Absorption of Bicozamycin and Its Ester Derivative in Yellowtail, *Seriola quinquergadiata*

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Abstract

Absorption of bicozamycin (BCM) and its ester derivative (FR2054) was compared in yellowtail, *Seriola quinquergadiata*. Since the maximum concentration (Cmax) of BCM in tissue following FR2054 dosing was 4.5 fold higher than that obtained by BCM dosing, an adequate tissue level could be achieved in the kidney and spleen.

This FR2054 was found in the blood and tissue in a short time after administration of FR2054 and its concentration decreased while BCM concentration increased inversely. In order to investigate the site of hydrolysis of FR2054 in fish body, the rate of hydrolysis in plasma and tissue homogenate was investigated. Most hydrolysis of FR2054 was occurred in the plasma, suggesting that the absorbed FR2054 is hydrolyzed gradually to BCM while circulating in the blood stream.

Outbreaks of pseudotuberculosis causing with the increase of drug resistant strains of *Pasteurella piscicida*1), there is a pressing need for the development of novel therapeutic agents. Bicozamycin2) has *in vitro* bactericidal activity against *Pasteurella piscicida* strains (mean MIC: 3.13 µg/ml) including resistant strains to marketed drugs1). However, absorption of orally administered BCM by mammals is poor3). The ester derivative of BCM, bicozamycin benzoate (FR2054)4), has no antibacterial activity but is hydrolyzed to BCM by esterase in vivo. Absorption of FR2054 is better than BCM itself4).

Since administration of drugs to yellowtail is usually done by mixing with feed, it is essential that an adequate concentration should be achieved in the fish body by this route. This study was conducted to investigate the pharmacokinetics of FR2054 in yellowtail as compared to BCM.

Materials and Methods

Drug

FR2054, the ester derivative of BCM and BCM were used. Their chemical structures are shown in Fig. 1. FR2054 itself has no antibacterial activity, but is hydrolyzed to the parent compound (BCM) by esterase *in vivo*4). The molecular weights of FR2054 and BCM are 406.39 and 302.28, respectively, so that 1 g potency of BCM is derived from 1.34 g of FR2054.

Fish

Twenty fish, mean body weight; 380 g, were used to
investigate the comparative hydrolysis activity from FR2054 to BCM in tissues. Two groups of 150 fish, mean body weight: 203 g, were used in the study of comparative pharmacokinetics in the blood after single oral administration of FR2054 and BCM. Hundred and fifty, mean body weight: 380 g, were also used to investigate tissue distributions (kidney and spleen) of drugs. All fish were healthy and had not been given any drugs for at least 2 weeks prior to put in the floating net pens (3 square meters) where they were kept for further weeks before the experiments started.

The pharmacokinetics and tissue distribution studies were done at water temperatures of 28 ± 1 °C and at 21 ± 1 °C, respectively.

Administration of the drugs

In the pharmacokinetic and tissue distribution studies the dose of BCM was 20 mg/kg. BW and of FR2054 was 20 mg (as BCM potency)/kg. BW. Drugs were mixed into the moist pelleted feed (raw fish mince: formulated feed = 7:3) and then administered once to fish.

Sampling

To investigate the pharmacokinetics, blood was sampled at 1, 3, 6, 9 and 12 h, and then at 1, 2, 3, 4 and 5 days after administration. Samples were taken from the individual hearts of 5 fish using a heparinized syringe. Since it was possible that FR2054 might be hydrolyzed to BCM by the esterase in the blood before the determination, an equal volume of acetonitrile was added to the blood immediately after taken in order to stop enzyme activity. In the tissue distribution study, individual kidneys and spleens were removed at 3, 6, 9, 24, 30 h, and 2, 3 and 4 days after administration from 10 fish at each sampling point. In both experiments, samples were frozen immediately after they were taken and stored at -80 °C until analyzed. In the hydrolysis study liver, kidney muscle, spleen, pyloric caeca and intestine were sampled from 5 fish and pooled. The hydrolysis study was performed within 2 h of taking the tissue.

The concentration of BCM and FR2054 in the samples were determined by high performance liquid chromatography

In vitro hydrolysis of FR2054

An aqueous solution of FR2054 (500 μg (as BCM potency)/ml) was added to 5 fold volume of plasma or 10 fold weight of tissue homogenate composed of equal volumes of tissue and phosphate buffer (pH 7.4). Samples and tissue homogenates were determined at 2 and 24 h after incubation and hydrolysis rate of FR2054 was calculated.

