催胆汁うっ滞因子の ^{125}I -immunoglobulin A 胆汁排泄への影響

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Jpn. J. Clin. Immun., 10(2): 161~170, 1987.

Effects of cholestatic factor on bile excretion of ^{125}I -dimeric immunoglobulin A

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【Summary】

The cholestatic factor, a lymphokine, has been shown to be produced from the lymph node cells of sensitized guinea pigs *in vitro* by stimulation with a specific antigen, PPD (purified protein derivatives). Two active fractions can be prepared from the supernatant of the medium by Sephadex G-75 gel filtration and DEAE cellulose column chromatography. When these fractions are injected into the mesenteric vein of normal rats, a marked reduction in bile flow is induced. Since dimeric immunoglobulin A (IgA) is richly contained in the bile and it is rapidly and actively transported from the blood to bile by hepatocytes, the authors investigated the effect of the cholestatic factor on dimeric IgA transport. As a result, this lymphokine induced a fall in dimeric IgA secretion, suggesting that it may also influence the vesicular transport system of the hepatocyte.

Key words : cholestatic factor,
lymphokine,
immunoglobulin A,
vesicular transport system

【概 要】

結核死菌感作モルモットのリンパ節細胞を精製ツベルクリン (purified protein derivatives, PPD) で刺激すると、催胆汁うっ滞因子 (cholestatic factor) が産生される。このリンホカインを Sephadex G-75 カラムによるゲル濾過および DEAE-cellulose カラムを用いるクロマトグラフィーで分画すると、2つの活性分画が得られ、これらの活性分画をラットの腸間膜静脈に注入すると、著明な胆汁排泄の抑制が認められた。この催胆汁うっ滞因子による胆汁排泄機構を研究する一環として、二量体 immunoglobulin A (IgA) の胆汁への排泄に催胆汁うっ滞因子がどのような影響を与えるかについて検討した。その結果、催胆汁うっ滞因子は IgA の胆汁への分泌を有意に抑制することが認められた。従って、催胆汁うっ滞因子は肝臓の vesicular transport system にも影響を及ぼすと推測された。

I. Introduction

Our previous investigations have suggested that a lymphokine, designated as cholestatic factor by us, might contribute to the development of intrahepatic cholestasis. This hypothesis was primarily proposed from our experiment in which the culture supernatant of antigen-stimulated lymphocytes from patients with drug-induced allergic hepatitis caused marked reductions in bile flow and bile excretion¹⁾. This was also evaluated in detail by using an animal model with tuberculin-sensitized guinea pigs²⁾. The cholestatic factor is a glycoprotein with a molecular weight of about 30,000 to 40,000 daltons³⁾, and it suppresses both bile acid-dependent and -independent bile flow⁴⁾. Previous studies have also suggested that this lymphokine markedly affects bile production in the bile canaliculi. This lymphokine-induced intrahepatic cholestasis was recently confirmed by Marbet et al.⁵⁾ who showed that the cholestatic factor was present in patients with alcoholic and acute viral hepatitis as well. In rats, plasma-derived dimeric immunoglobulin A (IgA) is transported through the liver parenchymal cells into the bile by a process involving receptor-mediated endocytosis, the intracellular vesicular transport system and subsequent exocytosis⁶⁻¹³⁾. Therefore, we designed the present study to investigate the effect of the cholestatic factor on biliary secretion of dimeric IgA.

II. Materials and methods

1. Preparation of the culture supernatant of activated lymphocytes

Outbred Hartley-strain guinea pigs were sensitized

to PPD (purified protein derivatives) as described previously⁴⁾. Lymphocytes obtained from the lymph nodes of these sensitized animals were suspended in Eagle MEM containing penicillin (100 u/ml) and streptomycin (100 µg/ml) at a cell concentration of 1×10^7 cells/ml. The cells were cultured with PPD (10 µg/ml) in a CO₂ incubator at 37°C for 48 hrs. The culture supernatants were then harvested for chromatographic fractionations.

2. Partial purification of the cholestatic factor

The culture supernatants of the activated lymphocytes were fractionated by gel filtration on a Sephadex G-75 column as reported before¹⁴⁾. Since cholestatic activity was predominantly recovered in one fraction (Fraction 4), this was further fractionated by DEAE-cellulose column chromatography and two cholestatic activity-containing fractions (Fraction 1 and 5) were obtained as described previously¹⁴⁾.

