Pharmacokinetics of sulphadimidine in carp (*Cyprinus carpio* L.) and rainbow trout (*Salmo gairdneri* Richardson) acclimated at two different temperature levels

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SUMMARY. *The influence of temperature (10° C and 20° C) on pharmacokinetics and metabolism of sulphadimidine (SDM) in carp and trout was studied.*
At 20° C a significantly lower level of distribution (Vd_{area}) and a significantly shorter elimination half-life ($T_{(1/2)}\beta$) was achieved in both species compared to the 10° C level. In carp the body clearance parameter ($Cl_{B(SDM)}$) was significantly higher at 20° C compared to the value at 10° C, whereas for trout this parameter was in the same order of magnitude for both temperatures.
N_4-acetylsulphadimidine (N_4-SDM) was the main metabolite of SDM in both species at the two temperature levels. The relative N_4-SDM plasma percentage in carp was significantly higher at 20° C than at 10° C, whereas there was in trout no significant difference.
In neither species was the peak plasma concentration of $N_{4\text{-}SDM}$ ($C_{max(N_4\text{-}SDM)}$) significantly different at two temperatures.
The corresponding peak time of this metabolite ($T_{max(N_4\text{-}SDM)}$) was significantly shorter at 20° C compared to 10° C in both carp and trout.
In carp at both temperatures, acetylation occurs to a greater extent than hydroxylation. Only the 6-hydroxymethyl-metabolite (SCH_2OH) was detected in carp, at a significant different level at the two temperatures. Concentrations of hydroxy metabolites in trout were at the detection level of the HPLC-method (0.02-μg/ml). The glucuronide metabolite (SOH-gluc.) was not detected in either species at the two temperatures.

INTRODUCTION

In intensive aquaculture, disease can cause tremendous economic losses. In bacterial infections, antimicrobial drugs such as oxytetracycline, sulphonamides and quinolones can be used for prophylactic and therapeutic purposes (2).
Applied drug treatment is often extrapolated from mammalian pharmacokinetic data. Consequently results of chemotherapy in aquaculture are often disappointing; i.e. the required MIC level for the pathogen concerned is not achieved and/or bacterial resistance is induced.
As stated by Grondel (16) a rational and optimal plan for antimicrobial therapy in aquaculture is needed. A prerequisite for a more fundamental approach is further development of specific pharmacokinetic studies in ectotherms. Pharmacokinetic studies in mammals pointed out that pharmacokinetic data are species and drug dependent.

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In poikilothermic vertebrates, temperature is one of the main environmental factors influencing the rate of all physiological and biochemical reactions affecting for instance metabolic rate, membrane lipid composition and immune system. Therefore, the influence of the water temperature must be taken into consideration in pharmacokinetic studies in ectotherms (7, 29, 35).
Sulphadimidine (SDM) is one of the sulphonamides often used in chemotherapy because of its broad antibacterial activity.
SDM can be metabolised by hydroxylation at the 5 and 6 position of the pyrimidine ring and by the acetylation-deacetylation pathway. After hydroxylation the metabolite may become glucuronidated and also acetylated (25). The hydroxy metabolites are microbiologically active and their activity can be potentiated by trimethoprim (21).
Recently the acetylation and hydroxylation reaction of SDM was demonstrated in carp. The main metabolite produced in this species was N_4-acetylsulphadimidine (16, 25). In this paper the influence of temperature on the pharmacokinetics and metabolism of sulphadimidine in carp and trout is described.

MATERIALS AND METHODS

Animals

Carp (*Cyprinus carpio* L) were bred at Zodiac, Agricultural University, Wageningen at 24° C in aquaria with aerated running tapwater. The fish were fed daily with pelleted dry food (Trouw & Co., Putten, The Netherlands) by means of 'Scharflinger' conveyerbelt automatic feeders.
Trout (*Salmo gairdneri* Richardson) were obtained from the Organisation for Improvement of Inland Fisheries (O.V.B., Lelystad, The Netherlands) and were subjected to a quarantine period of 6 weeks.

