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Pharmacokinetics and metabolism of miloxacin in cultured eel

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Abstract

Miloxacin (5,8-dihydro-5-methoxy-8-oxo-2*H*-1,3-dioxolo-[4,5-*g*]quinoline-7-carboxylic acid) is a synthetic antibacterial agent and is regulated in conformity with the Pharmaceutical Law in Japan. The pharmacokinetics and metabolism of miloxacin after intravascular and oral administration in cultured eel (*Anguilla japonica*) were examined by using our high-performance liquid chromatography (HPLC) system, which was developed as a reliable and precise method for simultaneous determination of miloxacin and its metabolite in this study. The kinetics of miloxacin was described by a two-compartment model after intravascular administration. The distribution half-life ($T_{1/2\alpha} = 0.86$ h) of miloxacin was shorter than the elimination half-life ($T_{1/2\beta} = 34.7$ h). The kinetics of orally administered miloxacin was fitted to a one-compartment model. Miloxacin was assimilated quickly ($T_{a1/2} = 3.5$ h) and cleared slowly ($T_{1/2} = 34.7$ h) after oral dosing. The bioavailability was calculated to be 87.9%. The tissue levels of miloxacin reached their peak levels within 1 day after oral administration. At their highest levels, the concentrations of miloxacin were observed in the order of kidney > muscle > liver. Miloxacin, its main metabolite 5,8-dihydro-8-oxo-2*H*-1,3-dioxolo-[4,5-*g*]quinoline-7-carboxylic acid (M-1) and the glucuronic acid conjugate of miloxacin and M-1 were detected, and a large amount of M-1 was still observed in bile at 20 days post dosing. As an application of pharmacokinetics, we attempted to evaluate the Japanese dosage regimens of miloxacin in cultured eel. A curve for predicting miloxacin levels was obtained by a computerized calculation, using various pharmacokinetics parameters that were experimentally determined. The curve was coincident with drug levels during the actual multiple oral dosing (60 mg/kg body weight) in this experiment. The serum levels of miloxacin were maintained above the MIC (for *Edwardsiella tarda*, 0.1 μg/ml).

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However, this seems to be a considerably excessive dosing because of the high value of the average steady-state serum concentration (Css: 55.4 μg/ml).

Keywords: Pharmacokinetics; Metabolism; Eel; Miloxacin; Bioavailability

1. Introduction

Miloxacin (5,8-dihydro-5-methoxy-8-oxo-2*H*-1,3-dioxolo-[4,5-*g*]quinoline-7-carboxylic acid) which is closely related to oxolinic acid in structure, has exhibited a broad spectrum of antibacterial activity and is especially active against gram-negative bacteria. Fig. 1 shows the chemical structure of miloxacin. The drug has been used for treatment of edwardsiellosis in cultured eel and is regulated in conformity with the Pharmaceutical Law in Japan. However, there are few papers concerning miloxacin in cultured eel, although there have been some studies of miloxacin in yellowtail (Ueno et al., 1985a,b).

The present paper deals with the pharmacokinetics and metabolism of miloxacin after administration in cultured eel. Miloxacin and its metabolite 5,8-dihydro-8-oxo-2*H*-1,3-dioxolo-[4,5-*g*]quinoline-7-carboxylic acid (M-1), which also has antibacterial activity (Izawa et al., 1978), were simultaneously determined with our newly developed high-performance liquid chromatographic (HPLC) method.

2. Materials and methods

2.1. Fish

Japanese eel *Anguilla japonica* were obtained from the Fisheries Research Institute in Aichi Prefecture, Japan. The average body weight was 175 g. The fish were kept in tanks with running filtered water. The average water temperature was 27°C.

2.2. Chemicals

Miloxacin and M-1 were obtained from Sumitomo Pharmaceutical, (Osaka, Japan). β-Glucuronidase (bovine liver, 78,000 Fishman units/g) was from Tokyo Kasei Kogyo (Tokyo, Japan). Other chemicals were of analytical or HPLC grade.