3. ¹²⁵I-labelling of dimeric IgA

Dimeric IgA was purchased from Cappel Co. and labelled with ¹²⁵I according to the method of Bolton and Hunter¹⁵⁾: Dimeric IgA was dissolved in 0.1 M borate buffer (pH 8.5) at a concentration of 2.5 mg/ml. Ten microliters of the IgA solution was added to ¹²⁵I-labelled hydroxyphenyl propionic acid (500 µCi), and the reaction mixture was agitated at 0°C for 30 min. After adding 0.5 ml of 0.2 M glycine and incubating at 0°C for another 5 min, 0.05 M phosphate buffer (pH 7.5) containing 0.25% gelatin was added to make a total volume of 1.0 ml. This solution was then applied to a Sephadex G-50 column (0.9×12 cm) equilibrated with 0.5 M phosphate buf-

Fig. 1 Gel filtration of ^{125}I -dimeric IgA on a Sephadex G-50 column (0.9×12 cm)

^{125}I -dimeric IgA was eluted in the first peak

fer (pH 7.5) containing 0.25% gelatin followed by elution with the same solution. Half a milliliter each of the eluate was collected to monitor optical density at 280 nm and to determine radioactivity using a γ counter (AL-201 type, Shimadzu Co.). Since ^{125}I -dimeric IgA was eluted in the first peak containing radioactivity as shown in Fig. 1, this fraction was used for the experiments^{16,17}.

4. Measurement of ^{125}I -dimeric IgA excretion into the bile

Twelve male Wistar rats weighing 250 to 350 g were fasted and anesthetized with pentobarbital (30 mg/kg body weight). After laparotomy, the common bile duct was cannulated with polyethylene tubing (PE-10, Clay Adams Co.). In order to determine the initial bile flow, the bile was collected for 30 min preceding the experiments. One milliliter of either Fraction 1 or Fraction 5, as obtained in Method 2, was then injected into the mesenteric vein, and 30 min later, 300 μl (15 μg protein) of ^{125}I -dimeric IgA (50 $\mu\text{Ci}/\text{mg}$) was injected intravenously. The bile was collected at 15 min intervals (0~15, 15~30, 30~45 and 45~60 min) and then at 30 and 60 min intervals (60~90 and 90~150 min). Bile volume and radioactivity of each fraction were then determined.

5. Estimation of ^{125}I -dimeric IgA clearance

Three hundred microliters (15 μg protein) of ^{125}I -dimeric IgA was intravenously injected into 6 rats 90 min after injection of either Fraction 1 or 5. Sixty minutes later, the bile was collected at 10 min intervals for 30 min, and the amount of bile and the radioactivity were measured. At about the middle of each bile collection, 200 μl of blood was drained and the radioactivity in the serum was counted. ^{125}I -dimeric IgA clearance was then calculated by the following equation:

$$\begin{aligned} &^{125}\text{I}\text{-dimeric IgA clearance (ml/min/100 g body weight)} \\ &= \text{amount of bile flow (ml/min/100 g body weight)} \\ &\quad \times \frac{\text{radioactivity in bile (cpm/50 } \mu\text{l)}}{\text{radioactivity in plasma (cpm/50 } \mu\text{l)}} \end{aligned}$$

III. Results

1. Effect of the cholestatic factor on ^{125}I -dimeric IgA excretion into the bile

In the control rats injected with the culture supernatant of non-stimulated lymphocytes prepared from tuberculin-sensitized guinea pigs, about a half of the administered ^{125}I -dimeric IgA was excreted into the bile within 150 min. In contrast, when the partially-purified cholestatic factor (either Fraction 1 or 5)