Experimental design

Carp and trout of both sexes were placed in flow-through aquaria in two temperature acclimation chambers. The water temperature in the aquaria was regulated in a storage tank.
The following temperature adaptation strategy was applied: animals of both species were adapted to the required temperature following an adaptation schedule of 2° C change per two days. Thus a group of carp (n = 6; body weight 386 ± 49 g) and trout (n = 7, body weight 288 ± 67 g) were adapted to 10° C and another group of carp (n = 6; body weight 421 ± 44 g) and trout (n = 6; body weight 259 ± 45 g) to 20° C.
After achieving these temperatures an acclimation period of 6 weeks was sustained for complete adaptation.
Sodium sulphadimidine® (33.3%; Aesculaap B.V., Boxtel) was dissolved in a phosphate buffered saline (PBS) solution pH 7.2 and administered intravenously (IV; caudal vein) at a dosage of 100 mg/kg to both species at the two different temperatures.
N_4-acetylsulphadimidine (N_4-SDM) was synthesised, and the hydroxy metabolites 6-hydroxymethylsulphadimidine (SCH_2OH) and 5-hydroxy-sulphadimidine (SOH) were isolated according to Vree *et al.* (37, 38).
Heparinised blood samples were taken from all 4 groups at regular time intervals from the caudal vein and centrifuged for 10 minutes at 800 x g. Directly afterwards plasma was frozen at -20° C pending HPLC analysis.

HPLC analysis

Deglucuronidation, sample preparation and HPLC analysis were performed at the RVV-District Nijmegen, the Netherlands as described by Nouws *et al.* (22, 23, 24).
SDM, the acetylation hydroxy metabolite (N_4-SDM) and the two metabolites SCH_2OH and SOH were determined simultaneously.

Pharmacokinetic and statistical analysis

Plasma antibiotic concentrations for each animal in the 4 experimental groups were analysed according to the standard procedures described by Baggot (3).
Elimination half-life ($T_{(½)}ß$, the apparent volume of distribution (Vd_{area}) and the body clearance $Cl_{B(SDM)}$ were calculated.
The peak concentration of the main metabolite ($C_{max(N4\text{-}SDM)}$) and corresponding peak time ($T_{max(N4\text{-}SDM)}$) for each animal were read from the collected concentration versus time data of the individual fish.
The area under the curve (AUC) for parent compound and metabolites was calculated following the trapezoid rule. From these AUC data the proportions of the metabolites with respect to the parent compound SDM was calculated as: % metabolite = 100 x [AUC(metabolite)/AUC(SDM)].
Statistical analysis was in case of normality performed with the two sample T-test and otherwise in a non-parametric way using the Wilcoxon rank sum test (36). A 95% confidence bounds was sustained.

RESULTS

Figures 1 and 2 show the plasma concentration (mean ± SE) versus time plots of SDM and the N_4-acetyl derivative at 10° C and 20° C in carp and trout respectively. Corresponding pharmacokinetic data and statistical comparison between temperatures within a species are presented in Tables 1 and 2.
In the 10° C carp group the curve of the parent compound SDM was described by a two compartment model. The distribution phase was 14 hrs ($T_{(½)\alpha}$: 10.0 ± 3.3 hrs) and the final elimination half-life ($T_{(½)}ß$) was 50.3 ± 6.6 hrs (interval: 14-288 hrs).
By contrast at 20° C the distribution phase was much faster and took place before the first sampling point (1 hr p.i.). Based on suitable data of a single fish an extrapolated zero-time SDM concentration (A°) of 837 µg/ml and a distribution half-life ($T_{(½)\alpha}$) of 0.27 hr was calculated for the distribution phase at this temperature.

