Effects of Antibiotics on Fibrinolytic Activity

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Abstract—Latamoxef, cefamandole, carbenicillin and cefotaxime were examined for their effects on fibrinolytic activity in vitro by means of the fibrin plate method, fibrin clot lysis time and euglobulin lysis time with human, rabbit and rat plasma. These antibiotics showed no fibrinolytic but weak antifibrinolytic activity at 1000 or 3000 $\mu\text{g/ml}$ in some assay systems.

Antibiotics have been reported to cause bleeding in some severely ill patients (1, 2), and although the mechanism is not yet known, a disorder of the vitamin K-dependent coagulation system (3, 4) and platelet dysfunction (5, 6) are suspected causes. Enhancement of fibrinolysis also causes bleeding (7), but the effect of antibiotics on fibrinolytic activity is not yet known. In this study, we examined the effects of some antibiotics on fibrinolytic activity in vitro by means of the fibrin plate method, fibrin clot lysis time and euglobulin lysis time. Latamoxef (LMOX) and cefotaxime (CTX) from the third generation, cefamandole (CMD) from the second, and carbenicillin (CBPC) from penicillin-type antibiotics were chosen, and the results with these compounds were compared with those for some reference compounds.

6-Amino-n-caproic acid (EACA) and trans-(aminomethyl)cyclohexanecarboxylic acid (AMCHA) were purchased from Nakarai Chemicals (Japan), sodium dextran sulfate from Pharmacia (Sweden), urokinase (UK) from Green Cross Co. (Japan), and fibrinogen from Seikagaku Kogyo (Japan). All the test compounds and euglobulin fractions were dissolved in 0.12 M acetate buffer (pH 7.4) unless otherwise mentioned.

Fibrin plates were prepared by the method of Norén et al. (8) and the effects of antibiotics and EACA on fibrinolytic activity of UK were examined using plasminogen-containing fibrin plates. The test compounds

were dissolved in 0.1 ml of 0.01 M phosphate buffer (pH 7.8) in various concentrations and mixed with 0.1 ml of UK saline solution (75 U/ml). An aliquot (5 μl) of the mixture was put into wells (3 mm in diameter) on the fibrin plates. The lysis area was measured after incubation at 37°C for 20 hr.

EACA inhibited the UK activity by 20% (lysis area) at the final concentration of 2000 $\mu\text{g/ml}$ (1.5×10^{-2} M), but LMOX, CMD, CBPC and CTX showed no inhibition at the concentrations of 0.64–2000 $\mu\text{g/ml}$.

Fibrin clot lysis time was measured with an aliquot (350 μl) of human fibrinogen solution, which was enriched with 2.5 μg of bovine plasminogen, transferred to a polystyrene tube, mixed in sequence with 50 μl of UK solution (20 U/ml), 50 μl of the test compound solution and 50 μl of thrombin solution (20 U/ml), and incubated at 37°C in a water bath. The time for complete lysis of the formed fibrin clot was measured (Table 1). LMOX and CBPC showed no effect on the fibrin lysis time, but it was increased significantly by DMD at 1000 $\mu\text{g/ml}$ and CTX at 3000 $\mu\text{g/ml}$. EACA prolonged the fibrin clot lysis time at 100 $\mu\text{g/ml}$ and AMCHA prolonged it at 30 $\mu\text{g/ml}$, confirming the report of Westlund et al. (9).