Table 1 Changes in ^{125}I -dimeric IgA secretion into the bile after infusion of the culture supernatant of non-stimulated lymphocytes prepared from tuberculin-sensitized guinea pigs (control)

intervals	Exp. 1			Exp. 2		
	radioactivity	bile volume	% of recovery in the bile	radioactivity	bile volume	% of recovery in the bile
15 min	0.80×10^5 cpm	150 μl /15 min	0.80	0.56×10^5 cpm	140 μl /15 min	0.51
30 min	2.82×10^5 cpm	310 μl /30 min	2.71	3.22×10^5 cpm	320 μl /30 min	3.11
45 min	10.84×10^5 cpm	440 μl /45 min	10.42	8.94×10^5 cpm	460 μl /45 min	8.62
60 min	17.86×10^5 cpm	580 μl /60 min	17.22	16.84×10^5 cpm	620 μl /60 min	16.23
90 min	34.06×10^5 cpm	910 μl /90 min	32.81	32.86×10^5 cpm	930 μl /90 min	31.63
150 min	44.42×10^5 cpm	1480 μl /150 min	42.72	47.84×10^5 cpm	1450 μl /150 min	46.11

intervals	Exp. 3			Exp. 4		
	radioactivity	bile volume	% of recovery in the bile	radioactivity	bile volume	% of recovery in the bile
15 min	1.06×10^5 cpm	140 μl /15 min	1.02	0.92×10^5 cpm	150 μl /15 min	0.91
30 min	4.78×10^5 cpm	350 μl /30 min	4.62	4.37×10^5 cpm	340 μl /30 min	4.21
45 min	14.98×10^5 cpm	450 μl /45 min	14.43	15.50×10^5 cpm	470 μl /45 min	14.91
60 min	26.00×10^5 cpm	600 μl /60 min	25.02	23.50×10^5 cpm	630 μl /60 min	22.62
90 min	40.98×10^5 cpm	920 μl /90 min	39.42	40.56×10^5 cpm	900 μl /90 min	39.00
150 min	45.96×10^5 cpm	1510 μl /150 min	53.82	56.99×10^5 cpm	1400 μl /150 min	54.81

mean $\pm$ SD

Table 2 Changes in ^{125}I -dimeric IgA secretion into the bile after infusion of the cholestatic factor (Fraction 1)

intervals	Exp. 1			Exp. 2		
	radioactivity	bile volume	% of recovery in the bile	radioactivity	bile volume	% of recovery in the bile
15 min	0.68×10^5 cpm	150 $\mu\text{l}/15$ min	0.62	0.47×10^5 cpm	140 $\mu\text{l}/15$ min	0.42
30 min	1.10×10^5 cpm	280 $\mu\text{l}/30$ min	1.00	0.99×10^5 cpm	260 $\mu\text{l}/30$ min	0.91
45 min	3.52×10^5 cpm	400 $\mu\text{l}/45$ min	3.22	5.83×10^5 cpm	420 $\mu\text{l}/45$ min	5.31
60 min	11.66×10^5 cpm	550 $\mu\text{l}/60$ min	10.61	11.33×10^5 cpm	520 $\mu\text{l}/60$ min	10.32
90 min	20.13×10^5 cpm	600 $\mu\text{l}/90$ min	18.28	18.59×10^5 cpm	650 $\mu\text{l}/90$ min	16.89
150 min	28.93×10^5 cpm	750 $\mu\text{l}/150$ min	26.32	29.81×10^5 cpm	800 $\mu\text{l}/150$ min	27.10
intervals	Exp. 3			Exp. 4		
	radioactivity	bile volume	% of recovery in the bile	radioactivity	bile volume	% of recovery in the bile
15 min	0.57×10^5 cpm	152 $\mu\text{l}/15$ min	0.51	0.83×10^5 cpm	145 $\mu\text{l}/15$ min	0.79
30 min	2.09×10^5 cpm	270 $\mu\text{l}/30$ min	1.90	2.75×10^5 cpm	280 $\mu\text{l}/30$ min	2.51
45 min	9.79×10^5 cpm	380 $\mu\text{l}/45$ min	8.91	10.45×10^5 cpm	380 $\mu\text{l}/45$ min	9.52
60 min	11.88×10^5 cpm	480 $\mu\text{l}/60$ min	10.79	15.40×10^5 cpm	520 $\mu\text{l}/60$ min	14.00
90 min	24.31×10^5 cpm	580 $\mu\text{l}/90$ min	22.11	23.98×10^5 cpm	600 $\mu\text{l}/90$ min	21.82
150 min	41.91×10^5 cpm	720 $\mu\text{l}/150$ min	38.11	33.55×10^5 cpm	780 $\mu\text{l}/150$ min	30.50
						mean $\pm$ SD
						0.59 ± 0.14
						$1.58 \pm 0.66^{**}$
						$6.74 \pm 2.59^{***}$
						$11.43 \pm 1.49^{***}$
						$19.78 \pm 2.25^{***}$
						$30.51 \pm 4.66^{***}$