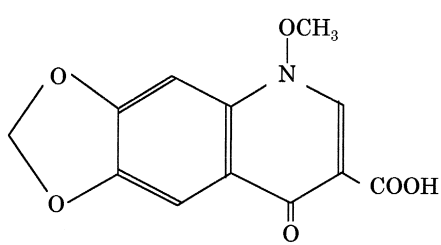

Fig. 1. Chemical structure of miloxacin.

2.3. Drug administration

Fish were anesthetized by placing them in ice-water for 5 min. For intravascular administration, miloxacin was dissolved in sterilized saline (100 mg/ml) and injected into the caudal vein at a dose of 30 mg/kg of body weight. The drug was mixed with a fish diet and was orally given to the fish by a catheter at a dosage of 60 mg/kg body weight. The drug was given either in a single dose in 1 day or in six doses over a 6-day period. Then, five fish were sampled at intervals ranging from 0.5 or 1 h to 20 days after the administration. The blood was sampled from the caudal vein with a syringe. The serum was obtained by centrifugation of the blood after storage overnight in a refrigerator and kept frozen at −40°C until analysis. Each sample was analyzed by HPLC. The muscle, liver, kidney, and bile were also collected. Samples of each type were pooled and stored at −40°C.

2.4. Assay procedure

Tissue samples (1 g muscle or 1 ml serum) were homogenized for 2 min in 30 ml of 0.1 M citrate buffer, pH 3.0: *N*,*N*-dimethylformamide (29:1) using a Physcotron (Nichi-On K.K., Tokyo, Japan). After centrifugation at 15,000 rpm for 20 min, the supernatant was transferred into a 500 ml-separatory funnel, and 5.0 g of NaCl and 15 ml of ethyl acetate were added to the solution. The funnel was then gently shaken for 5 min. After standing for a few minutes, the organic layer was pooled. The ethyl acetate treatment was repeated two more times, and then the aqueous layer was adjusted to pH 11–12 by 6 N NaOH and re-extracted twice with 15 ml of ethyl acetate. The aqueous layer was discarded. The pooled ethyl acetate layer was evaporated to dryness. The residue was dissolved in 1 ml of 1% Na_2CO_3 and the solution was injected into the HPLC.

Tissue samples (0.5 g liver or kidney, 0.3 ml bile) was homogenized and extracted by the same ethyl acetate treatment as described above. The aqueous layer was discarded. The pooled ethyl acetate layer was concentrated to ca. 30 ml in vacuo. Fifteen milliliters of saturated NaCl was added to the concentrate. The sample was shaken vigorously for 5 min and centrifuged. The aqueous layer was discarded, and the resulting organic layer was evaporated to dryness. The residue was dissolved in a mixture of 5 ml of 1% Na_2CO_3, 25 ml of 0.1 M citrate buffer, pH 3.0 and 2.0 g of NaCl. Fifteen milliliters of *n*-hexane was added to the solution. The sample was shaken vigorously for 5 min and centrifuged at 3,000 rpm for 5 min. The organic layer was discarded. The resulting aqueous layer was poured into a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA), which had previously been washed and wetted with 10 ml of methanol and 15 ml of water. The cartridge was washed with 10 ml of water, and then miloxacin was eluted with 20 ml of methanol. The eluate was evaporated to dryness, and the residue was dissolved in 1 ml of 1% Na_2CO_3 and the solution was injected into the HPLC.

The HPLC system consisted of a Gilson Model 802 pump and 311A UV detector (Gilson, France) and a Chromatopac C-R3A integrator (Shimadzu Seisakusho, Kyoto, Japan). The analytical column was a YMC-Pack C_{18} A-303 prepacked column (25 cm × 4.6 mm I.D., Yamamura Chemical Lab., Kyoto, Japan). The mobile phase was

0.1% trifluoroacetic acid: *N*,*N*-dimethylformamide:acetonitrile (72:1:27). The flow rate was 1.0 ml/min, and the UV detector was set at 254 nm. The injection volume was 20 μl. The column temperature was 30°C.

A standard solution containing of 100 μg/ml miloxacin and 100 μg/ml of M-1 was prepared in 1% Na_2CO_3. The solution was diluted to the required concentration with 1% Na_2CO_3 before use.