The effects of antibiotics on euglobulin lysis time were examined by preparing euglobulin fractions from human, rabbit and rat plasma according to the method of Kluft et al. (10). An aliquot (400 μl) of the euglobulin solution was mixed with 50 μl of

the test compound solution and 50 μ l of thrombin solution (25 U/ml) in a polystyrene test tube and incubated at 37°C for up to 600 min. Euglobulin lysis time was taken as the interval between addition of thrombin and complete lysis of the formed clot. As shown in Table 2, LMOX, CMD and CTX prolonged the euglobulin lysis time at 1000 μ g/ml, and CBPC prolonged it at 3000 μ g/ml. EACA

also increased the lysis time at 10 μ g/ml, but UK decreased the time at 0.3 U/ml. When rabbit euglobulin was used, the responses of EACA and UK were weaker than those with human euglobulin, and rat euglobulin gave weaker results than rabbit euglobulin. This species difference in the response agrees with the antifibrinolytic activities disclosed upon addition of UK to rat, rabbit and human whole

Table 1. Effects of some substances on fibrinolytic activity based on fibrin clot lysis time (min)

	No. of experiments	Concentration				
		0	100	300	1000	3000
Latamoxef ($\times 1$ μ g/ml)	3	87 $\pm$ 7 ^a	88 $\pm$ 7	90 $\pm$ 7	98 $\pm$ 5	121 $\pm$ 10
Cefamandol ($\times 1$ μ g/ml)	4	88 $\pm$ 5	91 $\pm$ 5	100 $\pm$ 6	117 $\pm$ 6*	211 $\pm$ 20*
Carbenicillin ($\times 1$ μ g/ml)	3	89 $\pm$ 7	90 $\pm$ 7	92 $\pm$ 7	94 $\pm$ 7	99 $\pm$ 7
Cefotaxime ($\times 1$ μ g/ml)	3	86 $\pm$ 6	87 $\pm$ 6	91 $\pm$ 7	106 $\pm$ 7	171 $\pm$ 19*
EACA ($\times 10^{-1}$ μ g/ml)	4	98 $\pm$ 5	89 $\pm$ 4	94 $\pm$ 5	115 $\pm$ 7*	194 $\pm$ 7*
AMCHA ($\times 10^{-2}$ μ g/ml)	4	88 $\pm$ 5	91 $\pm$ 4	93 $\pm$ 5	105 $\pm$ 6	143 $\pm$ 8*
Streptokinase ($\times 10^{-3}$ U/ml)	4	91 $\pm$ 5		53 $\pm$ 4	32 $\pm$ 1*	22 $\pm$ 0*
Dextran sulfate ($\times 10^{-4}$ μ g/ml)	4	90 $\pm$ 5		74 $\pm$ 7	61 $\pm$ 6*	51 $\pm$ 4*

EACA, 6-amino-n-caproic acid. AMCHA, trans-4-(aminomethyl)cyclohexanecarboxylic acid. ^aMean $\pm$ S.E. *Statistically significant difference compared with 0 concentration ($P < 0.05$).

Table 2. Effects of latamoxef, cefamandole, carbenicillin and cefotaxime on euglobulin lysis time in human, rabbit and rat plasma