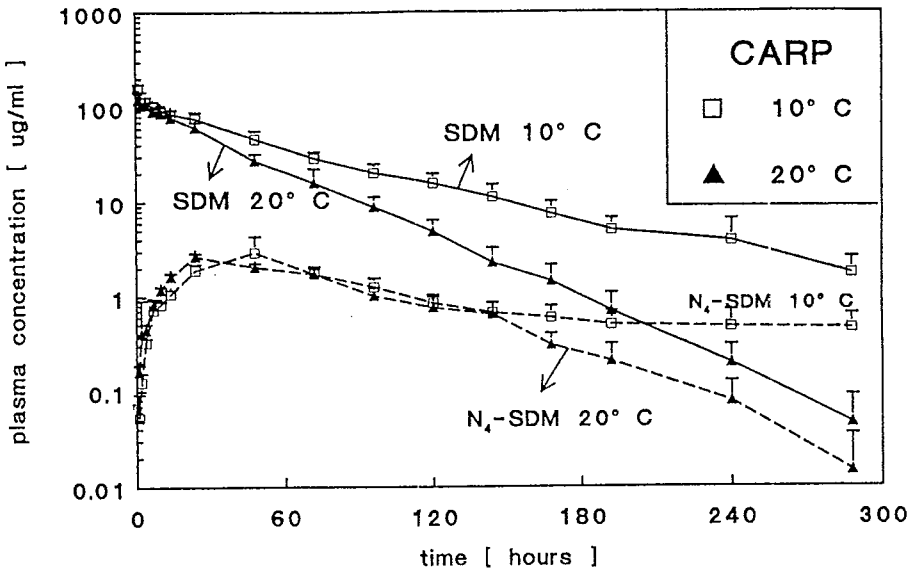

Figure 1. Mean ± (S.E.) plasma concentration-time profiles of sulphadimidine (SDM) and its main metabolite N_4-acetylsulphadimidine (N_4-SDM) at two different temperature levels (10° C and 20° C) in carp (*Cyprinus carpio* L.) following intravenous administration of SDM at a dosage of 100 mg/kg.

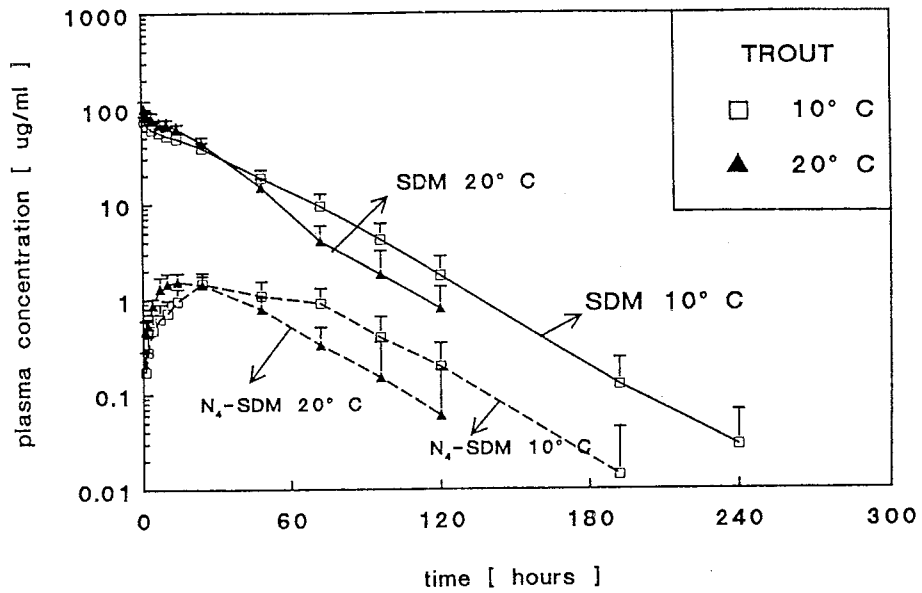

Figure 2. Mean ± (S.E.) plasma concentration-time profiles of sulphadimidine (SDM) and its main metabolite N_4-acetylsulphadimidine (N_4-SDM) at two different temperature levels (10° C and 20° C) in trout (*Salmo gairdneri* Richardson) following intravenous administration of SDM at a dosage of 100 mg/kg.

For trout the data collected did not allow characterisation of the distribution phase, because it took place within 1 hr p.i. Therefore the distribution half-life ($T_{(½\alpha)}$) was less than 0.5 hr. SDM showed a moderate distribution in the body of carp and trout. At 20° C a significantly lower level of distribution was achieved than at 10° C in both species (Tables 1 and 2).

Table 1. Pharmacokinetic values at two different temperatures (10 and 20° C) and the corresponding statistical comparison in carp (*Cyprinus carpio* L.) following intravenous (IV) administration of sulphadimidine (SDM).

