Studies on Oxacephem Antibiotics: Comparison of the Effects of 1-Oxa and 1-Thia Cephalosporins on Blood Coagulation Activities and Vitamin K Metabolism in Rats

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Abstract—Oxacephem antibiotics have been developed to increase the antibacterial activity of cephem antibiotics, but the effect of 1-oxygen replacement of cephem antibiotics on blood coagulation activities is not yet known. Therefore, latamoxef (LMOX), flomoxef (FMOX) and their 1-S congeners were examined for their effects on prothrombin time, activated partial thromboplastin time, plasma prothrombin and Factor VII levels, plasma and liver descarboxyprothrombin (PIVKA-II) levels, and liver microsomal vitamin K epoxide reductase activities in rats kept on a vitamin K-deficient diet. Under the vitamin-deficient states, LMOX, FMOX and their 1-S congeners inhibited the vitamin K epoxide reductase, although the effect of FMOX or its congener was much less than that of LMOX, and they decreased the blood clotting activities in rats fed a vitamin K-deficient diet. However, no difference was found in these effects between LMOX and its 1-S congener or between FMOX and its 1-S congener. This result suggests that the 1-oxygen replacement of cephem antibiotics is not responsible for the hypoprothrombinemic effect of the antibiotics.

1-Oxa cephalosporins (oxacephems) have been developed to increase the antibacterial activity of cephem antibiotics and some good results have been achieved demonstrated by the effects of latamoxef (1, 2) and flomoxef (3). The antibacterial activity of the oxacephem latamoxef was 4–16 times higher than that of the corresponding 1-S replaced latamoxef, and the activity of flomoxef was 2–8 times that of 1-S flomoxef.

However, hypoprothrombinemia and sometimes bleeding diathesis are noted during the treatment with some antibiotics (4); and their 3’-side chains, especially N-methyltetrazolethiol (NMTT), thiadiazolethiol (TDT) and methylthiadiazolethiol (MTDT), have been shown to be responsible for the hypoprothrombinemia (5, 6). These side chains affect neither γ-glutamylcarboxylase (7) nor vitamin K reductase (8), but inhibit vitamin K epoxide reductase (9, 10), resulting in an interruption of the reuse of vitamin K through the vitamin K cycle. Therefore, vitamin K deficiency is enhanced by the treatment with the cephalosporins containing these side chains, and synthesis of vitamin K dependent clotting factors are impaired, especially in malnutritional and debilitated patients.

Other than the observation indicating the adverse effect of the side chains, a number of workers feel that the effect of 1-oxygen replacement on blood coagulation is not yet known. Although we have recently shown that 1-oxygen replacement is not responsible for the inhibitory effect of latemoxef on platelet aggregation (11, 12), no comparison between 1-oxa and 1-thia compounds has been made with regards to blood clotting factor levels and clotting activities. In the present experiments, we examined this point with the use of latamoxef, flomoxef and their 1-S replaced derivatives.

Materials and Methods
Sprague-Dawley male rats, 8 weeks old,
obtained from Japan Clea Co. (Tokyo) were kept in an air-conditioned room (25±1°C, 50–60% humidity) lighted 12 hr a day (8.00 am to 8.00 pm). Commercially available diet (JCL-CA-1, Japan Clea Co., Ltd., Tokyo) was used as an ordinary diet which contained about 500 ng/g of vitamin K₁. A vitamin K-deficient diet containing 20–30 ng/g of vitamin K₁ was prepared with vitamin K-free casein (18.0%), sucrose (67.6%), a mixture of rapeseed and soybean oils (8.0%), salt mixture (Hegsted salt) (4.0%), fibers (1.5%) and adequate amounts of vitamins except vitamin K (13).