	No. of experiments	0	100	300	1000	3000
Human						
Latamoxef ($\times 1 \mu\text{g/ml}$)	4	359 $\pm$ 12	341 $\pm$ 10	386 $\pm$ 4	490 $\pm$ 12*	> 600
Cefamandol ($\times 1 \mu\text{g/ml}$)	4	355 $\pm$ 8	358 $\pm$ 11	376 $\pm$ 15	441 $\pm$ 14*	> 600
Carbenicillin ($\times 1 \mu\text{g/ml}$)	4	351 $\pm$ 14	338 $\pm$ 6	370 $\pm$ 23	379 $\pm$ 15	423 $\pm$ 23*
Cefotaxime ($\times 1 \mu\text{g/ml}$)	4	340 $\pm$ 12	335 $\pm$ 3	350 $\pm$ 15	416 $\pm$ 21*	> 600
EACA ($\times 10^{-2} \mu\text{g/ml}$)	4	361 $\pm$ 10	383 $\pm$ 2	417 $\pm$ 17	487 $\pm$ 29*	
Urokinase ($\times 10^{-4} \text{ U/ml}$)	3	336 $\pm$ 8		308 $\pm$ 9	272 $\pm$ 17	185 $\pm$ 27*
Rabbit						
Latamoxef ($\times 1 \mu\text{g/ml}$)	3	28 $\pm$ 2	29 $\pm$ 2	29 $\pm$ 1	31 $\pm$ 1	30 $\pm$ 3
Cefamandol ($\times 1 \mu\text{g/ml}$)	3	28 $\pm$ 1	28 $\pm$ 1	29 $\pm$ 1	30 $\pm$ 1	33 $\pm$ 1*
Carbenicillin ($\times 1 \mu\text{g/ml}$)	3	28 $\pm$ 1	29 $\pm$ 2	28 $\pm$ 1	30 $\pm$ 2	28 $\pm$ 1
Cefotaxime ($\times 1 \mu\text{g/ml}$)	3	29 $\pm$ 1	29 $\pm$ 1	30 $\pm$ 1	31 $\pm$ 1	34 $\pm$ 1*
EACA ($\times 10^{-1} \mu\text{g/ml}$)	3	28 $\pm$ 2	30 $\pm$ 2	31 $\pm$ 3	42 $\pm$ 2*	
Urokinase ($\times 10^{-3} \text{ U/ml}$)	3	27 $\pm$ 1		26 $\pm$ 1	23 $\pm$ 1	20 $\pm$ 0*
Rat						
Latamoxef ($\times 1 \mu\text{g/ml}$)	4	100 $\pm$ 4	100 $\pm$ 3	99 $\pm$ 2	102 $\pm$ 4	99 $\pm$ 2
Cefamandol ($\times 1 \mu\text{g/ml}$)	3	102 $\pm$ 5	104 $\pm$ 8	105 $\pm$ 9	111 $\pm$ 7	137 $\pm$ 17
Carbenicillin ($\times 1 \mu\text{g/ml}$)	4	99 $\pm$ 4	97 $\pm$ 4	98 $\pm$ 4	92 $\pm$ 4	88 $\pm$ 10
Cefotaxime ($\times 1 \mu\text{g/ml}$)	4	97 $\pm$ 4	103 $\pm$ 5	103 $\pm$ 4	110 $\pm$ 8	139 $\pm$ 7*
EACA ($\times 10^{-1} \mu\text{g/ml}$)	3	99 $\pm$ 3		102 $\pm$ 4	109 $\pm$ 4	236 $\pm$ 9*
Urokinase ($\times 10^{-2} \text{ U/ml}$)	3	95 $\pm$ 3	89 $\pm$ 4	77 $\pm$ 7	54 $\pm$ 6*	

EACA, 6-amino-n-caproic acid. AMCHA, trans-4-(aminomethyl)cyclohexanecarboxylic acid.

^aMean $\pm$ S.E. *Statistically significant difference compared with 0 concentration ($P < 0.05$).

blood samples (11).

The effects of the antibiotics on euglobulin-induced fibrinolysis were examined by the fibrin plate method. An aliquot (180 μ l) of the human euglobulin solution in 2.7 mM EDTA buffer (pH 7.8) was mixed with 20 μ l of the test compound solutions. A 20 μ l aliquot of the mixture or the preincubated mixture at 37°C for 30 min was put into wells (5 mm in diameter) of the fibrin plates and incubated at 37°C for 20 hr, after which the lysis area was measured. The effective doses of EACA and UK were nearly the same as those obtained with the euglobulin lysis time experiments (Table 2), and LMOX, CMD and CTX reduced the fibrinolytic activity of the human euglobulin fraction at 3000 μ g/ml. The antifibrinolytic activity of antibiotics found with the human euglobulin test was more pronounced than those with the other system, and the activity was about one tenth that of EACA when compared by the minimum effective molar concentration.

These results suggest that the four antibiotics studied show no fibrinolytic activity but show antifibrinolytic activity only at high concentrations (more than 1000 μ g/ml) which are not achievable in vivo.

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