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Absorption, distribution and excretion of ^{14}C-levofloxacin after single oral administration in albino and pigmented rats: binding characteristics of levofloxacin-related radioactivity to melanin in vivo

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Abstract

After single oral administration of ^{14}C-levofloxacin at a dose of 20 mg kg^{-1} under non-fasting conditions, the absorption, distribution and excretion of radioactivity were studied in albino and pigmented rats. Good penetration of radioactivity into tissues was indicated by higher concentrations in most tissues compared with serum and there were no quantitative differences in the distribution of radioactivity between albino and pigmented rats except for melanin-containing tissues such as the uveal tract of eyes and hair follicles. There was selective and strong binding of drug-related radioactivity to these tissues in pigmented rats. The uveal tract concentrations reached the maximum value (C_{max}) of 26.33 ± 0.75 μg eq. g^{-1} at 24 h after dosing and declined slowly with a terminal half-life of 468.1 h (19.5 days). The uveal tract concentration at 12 weeks was 0.73 ± 0.12 μg eq. g^{-1}, which is *c.* 1/36 of C_{max}. The $AUC_{0-\infty}$ for the uveal tract was 12.58 mg h^{-1} g^{-1}. The uveal tracts separated from one eye of each rat were extracted with 0.067 M phosphate buffer (pH 7.4) and 1 M HCl/EtOH (30:70), successively. In pigmented rats, approximately 85–48% of radioactivity bound to the uveal tract was released from the tissue by the washing procedures. Most of the eluted radioactivity was released with 1 M HCl/EtOH (30:70), indicating that the binding to melanin is reversible, and hydrophobic and electrostatic interactions play an important role in the binding of levofloxacin and/or its metabolites with melanin-containing ocular tissues. Only unchanged drug was detected in the extracts of the uveal tracts. The concentrations and half-life of radioactivity in the uveal tract after dosing of ^{14}C-levofloxacin were found to be much lower and shorter than those after dosing of ^{14}C-chloroquine. It is unlikely that levofloxacin causes toxicity because of its much lower affinity to melanin-containing ocular tissues and shorter duration of therapy compared to chloroquine.

Introduction

Fluoroquinolones represent a major class of antibacterials that have great therapeutic potential. The modification of the first-generation quinolones, such as nalidixic acid, has led to a considerable increase in their intrinsic antibacterial activity. The pharmacokinetic properties of these new fluoroquinolones are characterized by excellent oral bioavailability, extensive tissue penetration, low protein binding and a long elimination half-life. Levofloxacin is a fluoroquinolone antibiotic that is the active *S*-(–)-isomer of ofloxacin and demonstrates a broad range of activity against Gram-positive and -negative organisms and anaerobes (Hurst et al 2002).

Chloroquine is an antimalarial agent that is also used in the management of systemic lupus erythematosus and rheumatoid arthritis. Chloroquine is associated with benign corneal deposits and pigmentary retinopathy, which can lead to decreased visual acuity, visual fields and colour-vision defects as well as electroretinogram and electro-oculogram abnormalities (Jones 1999). Chloroquine has been shown to have distinct affinity for melanin-containing ocular tissues in pigmented animals (Lindquist & Ullberg 1972; Kasuya et al 1976). Several tissues, such as iris, skin, hair, inner ear and substantia nigra, contain melanin. The observation that long-term, high-dose chloroquine therapy produced chorioretinopathy (Hobbs et al 1959) has led to the awareness of an association

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between the toxic effects of some drugs and its high affinity for melanin. Since these early observations, melanin binding of drugs has been implicated not only in ocular toxicity but also in ototoxicity and pigment disturbances of skin and hair (Ings 1984; Larsson 1993; Salazar-Bookaman et al 1994). The drug accumulation in these pigmented tissues is of considerable interest not only from the pharmacological point of view but also toxicologically.

It has been reported that electrostatic forces play an important role in the binding of drugs to melanin. However, non-electrostatic contributions, including hydrophobic and van der Waals' interactions and charge-transfer reactions, also contribute to the binding for chloroquine (Larsson & Tjälve 1979; Tjälve et al 1981; Stepien & Wilczok 1982).

