Effects of Antibiotics on Fibrinolytic Activity In Vivo

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Abstract—The effects of latamoxef, cefamandole, carbenicillin and cefotaxime on fibrinolytic system in rats were examined under feeding of an ordinary diet or a vitamin K-deficient diet for eight days. No obvious change was observed in UK-induced plasma clot lysis time, euglobulin lysis time and antiplasmin level in plasma. These findings of the in vitro and ex vivo studies suggest that these antibiotics cause no enhancement of fibrinolytic activity.

Certain β-lactam antibiotics have been reported to cause prolongation of bleeding time in patients with vitamin K deficiency (1) and sometimes bleeding in severe cases (2). In a previous study (3), we examined the effects of latamoxef (LMOX), cefamandole (CMD), carbenicillin (CBPC) and cefotaxime (CTX) on fibrinolytic activity in vitro and reported that these antibiotics showed no fibrinolytic but weak antifibrinolytic activity. In this study, we examined the effects of these antibiotics on fibrinolytic activity in vivo using rats fed an ordinary or a vitamin K-deficient diet.

Urokinase (UK) was purchased from Green Cross Co. (Japan), thrombin from Sigma Chem. Co. (U.S.A.), and 6-amino-n-caproic acid (EACA) from Nakarai Chemicals (Japan). Male Sprague-Dawley rats (9–10 weeks of age) were housed in an air-conditioned room (25±1°C, 50–60% humidity) lighted 12 hr a day (8.00 a.m. to 8.00 p.m.). A rat chow diet (JCL-CA-1) was obtained from Japan Clea Co., Ltd. (Japan). A vitamin K-deficient diet was prepared in our laboratory from vitamin K-free casein (18.0%), sucrose 67.6%, a mixture of rapeseed and soybean oils (8.0%), a salt mixture (Hegsted salt, 4.0%), fibers (1.5%), and adequate amounts of vitamins except for vitamin K. Antibiotics were dissolved in distilled water (1 g titre/4 ml) and injected intravenously once a day for 8 days.

Blood was taken from the abdominal aorta with an injection syringe containing 1/10 volume of 3.8% sodium citrate solution under sodium pentobarbital anesthesia (60 mg/kg, i.p.), 24 hr after the last administration of the antibiotic. Plasma was obtained by centrifugation (1800xg) for 10 min. Part of the fresh plasma was used for the UK-induced plasma clot lysis time (UK-PCLT) assay and the remainder, which was stored at −20°C until use, for measurement of both the englobulin lysis time (ELT) and the antiplasmin level.

UK-PCLT was measured with a modification of the method reported by Gidron et al. (4). Plasma, 100 μl, was mixed 100 μl of 0.12 M acetate buffer (pH 7.4), 200 μl of UK solution (250–500 units/ml) and 100 μl of thrombin (15 units/ml), and incubated at 37°C in a water bath. The time for lysis of the formed clot was measured up to 300 min. When the effect of the antibiotics on UK-PCLT was examined in vitro, 50 μl of the test sample solutions and 50 μl of thrombin (25 units/ml) were added, and the lysis time was compared with the control which was mixed with the buffer instead of the test sample solutions.

Euglobulin lysis time (ELT) was measured by the method of Gallimore (5) after preparing the euglobulin precipitate by method of Kluft (6). A 1-ml portion of plasma was diluted to 10 ml with ice-cold water, and the mixture was adjusted to pH 5.9. The euglobulin fraction was obtained by centrifugation after cooling in an ice bath for 60 min and dissolved in 0.12 M acetate buffer. Next, 400 μl of the
Euglobulin solution was mixed with 100 μl of thrombin (15 units/ml) in a polystyrene tube, and the clot lysis time was measured at 37°C.

The antiplasmin level was measured by the method of Friberger (7). Plasmin solution (0.5 unit/ml) of 50 μl was added to 450 μl of the plasma diluted 100-fold with 0.05 M tris buffer (pH 7.4). This mixture was incubated at 37°C for 20 sec, and 2.0 ml of the prewarmed chromogenic substrate S-2251 solution (0.45 mM) in tris buffer was added. The change of the absorption (ΔA/min) at 405 nm was read 1 to 2 min later. The antiplasmin level in plasma (unit/ml) was calculated as follows:

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\frac{\Delta A_{\text{buffer}} - \Delta A_{\text{sample}}}{\Delta A_{\text{unit of plasmin}}} \times 100 \times \frac{1000}{450}
\]

Prothrombin time (PT) was measured with a photooptical clot sensing system, COAG-A Mate-X2 (Warner-Lambert Cr. Morris Plains, N.J.).