		10 °C	20 °C	P-value
Dose	(mg/kg)	100	100	-
No. of animals		6	6	-
Weight	(g)	386 ± 49	421 ± 44	P < 0.26
A°	(µg/ml)	49.4 ± 10.1	-	-
α	(h^{-1})	0.078 ± 0.027	-	-
$T_{(1/2)\alpha}$	(h)	10.0 ± 3.3	< 0.5#	-
B°	(µg/ml)	81.0 ± 9.06	113.8 ± 18.6	P < 0.005*
ß	(h^{-1})	0.014 ± 0.002	0.0273 ± 0.0026	P < 0.00001*
$T_{(1/2)ß}$	(h)	50.3 ± 6.6	25.6 ± 2.6	P < 0.0002*
$Cl_{B(SDM)}$	(ml/min/kg)	0.269 ± 0.044	0.411 ± 0.060	P < 0.002*
$AUC_{(SDM)}$	(µg.h/ml)	6379 ± 1096	4140 ± 591	P < 0.002*
Vd area	(l/kg)	1.15 ± 0.10	0.90 ± 0.07	P < 0.0009*
% metabolite**				
N_4-SDM	(%)	4.18 ± 1.31	5.62 ± 0.77	P < 0.047*
SCH_2OH	(%)	2.51 ± 0.67 (n=5)	0.67 ± 0.44	P < 0.001*
$C_{max(N4\text{-}SDM)}$	(µg/ml)	2.35 ± 0.54	2.69 ± 0.203	P < 0.23
$T_{max(N4\text{-}SDM)}$	(h)	40.0 ± 11.3	24.0 ± 0.0	P < 0.033*

Note: * = significant difference (P < 0.05) ** = 100 x [AUC(metabolite)/AUC(SDM)]
= estimated value - = not determinable in 5 fishes

Table 2. Pharmacokinetic values at two different temperatures (10 and 20° C) and the corresponding statistical comparison in trout (*Salmo gairdneri* Richardson) following intravenous (IV) administration of sulphadimidine (SDM).

		10 °C	20 °C	P-value
Dose	(mg/kg)	100	100	-
No. of animals		7	6	-
Weight	(g)	228 ± 67	259 ± 45	P < 0.4
A°	(µg/ml)	-	-	-
α	(h^{-1})	-	-	-
$T_{(1/2)\alpha}$	(h)	< 0.5#	< 0.5#	-
B°	(µg/ml)	107.1 ± 34.8	167.7 ± 65.0	P < 0.075
ß	(h^{-1})	0.035 ± 0.0068	0.049 ± 0.0097	P < 0.015*
$T_{(1/2)ß}$	(h)	20.6 ± 3.8	14.7 ± 2.9	P < 0.014*
$Cl_{B(SDM)}$	(ml/min/kg)	0.685 ± 0.118	0.665 ± 0.080	P < 0.74
$AUC_{(SDM)}$	(µg.h/ml)	2508 ± 437	2543 ± 295	P < 0.88
Vd area	(l/kg)	1.20 ± 0.18	0.83 ± 0.15	P < 0.0047*
% metabolite**				
N_4-SDM	(%)	4.05 ± 0.87	3.17 ± 1.09	P < 0.094
$C_{max(N_4\text{-}SDM)}$	(µg/ml)	1.47 ± 0.45	1.63 ± 0.40	P < 0.54
$T_{max(N_4\text{-}SDM)}$	(h)	24.0 ± 0.0	13.8 ± 5.2	P < 0.004*

Note: * = significant difference (P < 0.05) ** = 100 x [AUC(metabolite)/AUC(SDM)]
= estimated value - = not determinable

In both species elimination was significantly faster at 20° C than at 10° C. Besides this temperature influence, statistical comparison between species within temperatures showed a significant faster elimination half-life in trout at both temperatures ($P < 0.0001$).

In the carp at 20° C, the $Cl_{B(SDM)}$ was significantly higher than at 10° C, but in trout it was at both temperatures in the same order of magnitude, being approximately 1.5 to 2.5 times greater than for carp. There was a significant difference in $CL_{B(SDM)}$ between species ($P < 0.0001$). In carp at 10° C and 20° C N_4-SDM was the main product of metabolism of SDM resulting in 4.18% ± 1.31 and 5.62% ± 0.77 respectively. A significant temperature influence for the N_4-SDM plasma percentage was observed (Table 1).