2.5. Pharmacokinetic analysis

The most common method of pharmacokinetic evaluation is to assume that the drug concentration-time data can be described by one of several compartment models and to fit the data to an equation consistent with the assumed model using a non-linear least-squares regression. In our study, a pharmacokinetic analysis was applied assuming a one- or two-compartment model using the non-linear least-squares program MULTI (Yamaoka et al., 1981). Selection of models was judged by Akaike's information criterion (Yamaoka et al., 1978).

Wagner and Nelson (1964) reported that the drug absorption rate could be calculated from serum level vs. time data using the following equation when the behavior of the drug is expressed by a one-compartment model:

$$\text{Fraction absorbed} = \frac{A_t}{A_\infty} = \frac{C_t + \text{Ke}\int_0^t C\,\mathrm{d}t}{\text{Ke}\int_0^\infty C\,\mathrm{d}t}$$

where A_t is the cumulative amount of the drug absorbed up to time t, A_∞ is the amount of drug ultimately absorbed. C_t is the concentration at time t, and Ke is the first-order elimination rate constant (the value for the drug following intravascular administration).

This equation relates the cumulative amount of drug absorbed after a certain time to the amount of drug ultimately absorbed, rather than to the dose administered.

2.6. Consecutive oral administration

The serum level during multiple oral dosing of a constant dose (Cn) and average steady-state serum concentration (Css) can be estimated according to the formula:

$$\text{Cn} = \frac{F \cdot \text{Dose} \cdot \text{Ka}}{\text{Vd}(\text{Ka} - \text{Ke})}\left[\frac{1 - \mathrm{e}^{(-n \cdot \text{Ke} \cdot \tau)}}{1 - \mathrm{e}^{(-\text{Ke} \cdot \tau)}}\mathrm{e}^{(-\text{Ke} \cdot t)} - \frac{1 - \mathrm{e}^{(-n \cdot \text{Ka} \cdot \tau)}}{1 - \mathrm{e}^{(-\text{Ka} \cdot \tau)}}\mathrm{e}^{(-\text{Ka} \cdot t)}\right]$$

$$\text{Css} = \frac{F \cdot \text{Dose}}{\text{Vd} \cdot \text{Ke} \cdot \tau}$$

where F is the bioavailability, Ka is the first-order absorption rate constant, Vd is the apparent volume of distribution, τ is the dosage interval, n is the dosage time, and Ke is the first-order elimination rate constant (the value for the drug following oral administration).

2.7. Statistical moment analysis

The area under the concentration-time curve (AUC) was calculated by using the trapezoid rule including the terminal portion. The mean residence time (MRT) of the drug was obtained by a non-compartment analysis based on the statistical moment theory (Yamaoka and Tanigawara, 1983).

The bioavailability was calculated from the following equation:

$$F(\%) = \frac{\mathrm{AUC}_{\mathrm{p.o.}} \cdot \mathrm{dose}_{\mathrm{i.v.}}}{\mathrm{AUC}_{\mathrm{i.v.}} \cdot \mathrm{dose}_{\mathrm{p.o.}}} 100$$

where p.o. represents the oral administration, and i.v. represents the intravascular administration.

2.8. Presence of glucuronide conjugate

Some of the residues that were previously prepared for miloxacin and M-1 extraction were used for determination of glucuronide conjugates. These residues were from samples that were taken at the time of maximum serum concentration of miloxacin ($T_{\max}$) and at 20 days post dosing. Each residue was dissolved in 100 ml of 0.1 M citrate buffer, pH 3.0, and homogenized for 1 min. Five milligrams of β-glucuronidase and 1 ml of toluene were added to the homogenate as an antiseptic. The mixture was incubated at 37°C overnight. After incubation, the reaction mixture was concentrated to ca. 30 ml in vacuo. The released miloxacin and M-1 were re-extracted from the concentrate as described in the text.

3. Results

3.1. Examination of analytical procedures

Table 1 shows a comparison of the extractions of miloxacin and M-1 from eel muscle using different solvents. The recoveries of miloxacin and M-1 were 85% and 76%, respectively, for a mixture of 0.1 M citric acid, pH 3.0, and dimethylformamide (29:1).