 *—** : Significantly different ($p < 0.01$) from control

 *—*** : Significantly different ($p < 0.001$) from control

Table 3 Changes in ^{125}I -dimeric IgA secretion into the bile after infusion of the cholestatic factor (Fraction 5)

intervals	Exp. 1			Exp. 2		
	radioactivity	bile volume	% of recovery in the bile	radioactivity	bile volume	% of recovery in the bile
15 min	0.51×10^5 cpm	148 $\mu\text{l}/15$ min	0.54	0.54×10^5 cpm	150 $\mu\text{l}/15$ min	0.51
30 min	1.01×10^5 cpm	280 $\mu\text{l}/30$ min	1.06	1.42×10^5 cpm	280 $\mu\text{l}/30$ min	1.39
45 min	4.82×10^5 cpm	400 $\mu\text{l}/45$ min	4.80	3.91×10^5 cpm	400 $\mu\text{l}/45$ min	3.90
60 min	7.10×10^5 cpm	500 $\mu\text{l}/60$ min	7.08	6.19×10^5 cpm	540 $\mu\text{l}/60$ min	6.22
90 min	16.30×10^5 cpm	620 $\mu\text{l}/90$ min	16.30	14.61×10^5 cpm	600 $\mu\text{l}/90$ min	14.63
150 min	27.60×10^5 cpm	750 $\mu\text{l}/150$ min	27.58	28.32×10^5 cpm	780 $\mu\text{l}/150$ min	28.32
intervals	Exp. 3			Exp. 4		
	radioactivity	bile volume	% of recovery in the bile	radioactivity	bile volume	% of recovery in the bile
15 min	0.64×10^5 cpm	152 $\mu\text{l}/15$ min	0.62	0.73×10^5 cpm	145 $\mu\text{l}/15$ min	0.71
30 min	2.32×10^5 cpm	270 $\mu\text{l}/30$ min	2.31	3.24×10^5 cpm	280 $\mu\text{l}/30$ min	3.24
45 min	7.73×10^5 cpm	380 $\mu\text{l}/45$ min	7.72	9.92×10^5 cpm	410 $\mu\text{l}/45$ min	9.91
60 min	16.33×10^5 cpm	520 $\mu\text{l}/60$ min	16.32	17.92×10^5 cpm	500 $\mu\text{l}/60$ min	17.90
90 min	23.24×10^5 cpm	620 $\mu\text{l}/90$ min	23.20	28.62×10^5 cpm	580 $\mu\text{l}/90$ min	28.61
150 min	34.21×10^5 cpm	800 $\mu\text{l}/150$ min	34.20	35.33×10^5 cpm	720 $\mu\text{l}/150$ min	35.30
						mean $\pm$ SD
						0.60 $\pm$ 0.08
						2.00 $\pm$ 0.85**
						6.58 $\pm$ 2.38**
						11.88 $\pm$ 5.27**
						20.69 $\pm$ 5.59**
						31.35 $\pm$ 3.43***

*—** : Significantly different ($p < 0.01$) from control*—*** : Significantly different ($p < 0.001$) from control