We have previously reported the in-vitro binding characteristics of fluoroquinolones and chloroquine to synthetic melanin (Ono & Tanaka 2003). We have also reported the binding characteristics of chloroquine-related radioactivity to melanin-containing tissues such as the uveal tract in albino and pigmented rats after a single oral dosing of ^{14}C-chloroquine (Ono et al 2003). It was suggested that the binding mechanisms of chloroquine to melanin in vitro and those of chloroquine-related radioactivity to melanin containing tissue in vivo were similar even 24 weeks after dosing. The bindings were found to be not covalent but reversible and hydrophobic and/or electrostatic interaction plays a critical role in the melanin binding. To date, however, the nature of the interaction between melanin and fluoroquinolones still has not been fully characterized in vivo.

The objectives of this study were to investigate the differences in absorption, distribution and excretion after a single oral dosing of ^{14}C-levofloxacin between albino and pigmented rats and to define its in-vivo binding characteristics to melanin-containing ocular tissues. The ocular pharmacokinetics of ^{14}C-levofloxacin were compared with those of ^{14}C-chloroquine (Ono et al 2003).

Materials and Methods

Test materials

^{14}C-levofloxacin hemihydrate (Figure 1) with a specific activity of 2.88 MBq mg^{-1} was radiosynthesized at Daiichi Pure Chemicals Co. Ltd (Ibaraki, Japan). A radiochemical purity of 97.4% was established by TLC. Non-radiolabelled levofloxacin hemihydrate, levofloxacin *N*-oxide and desmethyl levofloxacin were synthesized by Daiichi Pharmaceutical Co. Ltd (Tokyo, Japan). All other reagents were of analytical grade and used without further purification.

Animals

The albino and pigmented animals used in this study were male SD:SLC rats (weighing 300–370 g, aged 7 weeks, n = 4 per time point; purchased from Japan SLC Inc., Shizuoka, Japan) and male BN/Crj rats (weighing 200–245 g, aged 7 weeks, n = 4 per time point; purchased from Charles River Japan Inc., Kanagawa, Japan), respectively. The animals were adapted to the laboratory conditions before the study at a temperature of 23 ± 2 °C and 55 ± 15% humidity. Animals were housed in stainless-steel cages, except for those in the excretion studies, which were housed singly in metabolism cages. Solid laboratory diet (F-2, Funabashi Farm Co. Ltd, Chiba, Japan) and tap water were available to all animals ad lib throughout the course of the study. All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daiichi Pharmaceutical Co. Ltd (Tokyo, Japan).

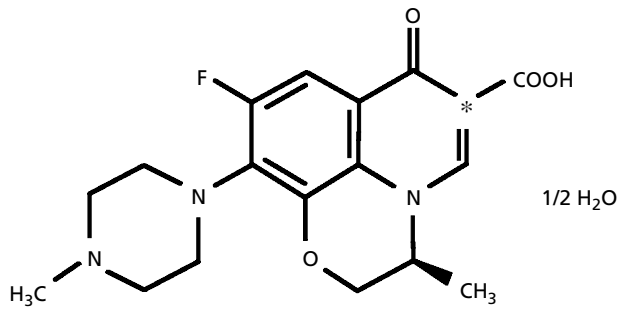

Figure 1 Chemical structure of ^{14}C-levofloxacin hemihydrate (*, ^{14}C-labelled position).

Dose formulation and administration

^{14}C-labelled and non-labelled levofloxacin hemihydrate were dissolved in distilled water to achieve 20 mg per 5 mL. The specific activity of the dosing solution was 213.4 KBq mg^{-1}. The dosing volume administered was 5 mL kg^{-1}. The dosing solution was given to rats via oral gavage.

Specimen collection

For collection of blood, tissue samples and excreta, seven groups of four male albino or pigmented rats received a single oral dose of ^{14}C-levofloxacin hemihydrate at a dose of 20 mg kg^{-1}. The animals were killed at 1 h, 24 h, 1, 2, 4, 8 and 12 weeks after oral dosing by ether inhalation and exsanguinated following incision of a carotid artery. Serum was obtained by centrifugation of blood samples. Liver, kidney, heart, lung, brain, skin, Harderian gland, hair and eyes were removed and weighed immediately. Eyes were dissected and the corpus vitreum and uveal tract were collected. The urine samples were collected at 0–8, 8–24, 24–48, 48–72 and 72–144 h after administration, and the feces were collected at 0–24, 24–48, 48–72 and 72–144 h after administration.