Table 1
Comparison of extracting solution (citrate buffer, pH 3.0: *N*,*N*-dimethylformamide) for miloxacin and M-1 from muscle of eel

Extracting solution (ml) 0.1 M citrate buffer: *N*,*N*-dimethylformamide		Recovery (%)	
		Miloxacin	M-1
30	0	58.5	56.8
29.5	0.5	88.3	68.1
29	1	84.5	75.6
25	5	58.1	47.1

Table 2
Effect of NaCl concentration in the extracting solution on the recovery of miloxacin and M-1

NaCl (g)	Recovery (%)	
	Miloxacin	M-1
0	89.8	78.4
2	94.7	77.5
5	93.5	86.4

As shown in Table 2, the recoveries of both drugs increased by the addition of NaCl to the solvent. The addition of NaCl had an effect on the partition of water and organic solvent.

The presence of miloxacin and M-1 in the liver, kidney, and bile could not be determined because of the presence of undesirable peaks in the chromatogram, even when the above procedure was used. Therefore, we attempted to further clean up the internal organ samples prepared with the ethyl acetate treatment by using a hexane treatment (for defatting) and a Sep-Pak C_{18} cartridge. These treatments were effective in eliminating interfering substances from liver, kidney, and bile (unpublished data). Finally, the analytical procedure was done as described above.

Miloxacin and M-1 were added to various tissues at a concentration of 2 μg/ml or g, and then the recovery rates were determined (Table 3). The recoveries and coefficients of variation of miloxacin and M-1 were 81–94% and 1.9–8.0% and 79–89% and 1.2–6.6%, respectively. The detection limits of miloxacin and M-1 at a signal-to-noise ration of 3 were 0.03 μg/ml or g for serum and muscle, 0.06 μg/g for liver and kidney, and 0.1 μg/ml for bile. The data reported in this study were not corrected for recovery. Fig. 2 shows typical chromatograms of miloxacin and M-1 obtained from various tissues in eel.

3.2. Pharmacokinetic analysis

3.2.1. Intravascular and oral administration

Fig. 3 shows the serum level vs. time plots of miloxacin after intravascular and oral administrations.

Table 3
Recovery of miloxacin and M-1 from various tissues of eel

Tissues	Recovery (%)	
	Miloxacin	M-1
Serum	94.4 (5.2)	79.4 (6.0)
Muscle	90.3 (4.3)	86.7 (6.6)
Liver	86.1 (2.4)	89.1 (1.7)
Kidney	81.0 (8.0)	79.8 (4.3)
Bile	90.6 (1.9)	83.9 (1.2)

The parentheses show the number of coefficients of variation.

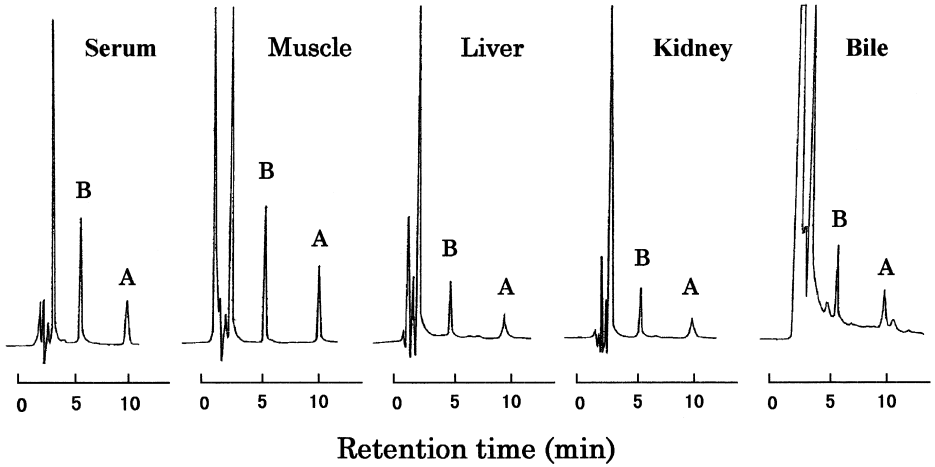

Fig. 2. Chromatograms of eel tissues spiked with miloxacin (A) and its metabolite (B).