The plasma fibrinolytic activity was examined by UK-PCLT and ELT at 0.5, 1 and 3 hr after a single administration of EACA (100 mg/kg, i.v.). ELT did not change during 0.5–3 hr, but UK-PCLT was markedly prolonged at 0.5 hr and later shortened to the level of non-treated rats at 3 hr. Thus, the antifibrinolytic activity of EACA was confirmed in rats by UK-PCLT. Next, the effect of LMOX (300 mg/kg) on plasma fibrinolytic activity was examined in the same way. LMOX caused no obvious change in either UK-PCLT or ELT. A slight but statistically significant prolongation of ELT was observed at 24 hr due to a small variation, but the change was not reproducible.

The four antibiotics described above were administered for 8 days, and the effects on the fibrinolytic system at 24 hr after the last administration are shown in Table 2. These antibiotics showed no apparent change in UK-PCLT, ELT or antiplasmin level. Since vitamin K deficiency causes a decrease in the factors related to blood coagulation, such as factors II, VII, IX, X and also those related to fibrinolysis, such as protein C and S, we examined the effect of antibiotics on plasma fibrinolytic activity in rats fed the vitamin K-deficient diet for 8 days. The prothrombin time was apparently prolonged as reported previously (8), but UK-PCLT was not changed by administration of these antibiotics. Also, the antiplasmin activity in plasma and the fibrinolytic activity of the euglobulin fraction, from which most of the major antiplasmins had been removed, were examined by the plasmin inactivation rate and ELT. No significant differences in fibrinolytic activity were detected in these experiments. These results suggest that prolongation of PT due to vitamin K deficiency, presumably induction of bleeding, was caused by reduction in coag-

| Time after administration | No. of experiments | UK250 U/ml | UK500 U/ml | ELT (min) |
|---------------------------|--------------------|------------|-------------|-----------|
| EACA (100 mg/kg, i.v.)    |                    |            |             |           |
| 0 hr (not treated)        | 4                  | 37±2       | 16±0        | 123±6     |
| 0.5                       | 4                  | 281±19*    | 247±53*     | 129±14    |
| 1                         | 5                  | 296±4*     | 48±3*       | 118±4     |
| 3                         | 5                  | 54±2*      | 20±1*       | 128±8     |
| Latamoxef (300 mg/kg, i.v.) |                  |            |             |           |
| 0 hr                      | 5                  | 29±1       | 13±0        | 115±4     |
| 0.5                       | 5                  | 40±7       | 13±1        | 139±21    |
| 1                         | 5                  | 32±2       | 12±0        | 142±13    |
| 3                         | 4                  | 37±7       | 13±1        | 124±16    |
| 24                        | 5                  | 28±1       | 13±0        | 148±7*    |

Plasma clot lysis was observed up to 300 min. If a clot did not lyse by 300 min, the clot lysis time was treated as 300 min. Values represent the mean±S.E. *Statistically significant compared with the 0 time (P<0.05). UK-PCLT: Urokinase-induced plasma clot lysis time.
Table 2. Effects of latamoxef, cefamandole, carbenicillin and cefotaxime on fibrinolytic activity in rats fed ordinary diet or vitamin K-deficient diet

|                   | Control          | Latamoxef | Cefamandole | Carbenicillin | Cefotaxime |
|-------------------|------------------|-----------|-------------|---------------|------------|
| Ordinary diet     |                  |           |             |               |            |
| No. of rats       | 5                | 5         | 5           | 5             | 5          |
| Body weight (g)   | initial          | 348±6     | 348±6       | 356±4         | 356±4      | 350±4      |
|                   | final            | 388±4     | 379±7       | 394±3         | 389±5      | 376±9      |
| UK-PCLT (UK250 U/ml) (min) | 43±4           | 44±5      | 46±3        | 44±3          | 47±2       |
| ELT (min)         | 112±9            | 118±8     | 132±6       | 113±8         | 120±7      |
| Antiplasmin (units/ml)* | 27.2±1.4     | 27.1±2.1  | 29.0±1.3    | 27.3±1.9      | 29.1±1.5   |
| PT (sec)          | 11.7±0.1         | 11.7±0.2  | 11.7±0.2    | 12.0±0.4      | 11.8±0.1   |