Radioactivity analysis

The measurement of the radioactivity concentrations in the blood, tissues and feces was conducted using the solubilization method. To each sample, 2 mL of a tissue solubilizer (Soluene-350, Packard Instrument Company, CT)

was added, and the resulting mixture was heated at 55 °C overnight. After solubilization, 0.4 mL of a toluene solution saturated with benzoyl peroxide was added and heated at 55 °C to bleach the samples. These samples were mixed with 12 mL of a liquid scintillator (Hionic-fluor, Packard Instrument Company, CT). Feces were prepared for scintillation counting by homogenization with water using a Polytron homogenizer (Brinkmann Co., Lucerne, Switzerland). The volume of total sample was measured and the aliquot of the homogenate (0.5 mL) was used for radioactivity analysis.

Serum and urine (0.2 mL) were mixed with 12 mL of a liquid scintillator (Aquasol, NEN Life Sciences, MA) and measured for radioactivity. Radioactivity was measured with a liquid scintillation counter (LSC700 or 900, Aloka Co. Ltd, Tokyo, Japan) using an external standard method.

Extraction study

The elution of radioactivity from the uveal tract separated from one eye of each rat was determined by extracting the tissue twice with 0.067 M phosphate buffer (pH 7.4, 1 mL) at 37 °C for 1 h, once with 0.067 M phosphate buffer (pH 7.4, 1 mL) at 37 °C for 16 h and then twice with 1 M HCl/EtOH (30:70, 1 mL) at 37 °C for 1 h. The supernatant concentrations of radioactivity eluted were determined by liquid scintillation counting after centrifugation at 11 000 rpm (*c.* 14 000 *g*) for 15 min. The radioactivity in the resulting tissue was measured using the solubilization method described above. The sum of the radioactivity in the supernatants and the resulting tissue was taken to be the total radioactivity (100%) in the uveal tract. The percentage of radioactivity eluted from the tissue was calculated for each extraction.

Metabolic pattern in the uveal tract

The uveal tract extracts were analysed by HPLC using a modification of the previously reported method to detect levofloxacin and/or metabolites (Tanaka et al 1993). The high-performance liquid chromatograph (Model 655A-11, Hitachi, Tokyo, Japan) was equipped with a variable-wavelength UV detector (Model 655A, Hitachi). The wavelengths were set at 295 nm. Separation was achieved on a reversed-phase column (TSK-GEL ODS-80 TM, 5 μm, 150 × 4.6 mm i.d., Tosoh, Tokyo, Japan) using a mobile phase consisting of tetrahydrofuran/50 mM phosphate buffer (pH 2.0)/1 M ammonium acetate (8:92:1, v/v) at a flow rate of 1 mL min^{-1}. Under these analytical conditions, levofloxacin was well separated from two major metabolites, levofloxacin *N*-oxide and demethyl levofloxacin.

Data analysis

The concentrations of radioactivity were expressed as microgram-equivalents (μg eq.) of levofloxacin hemihydrate per millilitre of fluid or gram of tissue. The net disintegration per minute (dpm) was determined as the dpm minus the background dpm. Samples having a net dpm less than the background value were considered to contain an amount of radioactivity below the limit of quantification (LOQ). The radioactivity in urine and feces was expressed as a percentage of the administered dose.

The radioactivity concentrations in the uveal tract vs time data in pigmented rats were analysed for non-compartmental methods using a SAG-CP software package (ASMedica, Osaka, Japan). The terminal phase rate constant (λ_z) was determined by least-square regression of the logarithm of concentration on time over the terminal phase. The terminal half-life was calculated to be $0.693/\lambda_z$. The area under the concentration–time curve (AUC) was determined to the last quantifiable concentration using the linear trapezoidal rule and extrapolated to infinity using the terminal phase rate constant.

Results

Radioactivity concentrations

The radioactivity concentrations in blood, serum and tissues after a single oral administration of ^{14}C-levofloxacin hemihydrate to albino and pigmented rats are shown in Tables 1 and 2, respectively.