In trout the N_4-acetyl derivative could also be considered as the main metabolite in the 10° C and 20° C group, but no significant difference in relative percentages were observed (Table 2).

In both species, the peak plasma concentration of N_4-SDM ($C_{max(N_4\text{-}SDM)}$) at the 10° C level was not significantly different from the value obtained for the 20° C level. On the contrary, the peak plasma concentration time of N_4-SDM ($T_{max(N_4\text{-}SDM)}$) at 20° C was significantly shorter in both species (Tables 1 and 2).

Statistical analysis between species within temperatures showed a significantly higher $C_{max(N_4\text{-}SDM)}$ for carp (10° C $P < 0.013$; 20° C $P < 0.0004$) and a significantly shorter $T_{max(N_4\text{-}SDM)}$ in trout (10° C $P < 0.033$; 20° C $P < 0.008$). For carp at 10° C the N_4-SDM plot was parallel to that of SDM from 48 to 144 hours and approached a steady-state level in the interval 192-288 hours. At 20° C the N_4-SDM/SDM percentage increased slowly until 192 hr p.i.; then the N_4-SDM plasma concentration vs time curve was parallel to that of SDM. In trout, the elimination half-life of N_4-SDM equalled that of SDM at 10° C and 20° C.

In carp, hydroxylation of SDM occurs to a smaller extent compared to acetylation at both temperatures. Only the 6-hydroxymethyl-metabolite (SCH_2OH) was detected in the carp groups at both temperatures giving 2.51 ± 0.67% at the 10° C and 0.67% ± 0.44 at the 20° C level. The amount of hydroxylation is significantly different between the two temperature groups.

It is not exactly clear to what extent hydroxylation occurs in trout because concentrations of hydroxy metabolites were at the detection level of the HPLC-method (0.02 μg/ml). The glucuronide metabolite (SOH-gluc.) was not detected in either species at the two temperatures.

DISCUSSION

Data revealed that the pharmacokinetics (distribution, metabolism and excretion) of SDM in carp and trout is dependent on the ambient temperature.

Distribution volume

A clear temperature influence was detected, the distribution volume being smaller at a higher temperature. This phenomenon can possibly be ascribed to changes in the cardiovascular system, alterations in vascularity or constitution of body tissues, and differences in binding capacity to blood proteins.
Barron *et al.* (4) demonstrated in trout the following physiological adaptations to an increased temperature: increased cardiac output, reduced blood flow to organs such as spleen, liver, kidney, gall-bladder and gastro-intestinal tract, and increased blood perfusion of white muscle. Johnston and Lucking (20) found the total number of pink and red muscle fibres increased significantly in goldfish following a cold acclimation period. At the same time, the temperature compensation process involves intrinsic biochemical adaptations e.g. a temperature induced lipid adaptation which might control membrane fluidity (18, 39). An increase in the degree of unsaturation of lipid components at a lower temperature has been reported (8, 18, 34). The better blood perfusion and alterations in membrane composition at 20° C may cause a relative change in drug transport process rates between blood and tissue and vice versa, which may affect the calculated distribution volume. Data elucidating a temperature influence on binding capacities of blood proteins are lacking.

Elimination

The elimination process involves metabolic transformation reactions and excretion mechanisms of the parent-compound and metabolites.
The final elimination half lives ($T_{(½)}\beta$) for SDM in carp were in accordance with earlier observations of Nouws *et al.* (25) 17.5 hrs and Grondel *et al.* (16) 17.5 $\pm$ 5.8 (n = 5) hrs at 20° C; while these parameters for trout corresponded to those of Borgan *et al.* (6) namely 51.7 $\pm$ 6.4 hrs at 7° and 24.6 $\pm$ 1.8 hrs at 14° C.