When miloxacin was administered intravascularly, the serum concentration reached T_{max} (43.9 mg/ml) just after dosing, and then decreased gradually to 0.69 mg/ml at 12 days. The concentration-time profiles showed a sharp distribution phase (α phase)

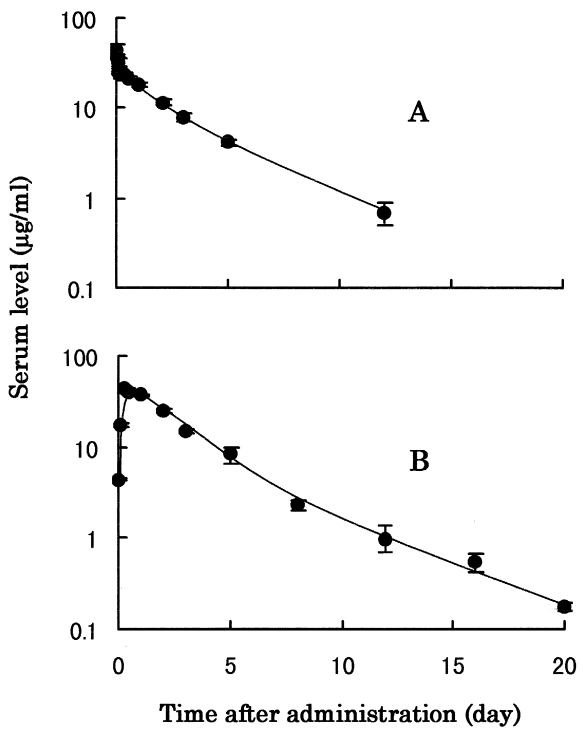

Fig. 3. Serum level vs. time plots of miloxacin after intravascular (A) and oral (B) administration. Symbols indicate the mean and standard deviations for four fish. The serum level scale is logarithmic.

Table 4
Pharmacokinetic parameters for miloxacin following intravascular administration

Parameters	
Dose (mg/kg)	30
Weight (g)	170
Water temperature (°C)	27
α (h^{-1})	0.81
β (h^{-1})	0.02
Ke (h^{-1})	0.03
K_{12} (h^{-1})	0.41
K_{21} (h^{-1})	0.39
$T_{1/2\alpha}$ (h)	0.86
$T_{1/2\beta}$ (h)	34.7
AUC ($\mu g \cdot h/ml$)	1860
MRT (h)	70.9
ClB (ml/kg/h)	16.1
Vss (l/kg)	0.81
Vc (l/kg)	0.56

within 5 h and a mild elimination phase (β phase) from 8 h to 12 days after dosing. The concentration-time profile could adequately be described by a two-compartment model.

$$C_t = 28.5[\exp(-0.81t)] + 25.0[\exp(-0.02t)]$$

The obtained pharmacokinetic parameters of miloxacin are shown in Table 4.

When miloxacin was administered orally, as shown in Fig. 3, T_{max} (43.4 μg/ml) was obtained at 6 h after dosing. Even at 20 days after dosing, miloxacin (0.18 μg/ml) was still measurable. The concentration-time profile could adequately be described by a one-compartment model with first-order absorption.

$$C_t = 57.5[\exp(-0.02t) - \exp(-0.20t)]$$

The obtained pharmacokinetic parameters of miloxacin are shown in Table 5.

Table 5
Pharmacokinetic parameters for miloxacin following oral administration

Parameters	
Dose (mg/kg)	60
Weight (g)	180
Water temperature (°C)	27
Ka (h^{-1})	0.2
Ke (h^{-1})	0.02
$T_{a1/2}$ (h)	3.47
$T_{1/2}$ (h)	34.7
AUC ($\mu g \cdot h/ml$)	3271
MRT (h)	76.8