In albino rats, the serum concentrations at 1 and 24 h after dosing were 2.91 ± 0.16 and 0.04 ± 0.01 μg eq. mL^{-1}, respectively. The serum concentrations were below LOQ thereafter. At 1 h after dosing, liver, kidney and heart showed higher concentrations than serum and the brain concentration was 0.22 ± 0.02 μg eq. g^{-1}, which is *c.* 1/13 of the serum level, indicating that ^{14}C-levofloxacin penetrated to the brain to a limited extent. The mean peak tissue concentrations are ranked as follows: kidney > liver > heart > serum > lung > skin > blood > Harderian gland > uveal tract > corpus vitreum > hair > brain.

In pigmented rats, serum concentrations at 1 and 24 h after dosing were 4.86 ± 0.16 and 0.07 ± 0.02 μg eq. mL^{-1}, respectively. The serum concentrations were below LOQ thereafter. At 1 h after dosing, the liver, kidney, heart and uveal tract showed higher concentrations than serum. The radioactivity concentrations in the uveal tract and hair reached maximum values at 24 h and 1 week after dosing, respectively. The brain concentration was 0.49 ± 0.03 μg eq. g^{-1}, which is *c.* 1/10 of the serum level, indicating that ^{14}C-levofloxacin penetrates to the brain to a limited extent. The main peak tissue concentrations are ranked as follows: uveal tract > kidney > liver > heart > serum > lung > hair > blood > skin > Harderian gland > brain > corpus vitreum. The uveal tract radioactivity concentrations are *c.* 376 times higher than those in serum at 24 h after dosing.

After oral administration to pigmented rats, the tissue distribution patterns were similar to those seen in albino rats. However, ^{14}C-levofloxacin showed a high affinity for melanin-containing ocular tissues. The radioactivity concentrations in uveal tract vs time profiles in albino and pigmented rats are shown in Figure 2. In pigmented rats, the uveal tract concentration at 1 h after oral dosing was 19.87 ± 2.09 and reached the maximum value (C_{max}) of 26.33 ± 0.75 μg eq. g^{-1} at 24 h. After C_{max} was reached, the concentrations declined slowly with a terminal half-life of

Table 1 Tissue concentrations of radioactivity after a single oral administration of ^{14}C-levofloxacin to albino rats at a dose of 20 mg kg^{-1}.

Tissue	Concentration (μg eq. levofloxacin g^{-1} or mL^{-1})						
	Time after administration (weeks)						
	0.006 (1 h)	0.143 (24 h)	1	2	4	8	12
Whole blood	2.28 ± 0.12	0.22 ± 0.09	0.14 ± 0.01	0.18 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	NT
Serum	2.91 ± 0.16	0.04 ± 0.01	ND	NT	NT	NT	NT
Liver	9.98 ± 0.45	0.12 ± 0.02	0.01 ± 0.00	NT	ND	ND	NT
Kidney	10.83 ± 0.95	0.18 ± 0.02	0.03 ± 0.00	ND	ND	ND	NT
Heart	3.01 ± 0.16	0.04 ± 0.00	0.00 ± 0.00	NT	ND	ND	NT
Lung	2.84 ± 0.15	0.03 ± 0.01	ND	NT	ND	ND	NT
Brain	0.22 ± 0.02	ND	ND	NT	NT	NT	NT
Skin	2.44 ± 0.11	0.13 ± 0.02	0.02 ± 0.01	ND	ND	ND	NT
Harderian gland	1.60 ± 0.06	0.01 ± 0.01	ND	NT	NT	NT	NT
Hair	0.25 ± 0.11	0.87 ± 0.01	0.48 ± 0.07	0.24 ± 0.00	0.42 ± 0.04	0.38 ± 0.23	NT
Corpus vitreum	0.46 ± 0.03	0.01 ± 0.01	ND	ND	ND	ND	NT
Uveal tract	1.38 ± 0.11	ND	ND	ND	ND	ND	ND

ND, not detectable, i.e. < LOQ (below limit of quantification); NT, not tested. Each value represents the mean ± s.e.m. for four rats.

Table 2 Tissue concentrations of radioactivity after a single oral administration of ^{14}C-levofloxacin to pigmented rats at a dose of 20 mg kg^{-1}.