Table 4 Clearance of ^{125}I -dimeric IgA in rats after infusion of cholestatic factor

	intervals	bile (cpm/50 μl)	time (min)	serum (cpm/50 μl)	bile volume (μl /10 min)	clearance (ml/min/ 100 g body weight)	mean $\pm$ SD
Control Exp. 1	0~10 min	80,182	5	1,269	100	1.58	1.59 $\pm$ 0.11*
	10~20 min	73,011	15	1,259	100	1.45	
	20~30 min	85,618	25	1,267	100	1.69	
Control Exp. 2	0~10 min	79,189	5	1,169	100	1.69	1.59 $\pm$ 0.11*
	10~20 min	61,065	15	1,068	100	1.43	
	20~30 min	84,518	25	1,267	100	1.67	
Fraction 1 Exp. 1	0~10 min	68,291	5	1,635	90	0.94	1.09 $\pm$ 0.15**
	10~20 min	50,740	15	1,269	90	0.90	
	20~30 min	74,894	25	1,359	90	1.24	
Fraction 1 Exp. 2	0~10 min	76,695	5	1,350	90	1.28	1.09 $\pm$ 0.15**
	10~20 min	60,790	15	1,370	90	1.00	
	20~30 min	73,831	25	1,431	90	1.16	
Fraction 5 Exp. 1	0~10 min	76,971	5	1,577	100	1.22	1.15 $\pm$ 0.08**
	10~20 min	59,324	15	1,236	90	1.08	
	20~30 min	63,294	25	1,249	90	1.14	
Fraction 5 Exp. 2	0~10 min	79,271	5	1,548	100	1.28	1.15 $\pm$ 0.08**
	10~20 min	69,324	15	1,436	90	1.09	
	20~30 min	64,021	25	1,349	90	1.08	

 *—** : Significantly different ($p < 0.01$) from the control

was injected 30 min before ^{125}I -dimeric IgA administration, a significant decrease in ^{125}I -dimeric IgA excretion was noted as shown in Table 1, Table 2 and Table 3. No significant difference in ^{125}I -dimeric IgA excretion was observed between the rats injected with Fraction 1 and 5, suggesting that they cause the reduction in ^{125}I -dimeric IgA excretion to the same extent.

2. Effect of the cholestatic factor on ^{125}I -dimeric IgA clearance

The ^{125}I -dimeric IgA clearance level was 1.59 ± 0.11 ml/min/100 g body weight in the control rats, while in the experimental groups injected with Fraction 1 and 5, it was 1.09 ± 0.15 and 1.15 ± 0.08 ml/min/100 g body weight, respectively ($p < 0.01$) (Table 4).

IV. Discussion

The murine liver can actively transport a considerably large amount of polymeric IgA from the plasma to the bile. Intravenously-injected polymeric IgA, though not monomeric IgA, is rapidly removed from the circulation and transported into the bile, where it appears to be bound to a secretory component^{6,18}. Therefore, it is assumed that rat hepatocytes synthesize the secretory component and transport it to their cell surface^{13,19}, and that the binding of polymeric IgA with this component results in their vesicular uptake and transport across the hepatocytes^{7,20}. Active plasma-to-bile transfer of polymeric IgA also occurs in rabbits²¹, but this cannot be generalized to all animals, especially to humans²². The present study was designed to determine the effect of the cholestatic factor on biliary secretion of dimeric IgA, because secretory IgA is one of the major protein constituent of the rat bile²³ and the important role of microtubules has been suggested in the IgA secretion by the intact liver⁸.

As shown in this study, a significant decrease in biliary secretion of dimeric IgA was observed when the partially-purified cholestatic factor was administered through the mesenteric vein of rats prior to dimeric IgA injection. An increased level of serum dimeric IgA was also detected by an injection of the cholestatic factor, indicating that the secretion of dimeric IgA was inhibited by this lymphokine.

A previous study has shown that bile-duct ligation of rats rapidly and selectively increases both the amount of polymeric IgA and secretory component in the serum²⁴. Although the concentration, source and significance of biliary IgA in man are still controversial, Nagra et al.²⁵ have found predominantly 11S secretory IgA in the bile but virtually negligible amounts of IgG and IgM. Also, an increase in the total serum IgA level has been reported in patients with liver cirrhosis²⁶. However, there are no data on biliary obstruction and nothing suggests a relationship between the high serum level of polymeric IgA in cirrhosis and the impaired plasma-to-bile transfer in the diseased liver, although a high level of the serum secretory component, putatively bound only to polymeric IgA, has been found in patients with liver diseases²⁷. Although serum IgA increases in patients with liver cirrhosis, its pathogenesis is different from the increase in IgA induced by the cholestatic factor. Therefore, the relation of this study on rats to the human secretory IgA system should be considered carefully.

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