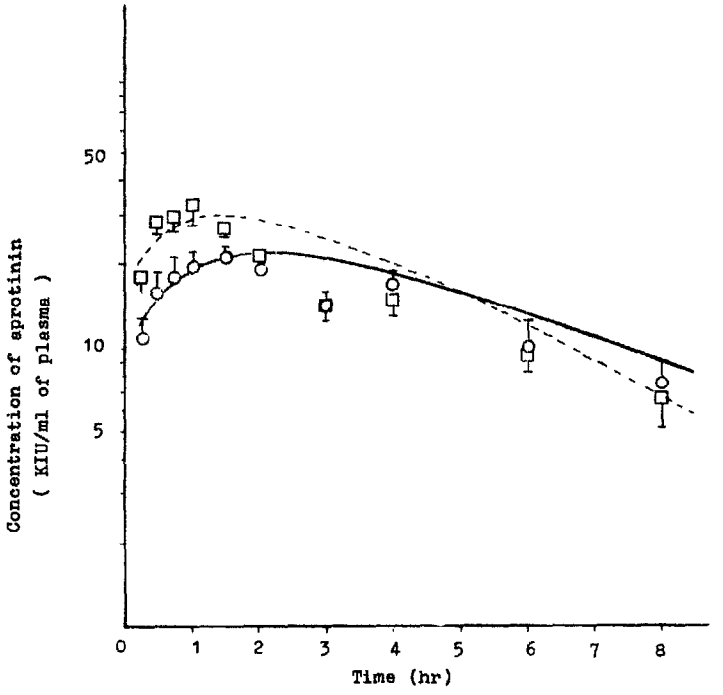


Fig. 4. Plasma level of aprotinin. ○, subcutaneous injection (100 000 KIU/head); □, intramuscular injection (100 000 KIU/head); ——— and ------, calculated curves of aprotinin (respectively, s.c. and i.m.) in a central compartment, obtained from Eqn. 3 in Scheme 1.

into the lymph and a small amount is transferred into the cerebrospinal fluid.

The curves in Fig. 4 show the plasma concentration of aprotinin after subcutaneous and intramuscular injections of the agent. In the case of subcutaneous and intramuscular

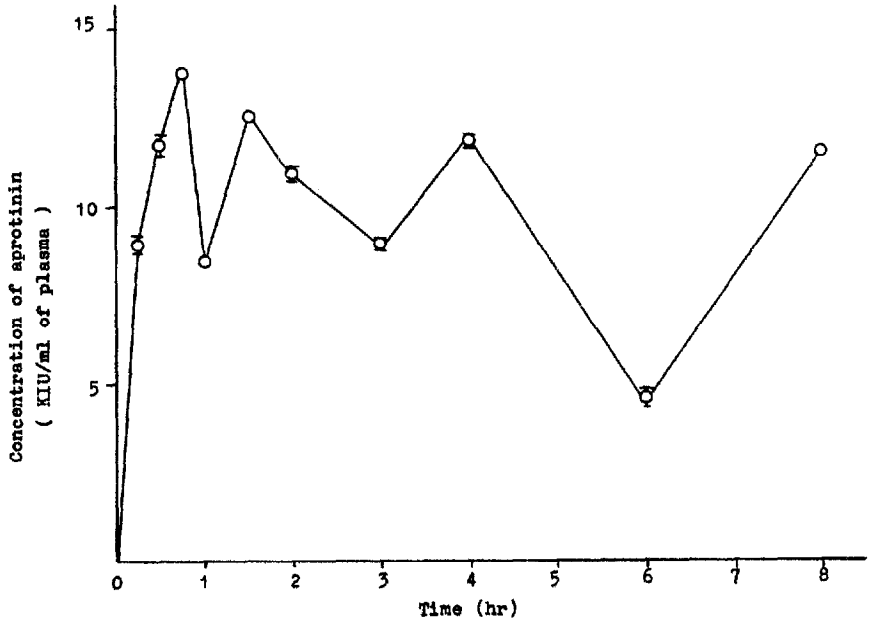


Fig. 5. Plasma level of aprotinin. ○, oral administration (300 000 KIU/head).

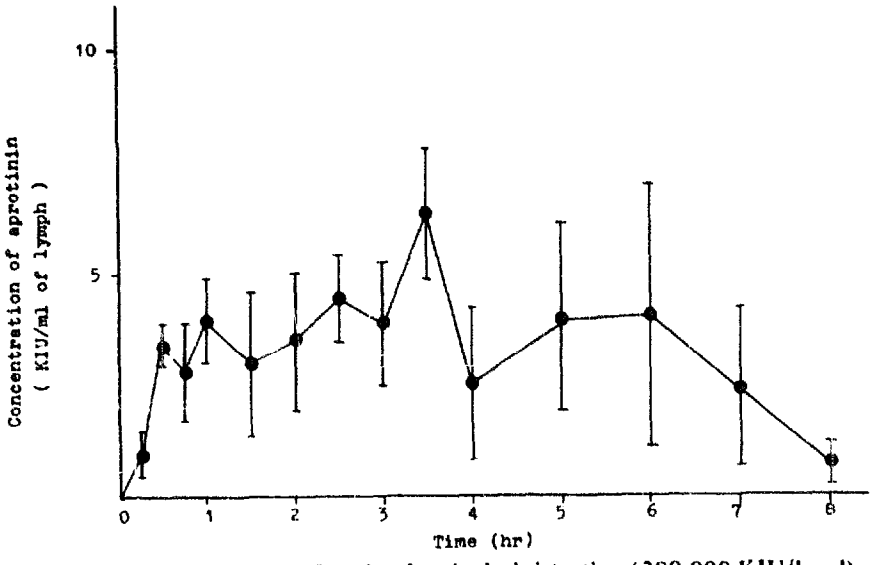


Fig. 6. Aprotinin in lymph after duodenal administration (300 000 KIU/head).