Tissue	Concentration (μg eq. levofloxacin g^{-1} or mL^{-1})						
	Time after administration (weeks)						
	0.006 (1 h)	0.143 (24 h)	1	2	4	8	12
Whole blood	3.61 ± 0.03	0.39 ± 0.03	0.18 ± 0.03	0.19 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	ND
Serum	4.86 ± 0.16	0.07 ± 0.02	ND	NT	NT	NT	NT
Liver	11.75 ± 0.43	0.14 ± 0.03	0.01 ± 0.00	NT	ND	ND	NT
Kidney	19.62 ± 1.41	0.31 ± 0.08	0.03 ± 0.00	ND	ND	0.00 ± 0.00	NT
Heart	5.09 ± 0.13	0.06 ± 0.02	ND	NT	ND	0.00 ± 0.00	NT
Lung	4.75 ± 0.27	0.06 ± 0.01	ND	NT	ND	ND	NT
Brain	0.49 ± 0.03	ND	ND	NT	NT	NT	NT
Skin	3.58 ± 0.09	1.11 ± 0.55	0.14 ± 0.05	ND	ND	0.00 ± 0.00	NT
Harderian gland	3.47 ± 0.13	0.05 ± 0.01	ND	NT	NT	NT	NT
Hair	0.32 ± 0.05	2.67 ± 0.18	4.69 ± 2.66	0.53 ± 0.10	1.59 ± 0.71	2.65 ± 1.05	2.02 ± 1.62
Corpus vitreum	0.42 ± 0.03	0.12 ± 0.01	0.04 ± 0.00	ND	ND	0.00 ± 0.00	NT
Uveal tract	19.87 ± 2.09	26.33 ± 0.75	16.00 ± 0.94	10.92 ± 0.53	5.17 ± 0.63	2.29 ± 0.09	0.73 ± 0.12

ND, not detectable, i.e. < LOQ (below limit of quantification); NT, not tested. Each value represents the mean ± s.e.m. for four rats.

468.1 h (19.5 days). The concentration at 12 weeks after dosing was 0.73 ± 0.12 μg eq. g^{-1}, which is about 1/36 of the C_{max}. The $AUC_{0-\infty}$ for the uveal tract was 12.58 mg h^{-1} g^{-1}.

In albino rats, the uveal tract concentrations were much lower than those in pigmented rats. The uveal tract showed a radioactivity concentration of 1.38 ± 0.11 μg eq. g^{-1} at 1 h after dosing and the concentrations were below LOQ thereafter.

Extraction of radioactivity from uveal tracts

The uveal tracts in albino and pigmented rats were extracted three times with 0.067 M phosphate buffer (pH 7.4) and twice with 1 M HCl/EtOH (30:70). The elution profiles for ^{14}C-levofloxacin-related radioactivity are shown in Table 3.

In albino rats, *c*. 97% of the radioactivity bound to the uveal tract was released by the first extraction with neutral phosphate buffer and no radioactivity was released by extraction with 1 M HCl/EtOH (30:70) at 1 h after dosing. The uveal tract radioactivity concentrations were below LOQ at 24 h and thereafter.

In pigmented rats, the uveal tract radioactivity concentrations were markedly higher than in the albino rats, and radioactivity was still measurable even at 12 weeks after dosing. About 75–85% of the radioactivity was released by the extraction procedures at 1 h to 8 weeks after dosing. At 12 weeks approximately 48% of the radioactivity was released. However, the concentration at 12 weeks was less