Chemical structures of the compounds used in the present experiments are shown in Fig. 1. They were synthesized by Narisada and his coworkers of our laboratories. These compounds were dissolved in saline and injected intravenously once a day at the dose of 30, 100 or 300 mg/kg for 3 or 7 days. At 3 or 24 hr after the last injection, the animals were placed under sodium methylhexabital anesthesia (125 mg/kg, i.p.), and blood was withdrawn from the abdominal aorta with a disposable syringe containing 1/10 volume of 3.8% sodium citrate. Then the liver was removed to obtain the microsome preparation for determination of vitamin K epoxide reductase activity.

Prothrombin time (PT), activated partial thromboplastin time (APTT), plasma concentrations of prothrombin, Factor VII and fibrinogen, and plasma and liver descarboxyprothrombin (PIVKA, proteins induced by vitamin K absence) levels were measured with a photo-optical clot sensing system, COAG-A-MATE-X2 (Warner Lambert Co., Morris Plains, N.J.). Plasma concentrations of prothrombin and both plasma and liver descarboxyprothrombin (PIVKA, proteins induced by vitamin K absence) levels were determined by the method of Shah et al. (14). The liver microsomal vitamin K epoxide reductase activity was measured by the procedures described by Kawamoto et al. (15). Plasma and liver vitamin K and vitamin epoxide levels were determined by the method of Hirauchi et al. (16, 17).

**Results**

The effects of LMOX and its 1-S congener on PT, APTT, plasma concentrations of prothrombin, Factor VII and fibrinogen, and plasma and liver PIVKA-II levels are given in Table 1. When LMOX was injected to rats fed an ordinary diet which contained a large amount of vitamin K, no change was found in plasma clotting activities. Therefore, the experiments were performed with the rats kept on a vitamin K-deficient diet. In comparison to the rats kept on the ordinary diet, the rats fed the vitamin K-deficient diet showed increases in PT, APTT and plasma and liver PIVKA-II levels and decreases in plasma prothrombin and Factor VII concentrations, but no change in plasma fibrinogen concentration.

The treatment with LMOX enhanced these changes of vitamin K deficiency; and especially the highest dose, 300 mg/kg, produced statistically significant changes compared with the vitamin K deficient control group.
Table 1. Effects of latamoxef (LMOX) and its 1-S congener on blood coagulation activities in rats fed a vitamin K-deficient diet

| Treatment    | Daily dose (mg/kg) | No. of rats | PT (sec) | APTT (sec) | Factor VII (%) | Fibrinogen (mg/dl) | Prothrombin (U/ml) | PIVKA-II                  |
|--------------|-------------------|-------------|----------|------------|---------------|---------------------|--------------------|------------------------|
| Control      | Saline            | 6           | 16.1±1.8 | 29.1±2.9  | 37.2±13.6     | 210±11              | 58.0±8.4           | 5.6±0.5                | 0.58±0.04               |
| LMOX         | 30                | 6           | 17.7±1.6 | 32.4±2.7  | 45.3±6.1      | 199±11              | 40.1±3.9           | 7.0±0.1*               | 0.64±0.03               |
|              | 100               | 6           | 20.7±12.8| 36.3±2.8  | 25.0±5.9      | 207±8              | 38.7±4.7           | 7.2±0.6                | 0.70±0.02*              |
|              | 300               | 6           | 26.1±4.0*| 39.9±1.8* | 17.3±3.7      | 238±13             | 30.1±2.7*          | 6.1±0.6                | 0.64±0.04               |
| 1-S LMOX     | 30                | 6           | 20.3±2.2 | 36.7±4.1  | 30.0±5.3      | 210±12             | 43.7±5.2           | 5.6±0.3                | 0.65±0.04               |
|              | 100               | 6           | 19.5±1.9 | 34.4±4.1  | 30.1±7.0      | 204±7              | 42.8±4.5           | 6.8±0.5                | 0.70±0.02*              |
|              | 300               | 6           | 24.1±2.0*| 44.2±3.7* | 25.3±3.1      | 203±8              | 31.2±3.2*          | 2.4±0.1                | 0.10±0.01               |

Rats were maintained on the vitamin K-deficient diet, and they were injected intravenously LMOX or its 1-S congener at the doses shown in the table, once a day for 7 days. Blood coagulation activities were determined 24 hr after the last injection. Each value represents the mean±S.E. *Statistically significant difference compared with the control (P<0.05).

