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PLASMA AND TISSUE LEVELS OF TRIMETHOPRIM IN THE RAINBOW TROUT, SALMO GAIRDNERI, AFTER ABSORPTION FROM FRESH AND SALT WATER

By
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BERGSJØ, T. & E. SØGNEN: *Plasma and tissue levels of trimethoprim in the rainbow trout, Salmo gairdneri, after absorption from fresh and salt water.* Acta vet. scand. 1980, *21*, 18—25. — This is a comparative study of uptake of trimethoprim from 1) fresh water, 2) salt water after 7 days of adaption and 3) salt water without previous adaptation. The rate and extent of absorption were found to vary significantly. The salt water adapted group reached a plasma concentration of approx. 1 μg/ml after 10 h, the unadapted salt water group after 24 to 48 h and the fresh water group did not reach this concentration. The results are discussed in relation to the non-ionic diffusion theory and to the alterations taking place in euryhaline species of fish during adaptation to salt water.

trimethoprim; rainbow trout; Salmo gairdneri; absorption; fresh water; salt water.

Previous investigations have shown that it is possible to establish efficient concentrations of sulphonamides in fish at an acceptable rate by adding the drug to water (*Bergsjø* 1974).

It has also been shown that the apparent rate of absorption is higher in salt than in fresh water. The pH of the fresh and salt water was 6.8 and 8.2, respectively. The sulphonamides used in the experiments were weak acids with pKa values of 7.4 and 10.4. Therefore the results did not conform with the accepted theories of passive diffusion of drugs through membranes (*Bergsjø & Bergsjø* 1978). Other explanations for the increased uptake of sulphonamides in salt water should be considered.

Firstly it would be of interest to examine the apparent rate of absorption of trimethoprim — a weak base with pKa 7.6 in fresh and salt water. This is also of considerable practical interest, since trimethoprim combinations with various sulphonamides are introduced also in the treatment of infections in fish.

The purpose of this experiment was to examine to which extent trimethoprim was taken up from the water and of what importance the salt content of the water would be. This was done by comparing uptake of trimethoprim from 1) fresh water, 2) salt water after adaptation and 3) salt water without previous adaptation.

MATERIAL AND METHODS

Healthy rainbow trout (Salmo gairdneri) bred at the Fish Research Station, Sunndalsøra, were used in the investigation. Average weight was 120 g, age approx. 12 months. At the station the fish were kept in 120-l plastic tanks and were not fed during the experiment. Air was supplied via a compressor. The natural pH of the salt water (salt concentration 32 ‰) and fresh water was 8.2 and 6.8, respectively, and the temperature was approx. 9°C.

Trimethoprim was added to fresh and salt water in equal amounts, 75 mg/l, the maximum that could be dissolved under the given conditions. One group of fish was placed in medicated fresh water, 1 group in medicated salt water after previous adaptation to salt water for 7 days and 1 group in medicated salt water without previous adaptation.

From each of the 3 tanks, 5 fish were removed for sampling at each of the following periods of exposure: 5, 10, 24, 48, 72 and 84 h. The fish were anaesthetized in a solution of benzocaine, 3 g in 10 l of water, for about 5 s. Blood samples were taken from the dorsal aorta with heparinized vacutainers. The blood was immediately centrifuged. Samples were also taken from muscle and liver. All samples were kept at —20°C until analyzed. After 84 h, when the absorption period was terminated, 8 fish from each tank were transferred to tanks with unmedicated fresh or salt water. Their clinical status was observed for 3 weeks.

Trimethoprim was assayed by microbiological plate assay on Oxoid "Sensitest" agar with Bacillus pumilus as indicator organism (Wellcome Research Laboratories, Great Britain). All

samples were taken in duplicates. The assay plates were incubated at 28°C, the zones of inhibition being read at 24 h. The zones were plotted against an average standard curve based on standards set up before, in the middle of and after the assay period. Standard solutions were placed on every assay plate to ensure that the test conditions were always identical.

Tissue samples from each batch of 5 fish were pooled. The samples were homogenized following addition of 0.9 % NaCl, 1:2. After homogenization the samples were disintegrated in an ultrasonic disintegrator and centrifuged. The supernatant fluid was then used for the assay.

Samples of plasma and tissue from fishes being anaesthetized but not bathed in trimethoprim were also assayed. These did not inhibit the growth of the test organism.

RESULTS

The results are given in Fig. 1 showing trimethoprim concentrations in plasma, muscle and liver.