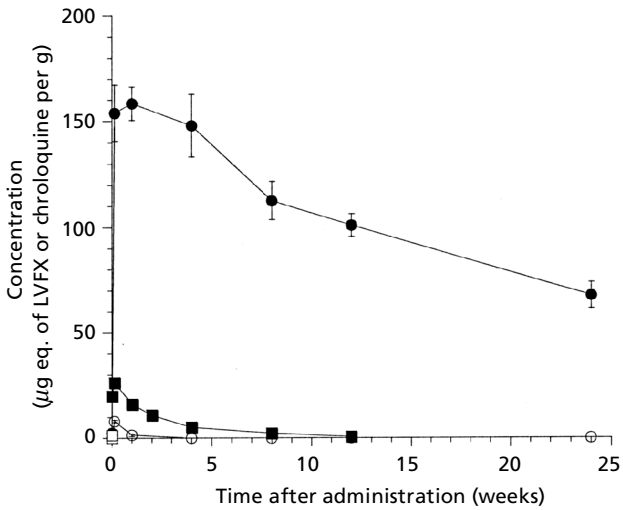

Figure 2 Radioactivity concentrations in uveal tract after single oral administration of ^{14}C-levofloxacin (LVFX) and ^{14}C-chloroquine (20 mg kg^{-1}) to pigmented and albino rats. Each value represents mean ± s.e.m. for four rats. ●, Chloroquine (pigmented rats); ○, chloroquine (albino rats); ■, levofloxacin (pigmented rats); □, levofloxacin (albino rats).

than 1 μg eq. g^{-1}. Most of the radioactivity was released from the uveal tract by extraction with 1 M HCl/EtOH (30:70).

Excretion of radioactivity

The mean percentage recovery of the total radioactivity given in albino and pigmented rats over 144 h is summarized in Table 4. In albino rats, 43.0 ± 1.6% of the dose given was excreted in the urine and 53.2 ± 1.0% in the feces, with total recovery being 96.2 ± 1.2%. The excretion pattern in pigmented rats was similar to that seen in albino rats with urinary, fecal and total excretion being 49.8 ± 1.8, 50.5 ± 3.7 and 104.4 ± 2.7% of the dose, respectively.

Metabolic pattern in uveal tract extracts

Only unchanged levofloxacin was detected on HPLC chromatograms of the uveal tract extracts with both 0.067 M phosphate buffer (pH 7.4) and 1 M HCl/EtOH (30:70) (data not shown).

Discussion

The remarkable capacity of melanin to bind various chemicals, including drugs, is proposed as one of the strongest retention mechanisms of the body. Melanins are polyanions with a relatively high content of negatively charged carboxyl groups and *o*-semiquinones (Ings 1984; Larsson 1993; Salazar-Bookaman et al 1994). Electrostatic forces have been reported to play an important role in the binding of drugs to melanin; however, non-electrostatic contributions, including hydrophobic and van der Waals' interactions and charge-transfer reactions, also contribute to the binding for such drugs as chlorpromazine and chloroquine (Larsson & Tjälve 1979; Tjälve et al 1981; Stepien & Wilczok 1982). Covalent binding has been suggested as the strong and partly irreversible binding of chlorpromazine and chloroquine to melanin (Larsson & Tjälve 1979; Ings 1984). However, the physiological function and binding mechanisms are not fully understood.

It has been also reported that fluoroquinolones such as ofloxacin, levofloxacin, lomefloxacin and moxifloxacin

Table 3 Radioactivity extracted (%) from uveal tract of albino (SD) and pigmented (BN) rats by washing with phosphate buffer (pH 7.4) and 1 M HCl/EtOH.

Strain	No. of washings	Radioactivity extracted from uveal tract (% of total radioactivity)						
		Time after administration (weeks)						
		0.006 (1 h)	0.143 (24 h)	1	2	4	8	12
SD	1	96.90 ± 1.79	ND	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND
	4	ND	ND	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND	ND
	Total	96.90 ± 1.79	ND	ND	ND	ND	ND	ND
BN	1	6.40 ± 0.73	3.00 ± 1.09	ND	ND	ND	ND	ND
	2	1.70 ± 0.99	0.80 ± 0.84	ND	ND	ND	ND	ND
	3	10.60 ± 1.06	10.50 ± 0.37	8.20 ± 0.68	11.90 ± 1.67	ND	ND	ND
	4	45.10 ± 1.40	52.30 ± 3.90	49.10 ± 4.05	39.90 ± 3.00	56.40 ± 5.20	48.80 ± 1.24	48.20 ± 16.08
	5	19.30 ± 1.49	18.60 ± 1.43	25.00 ± 1.38	23.90 ± 1.46	21.00 ± 1.88	26.50 ± 1.39	ND
	Total	83.20 ± 1.55	85.20 ± 4.00	82.20 ± 2.91	75.70 ± 2.51	77.30 ± 3.49	75.30 ± 2.12	48.20 ± 16.08

ND, not detectable, i.e. < LOQ (below limit of quantification). Washing solution: 1–3, 0.067 M phosphate buffer (pH 7.4); 4 and 5, 1 M HCl/EtOH (30:70). Each value represents the mean ± s.e.m. for four rats.