Results

Absorption of FR2054 and BCM

BCM concentration in the blood after oral administration of BCM at the dose of 20 mg/kg. BW is shown in Fig 2. Maximum concentration (Cmax) was 0.8 μg/ml and time to reach Cmax after administration (Tmax) was 1 day.

BCM concentration hydrolyzed from FR2054 in the blood after oral administration with FR2054 at the equimolar dose is shown in Fig. 3. Cmax and Tmax was 3.6 μg/ml and 1 day, respectively. The Cmax of BCM following FR2054 dosing was 4.5 fold higher than that obtained by BCM dosing. Area under the concentration

![Fig. 2. Bicozamycin concentration in the blood after single oral administration of bicozamycin at 20 mg/kg. BW in Seriola quinqueradiata.](image-url)
Absorption of bicozamycin

Fig. 3. Bicozamycin (●) and FR2054 (○) concentrations in the blood after single oral administration of FR2054 at 20 mg (as BCM potency)/kg. BW in Seriola quinqueradiata.

Fig. 4. Bicozamycin (●) and FR2054 (○) concentrations in the kidney after single oral administration of FR2054 at 20 mg (as BCM potency)/kg. BW in Seriola quinqueradiata.

curve (AUC) obtained by FR2054 and BCM dosing were respectively 130.0 and 33.0 µg.h/ml (3.9 fold). C\text{max} and T\text{max} of intact FR2054 were 3.5 µg (as BCM potency)/ml and 6h, respectively.

Drug distribution in tissues

Results of drug distribution by FR2054 dosing are shown in Fig. 4 and 5. The C\text{max} of BCM in the kidney and spleen were 3.8 and 2.2 µg/g, and T\text{max} was 30 h and 2 days, respectively. While C\text{max} of intact FR2054 in the kidney and spleen was 4.1 and 4.3 µg (as BCM potency)/g and T\text{max} was both 6 h.

Table 1. Hydrolysis of FR2054 in plasma and tissue homogenates originated from yellowtail

<table>
<thead>
<tr>
<th>Homogenates</th>
<th>Incubation period</th>
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<td>Plasma</td>
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<td>Intestine</td>
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In vitro hydrolysis of FR2054

As shown in Table 1, although 35.6% of FR2054 was hydrolyzed to BCM in plasma, only very little hydrolysis took place in the liver and kidney homogenates.

Discussion

BCM concentration after dosing did not reach even one third MIC (3.13 µg/ml) against Pasteurella piscicida in this study. This result suggesting that this would be ineffective as a therapeutic agent for pseudotuberculosis in yellowtail. By dosing FR2054 at an equimolar dose of BCM, almost equivalent to mean MIC level could be achieved in the kidney and spleen. When FR2054 is hydrolyzed to BCM, this drug shows antibacterial activity. Thus, FR2054 showed the possibility as a novel therapeutic agent for pseudotubercu-
loss in yellowtail.

Bacampicillin (BAPC) and pivampicillin (PVPC) are available as ester derivatives of ampicillin. It has been reported that no unchanged ester could be found circulating in the blood of volunteers receiving BAPC orally and PVPC is rapidly hydrolyzed in the intestinal tissue before being transferred into the portal blood stream. FR2054 was considered to have the same pathway as these drugs in yellowtail. In this study, intact FR2054 was found and its concentration decreased while BCM concentration increased inversely. Most hydrolysis of FR2054 was occurred in the plasma, suggesting that the absorbed FR2054 is hydrolyzed gradually to BCM while the circulating in the blood stream.

Acknowledgement

We are indebted to Dr. Valerie Inglis of Institute of Aquaculture, University of Stirling, for critical reading of the manuscript.

References


ビコザマイシンおよびその誘導体のプリ,

Seriola quinqueradiataにおける吸収

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プリ, Seriola quinqueradiataにおけるビコザマイシン（BCM）およびそのエステル誘導体（FR2054）の吸収を比較した。FR2054を投与したときのBCMの最高血液中濃度（C_{max})はBCMを投与したときよりも4.5倍高く、腎臓および肝臓においても充分な組織中濃度が得られた。FR2054投与後の血液および組織中のFR2054濃度は短時間に減少し、相反BCM濃度が増加した。FR2054の加水分解が魚体内のいずれの組織で行われるのかを推定するために、血漿および組織ホモジネート中での加水分解率を測定した結果、血漿中で最も加水分解が進んだ。このことから、吸収されたFR2054は血液循環中に徐々にBCMに加水分解されていくものと考えられた。