Table 2. Effects of flomoxef (FMOX) and its 1-S congener on blood coagulation activities in rats fed a vitamin K-deficient diet

| Treatment    | Daily dose (mg/kg) | No. of rats | PT (sec) | APTT (sec) | Factor VII (%) | Fibrinogen (mg/dl) | Prothrombin (U/ml) | PIVKA-II                  |
|--------------|-------------------|-------------|----------|------------|---------------|---------------------|--------------------|------------------------|
| Control      | Saline            | 6           | 15.5±1.1 | 29.5±2.3  | 59.1±12.3     | 201±7              | 56.4±6.0           | 4.9±0.4                | 0.42±0.02               |
| FMOX         | 30                | 6           | 16.2±0.8 | 32.7±2.0  | 36.7±8.6      | 192±2              | 44.8±3.7           | 5.4±0.4                | 0.43±0.02               |
|              | 100               | 6           | 21.3±2.4 | 39.0±2.4* | 28.8±5.5*     | 210±7              | 33.7±3.6*          | 5.6±0.6                | 0.44±0.03               |
|              | 300               | 6           | 20.8±1.7*| 41.1±3.7* | 29.2±10.5     | 209±7              | 31.9±6.6*          | 6.9±0.3                | 0.46±0.02               |
| 1-S FMOX     | 30                | 6           | 16.7±0.8 | 31.4±1.7  | 50.9±14.4     | 211±3              | 49.2±5.6           | 5.6±0.5                | 0.47±0.03               |
|              | 100               | 6           | 19.9±2.5 | 39.1±4.8  | 36.6±8.9      | 210±2              | 39.1±5.9           | 5.1±0.4                | 0.44±0.02               |
|              | 300               | 6           | 25.6±4.0 | 50.3±8.0  | 20.2±5.1*     | 215±9              | 25.4±4.3*          | 2.9±0.1                | 0.11±0.00               |

Experiments were carried out like those of Table 1, except that FMOX and its 1-S congener were injected instead of LMOX and its 1-S congener. Each value represents the mean±S.E. *Statistically significant compared with the control (P<0.05).
similar effect was produced by the treatment with LMOX 1-S congener, but no significant difference was observed between the effect of LMOX and that of the 1-S congener. The effects of FMOX and its 1-S congener were examined, and the results are given in Table 2. Higher doses of FMOX also enhanced vitamin K deficiency, but no difference was found between FMOX and the 1-S congener. These results suggest that although LMOX and FMOX enhance vitamin K deficiency, the effect is not solely due to their 1-oxygen since their 1-S congeners cause almost the same effect.

As shown in Fig. 2, when LMOX and FMOX were injected successively for 3 days, both the compounds inhibited liver microsomal vitamin K epoxide reductase dose-dependently, although the effect of FMOX was less than that of LMOX. Subsequently, the effects of the 1-S congeners on vitamin K epoxide reductase activity were compared with those of LMOX and FMOX (Table 3). The 1-S congeners also inhibited the enzyme, but their effects were almost comparable with those of the oxacephem.

Plasma vitamin K and vitamin K epoxide levels in these animals were also determined (Table 4), but no significant change was found.

**Discussion**

The present experiments demonstrate that oxacephems, LMOX and FMOX, and their corresponding 1-S congeners enhance vitamin K deficiency in rats fed vitamin K-deficient diet, but no difference is found between the oxacephem and its 1-S congener in their effects. The effects of LMOX and FMOX to enhance the vitamin K deficiency are due to the inhibition of vitamin K epoxide reductase (9, 10), but not to the inhibition of either γ-glutamylcarboxylase (7) or vitamin K reductase (8). Therefore, a single injection of vitamin K quickly normalizes the hypoprothrombinemia produced by these antibiotics in rats kept on a vitamin K-deficient diet (5, 7).