Absorption of trimethoprim from water took place in all the 3 groups, but the rate and degree of absorption varied significantly. The salt water adapted group reached a plasma concentration of approx. 1 µg/ml after 10 h. The unadapted salt water group reached this value after 24 to 48 h, while the fresh water group did not reach this concentration. As minimum inhibitory concentration (m.i.c.) of trimethoprim to some common fish-pathogenic bacteria is approx. 0.2 µg/ml (*McCarthy et al.* 1974 a), a minimum therapeutic concentration in plasma can be stipulated to 1 µg/ml (5 times the m.i.c.-value).

The concentrations in liver and muscle both exceed the plasma concentrations. The liver values are up to 12 times higher than the plasma values, the muscle values up to 3 times higher.

The fish which were transferred to clean water showed no clinical signs of injury.

DISCUSSION

When a drug is added to the water as in the present experiment, the absorption of the drug will be dependent inter alia on its degree of ionization and ability to pass a biological membrane like the gills. Trimethoprim is a weak base with pKa = 7.6. The pH of fresh water is 6.8, of salt water 8.2, pH of the fish blood

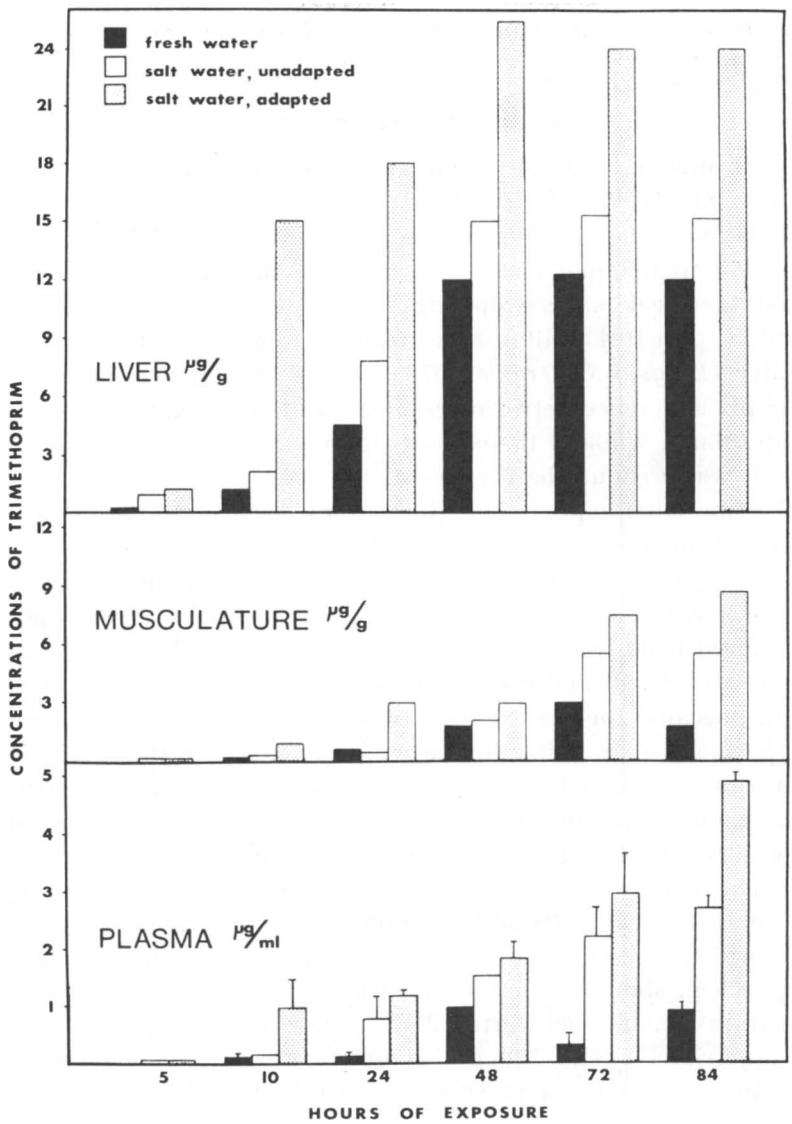

Figure 1. Concentration of trimethoprim in plasma and tissue of rainbow trout, Salmo gairdneri, after uptake of trimethoprim (75 mg/l) from fresh water, from salt water after adaptation to salt water and from salt water without previous adaptation.

is 7.4. According to *Shore et al.* (1957) the following formula can be used to calculate distribution of a weak base:

$$\frac{\text{C fish}}{\text{C water}} = \frac{1 + 10^{\text{pKa—pH fish}}}{1 + 10^{\text{pKa—pH water}}}$$

If we introduce the above-mentioned values, the ratio C fish/C water will be 0.35 for fresh water and 2.07 for salt water. The pH is thus favouring absorption from salt water. This cannot be of very great importance, however, as it has earlier been shown that the weak acids sulphadimidine (pKa 7.4) and sulphanilamide (pKa 10.4) will also be absorbed to a higher degree in salt water (*Bergsjø & Bergsjø* 1978). In the case of sulphanilamide the pH difference between fresh and salt water should be of no importance, while in the case of sulphadimidine absorption from fresh water should be favoured.