Data revealed that the final elimination half-life was longer at a lower water temperature in both species. According to Rasmussen (29) this phenomenon was also found for chloramphenicol in trout (33), ormetropim in catfish (7), oxytetracycline in trout (32), sulphadimethoxine in catfish (7) and trimethoprim in trout (32). Differences in elimination half-life are possibly the result of temperature adaptations, which have their impact on the activity of the fish, metabolism, cardiovascular system, tissue vascularisation, extent of the biotransformation process, and excretory capacity of gills and kidney.

Obviously elimination half-lives of SDM in carp and trout are longer than in mammals (25). This may be due to a) in freshwater teleostei the renal clearance of metabolites probably involves mainly passive diffusion (glomerular filtration) whereas in mammals active processes (glomerular and tubular secretion) are involved (14, 15, 19); b) the detoxication system in ectotherms have enzymes with lower optimal temperatures than comparable mammalian enzymes (9).

Metabolism

The major biotransformations such as hydroxylation, dealkylation, hydrolysis, and various conjugation reactions observed in mammals have also been observed in fish (5, 15, 17).

Theoretically, temperature may influence the metabolism of SDM in a qualitative or quantitative way.

There were no changes in metabolic pattern (qualitative effect) within species. In trout as well as in carp N_4-SDM was beyond question the main metabolite in both temperature groups whereas glucuronidation was not observed in any group. In carp, the percentage of N_4-SDM in plasma was relatively higher at 20° C than at 10° C. The 6-hydroxymethylmetabolite (SCH_2OH) was also detected in both temperature groups of carp. Although the detection of 5-hydroxysulphadimidine (SOH) has been reported in former reports (16, 25), it is doubtful this metabolite really is formed. Data collected over a long interval (288 hrs, this study) indicated that the concentration of this assumed metabolite was at a very low steady-state level and independent of the concentration of the parent compound. Besides, administration of SDM at varying dosages to carp did not influence the amount of 5-hydroxysulphadimidine formed (unpublished results).

Quantitatively, there are some minor differences between the metabolites formed in the temperature groups, which were not significant in trout (Tables 1 and 2). It is difficult to ascribe the observed quantitative differences to the separate individual processes of metabolism, distribution or elimination. Observed differences are the net result of these processes.

Species differences

Clear differences between the two species were observed for the elimination half-time ($T_{(½)\beta}$) and body clearance ($Cl_{B(SDM)}$), both indicating trout had a faster elimination than carp. This agrees with a comparative study including carp (20° C) and trout (12° C using ciprofloxacin (26) and oxytetracycline (14, 15). This clear difference between the two species can be attributed to species dependent physiological differences in renal function, muscle constitution, activity, vascularisation etc. The metabolism of SDM also showed species dependent differences. Obviously the peak concentration of the main metabolite N_4-SDM ($C_{max(N4\text{-}SDM)}$) was for both temperatures higher in carp than in trout, whereas hydroxylation was negligible in trout. Besides, the maximal N_4-SDM concentration in plasma was achieved faster in trout than in carp. In the carp a gradual increase of the N_4-SDM/SDM percentage was observed until 192 hrs p.i., in contrast to trout. An explanation, may be, that in the carp a stimulation of acetylation occurs in the presence of SDM. In Chambers review (9) two clear examples of enzyme induction are given: a significant increase in activity of 3,4-benzpyrene hydroxylase in brown trout and capelin by crude oil (27) whereas stimulation of this enzyme occurred in rainbow trout by 3-methylcholanthrene (28).

In general, differences in amount and composition of biotransformation enzymes in various fish species can probably be related to evolutionary history (9).

Because of the bacteriostatic action of SDM, successful therapy is dependent on the status of the immunological defense mechanisms of the host. For oxytetracycline an immunosuppressive effect has been demonstrated (11, 12, 13, 30, 31). In the case of SDM, it is not clear to what extent an immunomodulating effect occurs. It is established that temperature modulates immunological reactions in ectothermic animals (1, 10).

In conclusion, pharmacokinetic studies in fishes are complicated by environmental temperature. From a theoretical point of view it is essential to increase the insight into the physiological and biochemical adaptations to environmental temperature;

from a practical point of view it is worth developing directly applicable terms e.g. the use of degree days in relation to detected concentrations of drug residues and withdrawal periods (7, 29)

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