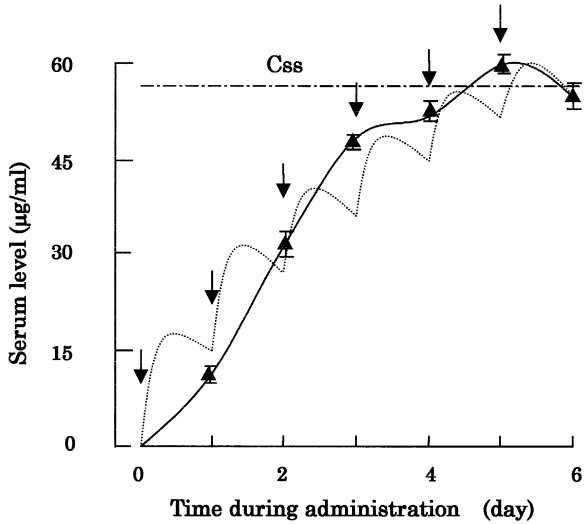

Fig. 4. Serum level of miloxacin in eel during 6-days consecutive oral administration at a dose of 60 mg/kg. ——: Serum level during the multiple posing (Symbols indicate the mean and standard deviations for four fish.). ------: A predicted curve. The arrow: time of dosing, –·–·–: Css (Average steady-state serum concentration).

3.3. Consecutive oral administration

Fig. 4 shows the serum level of miloxacin after 6 days of consecutive oral administration. A curve for predicting miloxacin levels was obtained by a computerized calculation, using various pharmacokinetic parameters that were obtained experimen-

Table 6
Tissue levels of miloxacin in eel after oral administration at a dose of 60 mg/kg

Time after administration	Tissues			
	Muscle	Liver	Kidney	Bile
1 h	0.48	3.62	1.73	34.7
3 h	2.67	4.91	20.6	29
6 h	3.35	4.32	8.43	24.8
9 h	4.99	4.97	7.4	25
12 h	16.3	23	22.3	108
1 day	28.4	24.8	37.3	390
2 days	18.5	9.19	11.5	330
3 days	12.5	8.28	11.9	161
5 days	5.49	3.12	3.61	213
8 days	2.33	1.2	1.14	177
12 days	0.83	0.43	0.56	52.6
16 days	0.08	ND	ND	0.35
20 days	0.07	ND	ND	0.25

The unit of the experimental number is μg/ml or g. ND: not detected.

Table 7
Tissue levels of M-1 in eel after oral administration at a dose of 60 mg/kg

Time after administration	Tissues			
	Muscle	Liver	Kidney	Bile
1 h	ND	3.39	0.45	1.52
3 h	0.03	6.01	3.81	2.64
6 h	0.03	5.31	2.45	12.5
9 h	0.06	2.77	3.17	20.4
12 h	0.08	12	7.63	40.3
1 day	0.26	7.66	9.22	236
2 days	0.15	7.32	11	289
3 days	0.09	4.89	3.39	127
5 days	0.05	3.29	3.05	292
8 days	0.03	1.09	1.89	179
12 days	ND	0.46	0.5	128
16 days	ND	ND	ND	1.22
20 days	ND	ND	ND	1.01

The unit of the experimental number is μg/ml or g. ND: not detected.

tally. The curve was coincident with drug levels during the actual multiple oral dosing (60 mg/kg body weight) in this experiment. The serum level of miloxacin was sharply increased at every dosing, and T_{max} (51.4 μg/ml) was observed on the 6th day during oral dosing.

Table 8
Level of conjugated and non-conjugated miloxacin in eel after oral administration

Tissues	Time	Miloxacin	Glu–Miloxacin	M-1	Glu–M-1
Serum	Cmax	39.2	0.14	2.8	0.32
		92.3%	0.3%	6.6%	0.8%
	20 days	ND	ND	ND	0.11
Muscle	Cmax	28.4	1.51	0.26	1.63
		89.3%	4.7%	0.8%	5.1%
	20 days	0.07	ND	ND	0.2
Liver	Cmax	24.8	1.12	7.66	2.25
		69.2%	3.1%	21.4%	6.3%
	20 days	ND	ND	ND	2.1
Kidney	Cmax	37.3	2.41	9.22	2.34
		72.8%	4.7%	18.0%	4.6%
	20 days	ND	ND	ND	0.32
Bile	Cmax	390	1056	236	123
		21.6%	58.5%	13.1%	6.8%
	20 days	0.25	0.36	1.01	0.34

The unit of the experimental number is μg/ml or g. The percentage shows the ratio of miloxacin and its glucuronide at Cmax. Cmax: maximum concentration of miloxacin post dosing. ND: not detected. Glu-: glucuronide conjugate.

