Short Paper

Bioavailability of Ultra-fine Preparation of Oxolinic Acid in Red Sea Breams

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Oxolinic acid shows a wide spectrum of antibacterial activity against gram negative bacteria and part of gram positive bacteria. Since oxolinic acid has a very low solubility, an ultra-fine preparation of oxolinic acid was developed for enhancing bioavailability.

In this paper, oxolinic acid in red sea bream serum was assayed by high performance liquid chromatography (HPLC) after oral administration of ultra-fine and ordinary preparations of oxolinic acid and the difference in absorption by the red sea bream between the two preparations of oxolinic acid was studied.

Ultra-fine (1.0 μm in diameter) and ordinary (6.4 μm in diameter) oxolinic acid were mixed with a diet for yellowtail in a suspension and orally administered at a dose of 30 mg/kg to red sea breams weighing an average of 530 g. Blood samples were drawn by heart puncture from five individual fish in each group 1, 2, 4, 6, 8, 12 and 24 h after administration and serum samples were kept frozen at -20°C until analysis. These samples were collected in Oct. to Nov., 1985, and the average of water temperatures during the examination was 22.5°C .

One ml of 0.01 N sodium hydroxide containing nalidixic acid (2 $\mu\text{g}/\text{ml}$) as an internal standard, 10 ml of 0.2 M sodium acetate-hydrochloric acid buffer (pH 2.0) and 10 ml of ethyl acetate were added to each 1 ml of the serum samples in a glass-stoppered 30 ml centrifuge tube. The tube was shaken for 20 min and centrifuged for 10 min at 2,500 rpm. The organic layer (8 ml) was transferred to another tube containing 0.1 g of magnesium hydroxide and extracted with 5 ml of 0.1 N sodium hydroxide for 20 min. After centrifuging for 10 min at 2,500 rpm, 4.5 ml of the alkaline aqueous layer was transferred to another tube containing 1 ml of 2 N hydrochloric acid and the mixture was extracted with 4 ml of chloroform for 20 min. After centrifuging for 10 min at 2,500 rpm, the aqueous layer was removed and the organic layer was evaporated to dryness at 40°C .

The residue was dissolved in 1 ml of the mobile phase for HPLC, i.e., a mixture of 0.015 M phosphate buffer (pH 7.0) and acetonitrile (65:35) and this solution was

Fig. 1. Serum level-time curve of oxolinic acid in red sea bream following single oral administration of ultra-fine (○) and ordinary (●) preparations of oxolinic acid at dose of 30 mg/kg. Each point represents the mean of five fish.

subjected to HPLC. Oxolinic acid in red sea bream serum was determined by the HPLC methods reported by Kasuga *et al.*^{1,2)} and Ueno *et al.*³⁾

Serum levels of oxolinic acid are shown in Fig. 1. Both drugs appeared in the blood within 1 h and reached a maximum concentration (T_{max}) at 4 h after administration, but the maximum concentration (C_{max}) of the ultra-fine oxolinic acid was 4.24 $\mu\text{g}/\text{ml}$ and 2.8 times higher than that (1.53 $\mu\text{g}/\text{ml}$) of the ordinary oxolinic acid. Thereafter, serum levels of both drugs decreased gradually, and the half-lives of the ultra-fine and ordinary oxolinic acid were calculated to be 11.5 and 14.9 h, respectively, by the least square method from concentrations determined during the elimination phase, which was from 8 h to 24 h after administration and regarded as a linear part of the logarithmic serum concentration-time curve. The area under serum concentration-time curve from 0 to infinite time ($\text{AUC}_{0-\infty}$) after administration was calculated as follows:

$$\text{AUC}_{0-\infty} = \text{AUC}_{0-24} + C_{24}/\text{Kel}$$

where AUC_{0-24} from 0 to 24 h (the last sampling time) is calculated using the trapezoidal rule, Kel is an elimination rate constant and C_{24} is serum concentration of oxolinic acid obtained by the theoretical formula for calculating Kel. The $\text{AUC}_{0-\infty}$ of the ultra-fine oxolinic acid was 1.35 times greater than that of the ordinary oxolinic acid (54.99 versus 40.82 $\mu\text{g}\cdot\text{h}/\text{ml}$). The increased C_{max} and $\text{AUC}_{0-\infty}$ are probably due to enhanced dissolution of oxolinic acid resulting from micronization. Therefore, it was concluded that the bioavailability of oxolinic acid in red sea breams was enhanced by micronization as observed with beagles.³⁾

References

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