| Vitamin K-deficient diet |                  |           |             |               |            |
|--------------------------|------------------|-----------|-------------|---------------|------------|
| No. of rats              | 4                | 4         | 4           | 4             | 5          |
| Body weight (g)          | initial          | 326±7     | 326±3       | 326±6         | 326±4      | 327±4      |
|                          | final            | 349±8     | 333±5       | 326±8         | 335±6      | 333±6      |
| UK-PCLT (UK250 U/ml) (min) | 42±20           | 47±4      | 46±2        | 74±27         | 62±10      |
| ELT (min)                | 139±20           | 156±26    | 138±8       | 184±12        | 148±7      |
| Antiplasmin (units/ml)   | 31.7±2.5         | 30.9±3.0  | 34.0±1.2    | 36.3±4.9      | 39.5±2.7   |
| PT (sec)                 | 15.6±1.2         | 26.8±1.0* | 25.5±2.4*   | 20.8±2.7      | 20.6±1.5*  |

Antibiotics (300 mg/kg, i.v.) were administered once a day for 8 days. Blood was drawn 24 hr after the final administration. Values represent the mean±S.E. *This value corresponds to units of plasmin consumed by one milliliter of the plasma. *Statistically significant difference compared with the control (P<0.05). UK-PCLT: Urokinase-induced plasma clot lysis time, ELT: Euglobulin lysis time, PT: Prothrombin time.

ulation factors II and VII and not related to antiplasmin and plasminogen activator levels in plasma.

When the effects of these antibiotics on fibrinolytic activity were additionally examined by an in vitro assay (UK-PCLT), these antibiotics showed antifibrinolytic activity in the range of 1000–3000 µg/ml, which was nearly the same as those estimated by fibrin clot lysis time and rat ELT (3). These in vitro data suggest that these antibiotics examined had a weak inhibitory effect on plasmin or urokinase activity.

From these findings, we conclude that LMOX and the other antibiotics cause no enhancement of fibrinolytic activity in vivo.

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References
1 Weitekamp, R.M. and Aber, R.C.: Prolonged bleeding times and bleeding diathesis associated with moxalactam administration. JAMA 249, 69–71 (1983)
2 Hooper, C.A., Haney, B.B. and Stone, H.H.: Gastrointestinal bleeding due to vitamin K deficiency in patients on parenteral cefamandole. Lancet i, 39–40 (1980)
3 Okuno, I., Fujiharu, Y. and Uchida, K.: Effects of antibiotics on fibrinolytic activity. Japan. J. Pharmacol. 42, 150–152 (1986)
4 Gidron, E., Margalit, R. and Shalitin, Y.: A rapid screening test for reduced fibrinolytic activity of plasma: streptokinase activated lysis time. J. Clin. Pathol. 31, 54–57 (1978)
5 Gallimore, M.J., Tyler, H.H. and Shaw, J.T.B.: The measurement of fibrinolysis in the rat. Thromb. Diath. Haemorrh. (Stuttg.) 26, 296–310 (1971)
6 Kluit, C., Brakman, P. and Veldhuizen-Stolk, E.C.: Screening of fibrinolytic activity in plasma euglobulin fractions on the fibrin plate. In Progress in Chemical Fibrinolysis and Thrombosis, Edited by Davidson, J.F., Samana, M.M. and Desnoyers, P.C., Vol. 2, p. 57–65, Raven Press, New York (1976)
7 Friberger, P., Knös, M., Gustavsson, S., Aurell, L. and Cleason, G.: Methods for determination of plasmin, antiplasmin and plasminogen by means of substrate S-2251. Haemostasis 7, 138–145 (1978)
8 Uchida, K., Nomura, Y., Takase, H., Haruachi, T., Yoshizaki, T. and Nakao, H.: Effects of vitamin K-deficient diets and fasting on blood coagulation factors in conventional and germ-free rats. Japan. J. Pharmacol. 40, 115–122 (1986)