Table 4 Cumulative urinary and fecal excretion of radioactivity after a single oral administration of ^{14}C-levofloxacin to albino (SD) and pigmented (BN) rats at a dose of 20 mg kg^{-1}.

Time (h)	Excretion of radioactivity (%)					
	SD			BN		
	Urine	Feces	Total	Urine	Feces	Total
0–8	26.3 ± 2.8	—	26.3 ± 2.8	34.8 ± 3.0	—	34.8 ± 3.0
0–24	40.0 ± 0.9	39.2 ± 7.5	79.2 ± 7.3	46.7 ± 1.7	41.8 ± 1.7	88.5 ± 0.7
0–48	42.7 ± 1.6	51.7 ± 1.4	94.4 ± 0.8	49.4 ± 1.7	48.7 ± 3.6	98.1 ± 2.6
0–72	42.9 ± 1.6	52.9 ± 1.1	95.8 ± 1.0	49.7 ± 1.8	50.2 ± 3.7	99.9 ± 2.7
0–144	43.0 ± 1.6	53.2 ± 1.0	96.2 ± 1.2	49.8 ± 1.8	50.5 ± 3.7	104.4 ± 2.7

Each value represents the mean ± s.e.m. for four rats.

show high affinity to melanin and pigmented tissues (Fukuda & Sasaki 1989, 1990; Kurata et al 1991; Siefert et al 1999; Fukuda et al 2000). To date, however, the nature of the interaction between melanin and fluoroquinolones has still not been fully characterized.

We have previously reported the in-vitro binding characteristics of fluoroquinolones, including levofloxacin, to synthetic melanin (Ono & Tanaka 2003). During the course of this study, we found electrostatic forces mainly participate in the formation of a chloroquine–melanin complex, whereas van der Waals' and hydrophobic interactions are involved in the levofloxacin–melanin complex in addition to electrostatic forces. We also previously reported the binding characteristics of chloroquine to melanin-containing tissues such as the uveal tract in albino and pigmented rats after a single oral dosing of ^{14}C-chloroquine (Ono et al 2003). It was found that the binding mechanisms of chloroquine to melanin in vitro and in vivo were similar even at 24 weeks after dosing.

In the present study, the absorption, distribution and excretion of radioactivity in albino and pigmented rats after single oral administration of ^{14}C-levofloxacin were investigated and the disposition of radioactivity and its in-vivo binding characteristics to melanin-containing ocular tissues up to 12 weeks after dosing were compared. The ocular pharmacokinetics of ^{14}C-levofloxacin were also compared with those of ^{14}C-chloroquine (Ono et al 2003).

Good penetration of ^{14}C-levofloxacin-related radioactivity into tissues is indicated by higher or similar concentrations in most tissues compared with serum both in albino and pigmented rats. On the other hand, at 1 h after dosing, the radioactivity concentrations in brain were much lower than the serum concentrations, indicating that the radioactivity crosses the blood–brain barrier to a limited extent only. No qualitative differences in the distribution of radioactivity were found between albino and pigmented rats except for the eyes and hairs. The concentrations in the uveal tract and hair of pigmented rats are much higher and declined much more slowly than those of albino rats (Figure 2 and Table 3). This difference in the distribution pattern was interpreted as reflecting the high affinity of ^{14}C-levofloxacin to melanin. In pigmented rats, the half-life of uveal tract radioactivity after dosing of ^{14}C-levofloxacin was 19.5 days, which was much shorter than that of ^{14}C-chloroquine (187 days) and the radioactivity concentrations in the uveal tract after dosing with ^{14}C-levofloxacin were much lower than after dosing with ^{14}C-chloroquine (Figure 2). The C_{max} for ^{14}C-levofloxacin was 26.33 μg eq. g^{-1}, which is *c.* 1/6 of that for ^{14}C-chloroquine (158.42 μg eq. g^{-1}) and the $AUC_{0-\infty}$ was 12.58 mg h^{-1} g^{-1}, which is *c.* 1/67 of that for ^{14}C-chloroquine (842.3 mg h^{-1} g^{-1}) (Ono et al 2003). Levofloxacin showed much lower affinity and capacity to synthetic levodopa melanin than chloroquine in vitro (Ono & Tanaka 2003). This difference in ocular pharmacokinetics between ^{14}C-levofloxacin and ^{14}C-chloroquine was interpreted to reflect much stronger binding of ^{14}C-chloroquine to synthetic melanin in vitro.