3.4. Tissue distribution of miloxacin after oral administration

Table 6 shows the tissue level of miloxacin in eel after oral administration. Miloxacin was detected in the non-alimentary tissues of all fish within 1 h after administration of an oral dose. The tissue levels of miloxacin reached their peak levels within 1 day after administration. At their highest levels, the concentrations of miloxacin were observed in the order of kidney (37.3 μg/g) > muscle (28.4 μg/g) > liver (24.8 μg/g). A considerable amount of miloxacin (390 μg/ml) was observed in the bile. The tissue levels of M-1 in eel after oral administration were examined (Table 7). The peak levels of M-1 were observed in all tissues between 1 and 2 days post dosing. The bile levels remained above 100 μg/ml for up to 12 days.

3.5. Metabolites of miloxacin

Table 8 shows the levels of conjugated and non-conjugated miloxacin after oral administration in eel. Miloxacin, M-1 and their glucuronic acid conjugates were detected, and M-1 was still observed in bile at 20 days post dosing. At the T_{max}, the percentage of miloxacin that was in miloxacin and its metabolites was high in serum (92%) and muscle (89%). However, a high concentration of the glucuronic acid conjugate of miloxacin was observed in the bile.

4. Discussion

Only one previous study has investigated the assay of miloxacin and M-1 in fish by HPLC (Ueno et al., 1985a). In that study, we reported that these drugs could not be detected in internal organs such as liver, kidney, and gall bladder because of the presence of interfering substances, and that M-1 showed very low recovery (11–22%) from various tissues in fish. Thus, the present study was conducted to develop an analytical procedure that detects miloxacin and M-1 even in the internal organs of fish. As the preceding results show, we have developed a selective, reliable, and precise method for the simultaneous determination of miloxacin and its metabolite in cultured eel tissues.

We showed that the pharmacokinetics of miloxacin after intravascular administration in eel could be described by a two-compartment model. Oxolinic acid and nalidixic acid are closely related quinolones that are structurally similar to miloxacin. Björklund and Bylund (1991) and Kleinow et al. (1994) reported a two-compartment model for oxolinic acid after intravascular administration in rainbow trout. Our previous paper (Uno et al., 1996) also reported a two-compartment model for nalidixic acid in rainbow trout.

In eel after intravascular administration, the half-life for serum distribution ($T_{1/2\alpha}$) was 0.86 h, and the half-life for elimination ($T_{1/2\beta}$) was 34.7 h. For comparison, rainbow trout, after given a dose of oxolinic acid, exhibited $T_{1/2\alpha}$ and $T_{1/2\beta}$ values of 0.15 and 81.3 h (Kleinow et al., 1994) and 0.31 and 69.7 h (Björklund and Bylund, 1991), respectively. That is, the rate of absorption of oxolinic acid in rainbow trout is faster than the rate of absorption of miloxacin in eel, and the rate of elimination of

oxolinic acid in rainbow trout is slower than the rate of elimination of miloxacin in eel. However, Uno et al. (1996) reported that, in rainbow trout, the absorption of nalidixic acid ($T_{1/2a} = 1.35$ h) was slower than the absorption of oxolinic acid and miloxacin, and that the elimination of nalidixic acid ($T_{1/2b} = 12.8$ h) was faster than the elimination of oxolinic acid and miloxacin.