Some other explanations than alteration in non-ionic diffusion through membranes ought to be discussed.

The physiological changes that occur when a euryhaline fish (fish that can adapt to a change in the salt content of the water) is moved from fresh to salt water are extensive. Both intake and excretion of salt and water will be subjected to profound alterations, because the fish changes from being hyperosmotic to being hyposmotic in relation to the environment. One important implication of the adaptation is that the fish will start to drink water, and consequently drugs may be taken up not only through the gills but also through the gastrointestinal tract.

The amount of water ingested varies with the species. For rainbow trout it is found to be approx. 5 ml/kg/h (*Conte* 1969). According to this, the salt water fish in this experiment, weighing 120 g, should be supplied with 1 mg trimethoprim via the gastrointestinal tract during 24 h. *McCarthy et al.* (1974 b) gave 2 mg trimethoprim/100 g fish orally to a rainbow trout and attained a maximum plasma concentration of 7.5 µg/ml. Compared to this, the drug taken up from the swallowed water probably represents a substantial addition to what is absorbed through the gills.

The higher plasma and tissue concentrations attained in salt water might also partly be a result of reduced renal excretion of the drug. The kidney functions and the urine production are known to change profoundly by transfer of fish from fresh to salt water. *Holmes & McBean* (1963) found the glomerular filtra-

tion rate to fall from 156 ml/kg/day to 10 ml/kg/day after 10 days of adaptation, and the amount of urine decreased from 82.5 ml to 0.74 ml/kg/day during the same period of adaptation (*Hichman & Trump* 1969).

Many factors concerning the excretion of trimethoprim in fish under different conditions are unknown, but a reduction in the glomerular filtration rate by more than 15 times is likely to affect the renal excretion of the drug. To which extent the total excretion of the drug is influenced depends upon the part played by other excretory pathways such as the gills, the skin and the liver. The latter is likely to play an important role in the elimination of trimethoprim as judged by autoradiographic studies (*Bergsjø et al.* 1979).

The distribution of trimethoprim between plasma and tissues follows the same pattern as in various species of mammals, with higher concentrations in tissues than in plasma. For the liver the concentration of trimethoprim is 3 times higher than in plasma in the mouse (*Bushby & Hitchings* 1968), 4 times higher in the goat, 5 times higher in the cow (*Nielsen & Rasmussen* 1975) and 12 times higher in the rainbow trout as shown in the present experiment.

Whether the concentration of trimethoprim in the liver reflects the magnitude of biliary excretion of the drug is not possible to discuss in further detail before specific excretory studies are carried out.

CONCLUSION

It remains to be seen whether salt water adaptation can be utilized in practical drug administration to fish. It is, however, a fact that in the stage of development when the euryhaline species of fish can be adapted they are subject to several diseases, such as vibriosis and infections of the gills and the skin. In this period it is almost impossible to administer the drug with the feed. Provided that adequate oxygenation is established, it is possible to treat a large number of fish in a comparatively small volume of water. This procedure is economic and allows the user to dispose of the remains of the drug in question in a controlled manner.

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SAMMENDRAG

Plasma- og vevskonsentrasjoner av trimethoprim hos regnbueørret, Salmo gairdneri, etter absorpsjon fra ferskvann og saltvann.

Det er foretatt en undersøkelse over i hvilken grad regnbueørret, Salmo gairdneri, kan absorbere trimethoprim fra ferskvann, fra saltvann etter forutgående adaptasjon og fra saltvann uten adaptasjon. En viss absorpsjon fant sted i alle tre grupper. Fra ferskvann gikk imidlertid absorpsjonen så langsomt at man ikke nådde opp i tera-

peutiske plasma-konsentrasjoner i løpet av de 84 timer forsøket varte. Ved opptak fra saltvann uten adaptasjon ble det oppnådd terapeutiske konsentrasjoner etter ca. 24 timer, og ved opptak fra saltvann med adaptasjon etter ca. 12 timer. Mulige årsaker til forskjellene mellom de tre grupper blir diskutert.

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