We investigated the binding nature of ^{14}C-levofloxacin-related radioactivity to melanin-containing uveal tracts obtained at 1 h to 12 weeks after dosing. The extraction of the melanin–drug complexes with various solutions makes possible the explanation of the binding nature between the drugs and melanin (Larsson & Tjälve 1979; Tjälve et al 1981; Stepien & Wilczok 1982). The elution of the drug by neutral buffer solution suggests participation of van der Waals' forces. Recovery of the drug by strongly acidic 1 M HCl/EtOH (30:70) indicates that electrostatic and/or hydrophobic interactions can play an important role in the formation of the complex.

In albino rats, almost all of the radioactivity was released with neutral phosphate buffer, suggesting that the binding is weak and reversible, and that van der Waals' interactions mainly contribute to the binding. In pigmented rats, 75–85% of the radioactivity was released by extraction procedures up to 8 weeks after dosing. Most of the radioactivity was released by extraction with 1 M HCl/EtOH (30:70), indicating that electrostatic and/or hydrophobic interactions as well as van der Waals' interactions are involved in the levofloxacin–melanin complex in vivo. It has been suggested that the binding mechanisms in vitro and in vivo are similar (Ono & Tanaka 2003). The recovery of the radioactivity from the uveal tract was lower (*c.* 48%) at 12 weeks after dosing. However, the mean concentrations in the uveal

tract were below 1 μg eq. g^{-1}. The uveal tract extracts were analysed by HPLC using a UV detection method. Only unchanged levofloxacin, not levofloxacin *N*-oxide and desmethyl levofloxacin, was detected on HPLC chromatograms of the extracts.

Approximately 96 and 104% of the radioactivity given orally was recovered in urine and feces up to 144 h after dosing in pigmented and albino rats, respectively. Excretion was almost completed at 48 h after dosing. The excretion pattern in pigmented rats was similar to that seen in albino rats.

It has been reported that prolonged high dosage (0.25 g or more daily for several years) is usually necessary before ocular toxicity of chloroquine develops (Böke et al 1967). The low dosage associated with antimalarial treatment does not normally produce noticeable ocular changes (Goldman & Preston 1957; Hobbs et al 1959). Leblanc et al reported that drug-related toxic effects on the retina described in humans and animals were unrelated to melanin binding and that melanin binding and retinal toxicity were two separate entities, the latter being related to the intrinsic toxicity of the compound rather than its ability to bind (Leblanc et al 1998). It is unlikely that levofloxacin will cause toxicity because of its much lower affinity to melanin-containing ocular tissues and its shorter duration of therapy compared to chloroquine.

Conclusion

In summary, we have demonstrated the disposition of ^{14}C-levofloxacin after single oral administration in albino and pigmented rats. ^{14}C-levofloxacin showed good tissue distribution in most tissues. There were no marked differences in the distribution pattern except for the melanin-containing tissues such as the eye and hair. In pigmented rats, ^{14}C-levofloxacin showed high affinity to the uveal tract and hair. The radioactivity concentrations in the uveal tract and hair were measurable by liquid scintillation counting up to 12 weeks after dosing. However, most of the radioactivity in the uveal tract was released from the tissue with neutral phosphate buffer and 1 M HCl/EtOH (30:70), indicating that the binding is reversible and that electrostatic forces as well as van der Waals' and hydrophobic interactions are involved in the binding of levofloxacin with the melanin-containing ocular tissues. Only unchanged levofloxacin was detected in the uveal tract extracts by HPLC. The concentrations and the half-life of radioactivity in the uveal tract after dosing with ^{14}C-levofloxacin were found to be much lower and shorter than those after dosing with ^{14}C-chloroquine.

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