An apparent steady-state distribution (Vss: 0.81 l/kg) was found for miloxacin in eel. A similar Vss value (1.01 l/kg) for nalidixic acid was obtained in rainbow trout (Uno et al., 1996). In rainbow trout, the Vss for oxolinic acid was two times higher than that for miloxacin (Björklund and Bylund, 1991; Kleinow et al., 1994). The total body clearance (Cl_B), defined as the total volume in the body (including blood, kidney, and liver) that is completely cleared of a drug per unit time, is an important parameter for characterizing drug disposition. The Cl_B of miloxacin was similar to that of oxolinic acid in rainbow trout (16.9 and 20.2 ml/kg/h) as reported by Björklund and Bylund (1991) and Kleinow et al. (1994). The clearance time for miloxacin from the body tissues in eel, obtained from Cl_B/Vss, was 50 h. In rainbow trout, the clearance time for oxolinic acid, calculated from the data of Björklund and Bylund (1991), was 94 h. Similarly, using the data of Kleinow et al. (1994) and Uno et al. (1996), the clearance times of oxolinic and nalidixic acids were calculated to be 106.5 and 18.4 h, respectively. Therefore, among members of the quinolone group, nalidixic acid has the fastest terminal elimination rate, though there are some differences among fish species.

The pharmacokinetics of miloxacin in eel after oral administration was described by a one-compartment model with first order absorption. Our previous papers have reported one-compartment models with first order absorption for nalidixic acid in rainbow trout (Uno et al., 1992b).

The bioavailability of miloxacin following oral administration to eel was calculated to be 87.9%. Although there are no published bioavailability values for miloxacin in fish, our values in eel are considerably higher than those previously reported for oxolinic acid (13.6–38.1%) (Björklund and Bylund, 1991; Cravedi et al., 1987). The bioavailability of nalidixic acid was reported to be 89.6% in rainbow trout (Uno et al., 1996).

The mean absorption time (MAT), defined as $MRT_{p.o.} - MRT_{i.v.}$, was 5.9 h. The time required for drug absorption (TDA) is defined as the time for absorption to reach 90% of the maximum level. TDA, calculated by the Wagner–Nelson method, was 8 h. For comparison, the TDAs for nalidixic acid and oxolinic acid in rainbow trout were found to be 120 and 144 h, respectively (unpublished data). Thus, miloxacin is absorbed in eel much more quickly than are nalidixic acid and oxolinic acid in rainbow trout.

Several drugs have been shown to be rapidly assimilated in the stomach in fish (Ueno et al., 1988a,b, 1995; Droy et al., 1990; Uno et al., 1992a,b, 1993). In yellowtail, the peak levels of miloxacin in all tissues were attained at 1–3 h post dosing, and at that time, the concentrations of miloxacin were observed in the order of liver (1.48 μg/ml) > muscle (1.13 μg/g) > serum (0.66 μg/g) (Ueno et al., 1985a,b). Miloxacin is much less available and more quickly assimilated in yellowtail than in eel.

In mammals, miloxacin is biotransformed to at least seven metabolites: M-1, 1,4-dihydro-7-hydroxy-1,6-dimethoxy-4-oxoquinoline-3-carboxylic acid (M-2), 1,4-dihydro-6,7-dihydroxy-4-oxoquinoline-3-carboxylic acid (M-3), and the glucuronides of miloxacin, Ms-1, -2, and -3 (Yoshitake et al., 1978a,b,c, 1979, 1980). M-1 and the

glucuronides of miloxacin and M-1 were observed in yellowtail (Ueno et al., 1985a,b), and the same metabolites were also found in eel in the present study. The M-1 level was much higher in the internal organs, especially the gall bladder (i.e., bile). The metabolism of miloxacin seems to be less complex in fish than in mammals.

As an application of pharmacokinetic studies, we attempted to evaluate the Japanese dosage regimens of miloxacin in cultured eel. As shown in Fig. 4, the serum levels of miloxacin were maintained above the MIC (for *Edwardsiella tarda*, 0.1 μg/ml; Ito, 1978). However, this seems to be an excessive dose because of the high value of Css (55.4 μg/ml). An excessive dose not only raises costs for fish farms, but also causes environmental pollution and outbreaks of drug-resistant bacteria (Björklund et al., 1990, 1991). Further investigations are needed to compare these theoretical calculations with results obtained under actual conditions of the dosage regimens. Such studies should include determinations of the rate of diffusion of feed into the pond water, the amount of residual feed, the compounding ratio of feed, the chemical types of drugs and water temperature used in the fish farms.

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