![Graph](image)

**Table 3.** Effects of latamoxef (LMOX), flomoxef (FMOX) and their 1-S congeners on rat liver microsomal vitamin K epoxide reductase activity

| Treatment     | Vitamin K epoxide reductase activity (pmol/min/mg protein) |
|---------------|------------------------------------------------------------|
| Control       | 138.6±0.8                                                  |
| LMOX          | 113.2±1.6*                                                 |
| 1-S LMOX      | 114.2±8.1*                                                 |
| Control       | 133.8±4.8                                                  |
| FMOX          | 112.9±3.9*                                                 |
| 1-S FMOX      | 118.7±2.9*                                                 |

Animals maintained on an ordinary diet were given intravenously LMOX, FMOX or their 1-S congeners at 300 mg/kg, once a day for 3 days; and their livers were obtained 3 hr after the last injection. Each value represents the mean±S.E. of 3–4 rats. *Comparison between LMOX (or FMOX) and the 1-S congener. *Statistically significant difference compared with the control (P<0.05).
Table 4. Effects of latamoxef (LMOX), flomoxef (FMOX) and their 1-S congeners on plasma vitamin K and vitamin K epoxide levels in rats

| Treatment   | Vitamin K (ng/ml) | Vitamin K epoxide (ng/ml) |
|-------------|-------------------|---------------------------|
| Control     | 1.19±0.17         | 0.05±0.02                 |
| LMOX        | 1.08±0.29         | 0.04±0.03                 |
| 1-S LMOX    | 0.62±0.17         | 0.03±0.03                 |
| FMOX        | 0.74±0.08         | 0.04±0.04                 |
| 1-S FMOX    | 1.04±0.15         | 0.04±0.03                 |

Animals fed an ordinary diet were injected intravenously LMOX, FMOX or their 1-S congeners once a day for 3 days (300 mg/kg/day). Blood samples for the determination of vitamin K and its epoxide were withdrawn 3 hr after the last injection. Each value represents the mean±S.E. of 4 rats.

Oxacephem and Blood Coagulation

The 3'-side chains, NMTT, TDT and MTDT, have been shown to be responsible for the hypoprothrombinemic effect (5, 6) and the antabuse-like effect (20, 21), the inhibition of aldehyde dehydrogenase, of the antibiotics possessing these side chains in their structures. The side chain of FMOX, N-hydroxyethyltetrazolethiol (HTT), does not inhibit aldehyde dehydrogenase (22, 23) and is less inhibitory towards vitamin K epoxide reductase than NMTT (Fig. 2). In the present experiment, we compared the effects of antibiotics and their 1-S congeners by treating rats for 3 or 7 days, because the hypoprothrombinemic effects of antibiotics on blood coagulation factors and vitamin K epoxide reductase activity were detectable by the successive administration for 3 days or more (24). In addition, inhibition of vitamin K epoxide reductase activity by antibiotics were detectable similarly in both vitamin K-deficient and -sufficient rats (24). Thus, we can evaluate the action of antibiotics under the present experimental conditions.

Yoshida (1) and Narisada et al. (2) have reported that the substitution of an oxygen atom at the 1-position of cephalosporins increases the hydrolysis rate in solution, compared with the original compounds. This suggests that liberation of the side chain is faster for oxacephems than for the corresponding 1-S congeners. In fact, slightly higher blood level of HTT was observed in FMOX-injected rats compared with that in 1-S congener-treated rats (K. Mizoziri, unpublished results). Therefore, we compared the in vivo effect of both the compounds in the present experiments, but no significant difference was found between 1-oxa and 1-thia compounds. This finding suggests that although the liberation of the side chain is slightly faster for the oxacephem than the corresponding 1-S compound, there is no practical difference with regards to the effect on blood coagulation activities between the oxacephem and its 1-S congener.

In addition, no difference was found in the accumulation of vitamin K epoxide between LMOX and 1-S LMOX or FMOX and 1-S FMOX, suggesting that the 1-oxygen replacement does not enhance the inhibitory action of antibiotics on vitamin K epoxide reductase.

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