injections, a voluntary value was substituted for Eqn. 3 in Scheme 1 and the values nearest the observed values were regarded as K_a (absorption rate constant), based on the trial and error method – $K_a = 0.28\ h^{-1}$ and $K_a = 0.48\ h^{-1}$, respectively. However, it seems that aprotinin might be transferred into the lymph and cerebrospinal fluid because the observed values were lower than the values calculated from Eqn. 3 about 2 h after injection, as shown in Fig. 4. This possibility should be studied in detail in future.

Aprotinin concentrations in the plasma, lymph and cerebral fluid after injection of the agent into the duodenum are shown in Figs. 5, 6 and 7. Because none of the

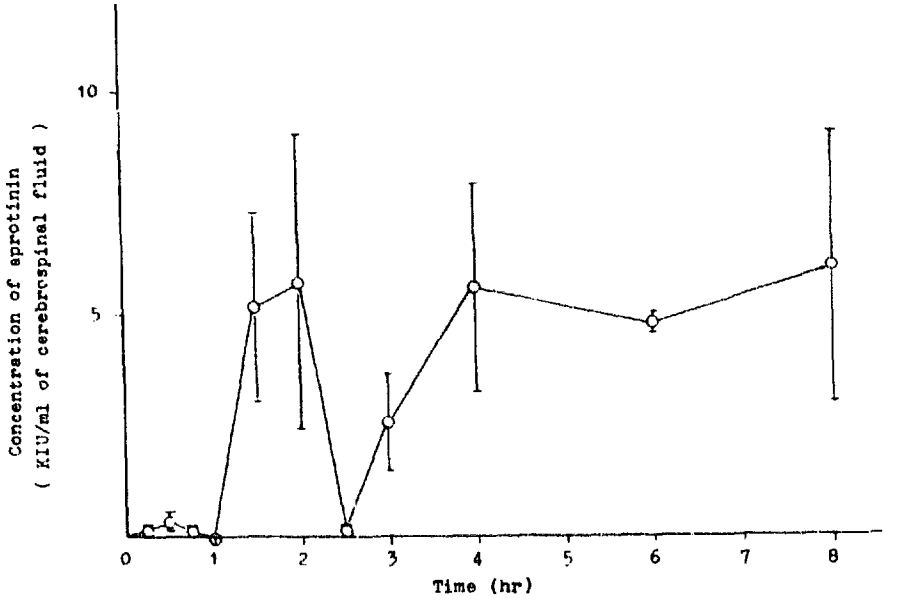


Fig. 7. Aprotinin in cerebrospinal fluid after duodenal administration (300 000 KIU/head).

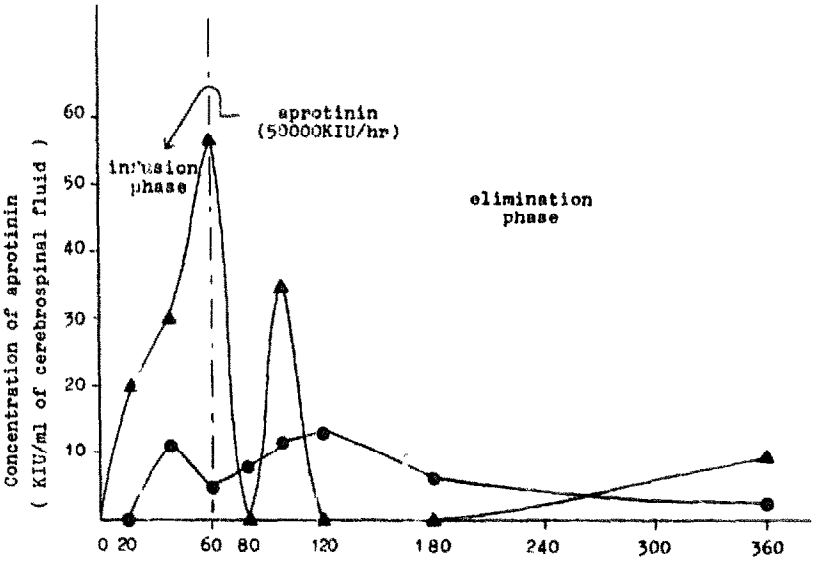


Fig. 8. Aprotinin in cerebrospinal fluid of patients. •, Patient D; ▲, Patient K.

concentration curves of aprotinin were smooth, it was not possible to calculate any pharmakokinetic parameters.

This experiment revealed that a small amount of aprotinin was absorbed from the digestive tract and transferred into the lymph and cerebrospinal fluid. It is incomprehensible that the concentration of aprotinin, especially in the cerebrospinal fluid, decreased temporarily; however, it increased again and was present even 8 h after administration.

Samples of cerebrospinal fluid from only two patients having ventricular drainage could be obtained and on whom drip infusion of aprotinin was performed. Fig. 8 shows aprotinin concentration in the cerebrospinal fluid. It is of interest that the concentration of aprotinin in human cerebrospinal fluid also tends to decrease temporarily and to increase again. The behavior of aprotinin in cerebrospinal fluid will be further studied.

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