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

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Abstract

Chloroquine is an antimalarial agent that has been reported to have distinct affinity to melanin. After single oral administration of ^{14}C-chloroquine at a dose of 20 mg kg^{-1} under non-fasting conditions, the absorption, distribution and excretion of ^{14}C-chloroquine-related radioactivity were studied in albino and pigmented rats. The objectives of the study were to investigate differences in the disposition of chloroquine between albino and pigmented rats and to define its in-vivo binding characteristics to melanin-containing ocular tissues. Extensive uptake of radioactivity into tissues was indicated by higher concentrations in most tissues compared with serum and there was no quantitative differences in the distribution of radioactivity found between albino and pigmented rats except for melanin-containing tissues, such as the uveal tract of the eye and perhaps hair follicles. There was selective and strong binding of drug-related compounds to these tissues in pigmented rats. The uveal tract concentrations reached the maximum value of 158.42 ± 7.86 μg equiv g^{-1} (mean ± s.e.) at 1 week and decreased very slowly with a terminal half life of 4476 h (187 day). The uveal tract concentrations at 24 weeks were still high (67.75 ± 6.19 μg equiv g^{-1}). The AUC for uveal tract was 842.3 mg·h g^{-1}. A relatively high concentration was still determined in the uveal tract even at 48 weeks after single oral dosing by whole-body autoradiography. 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, almost all radioactivity was released from the tissue with 1 M HCl–EtOH (30:70), indicating that the strong binding by melanin was reversible, and that hydrophobic or electrostatic interaction would play a critical role in the binding of chloroquine and its metabolites with the melanin-containing ocular tissues. Approximately 70% of the radioactivity given was recovered in urine and faeces up to 144 h after dosing both in pigmented and albino rats. The excretion pattern in pigmented rats was similar to that seen in albino rats.

Introduction

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 (Hobbs et al 1959) produced chorioretinopathy led to the awareness of an association between the toxic effects of some drugs and their 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 the skin and hair (Ings 1984; Larsson 1993; Salazar-Bookaman et al 1994). The distribution and accumulation of drugs in these pigmented tissues is of considerable interest mainly from a toxicological point of view.

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It was 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 reaction would also contribute to the binding for chloroquine (Larsson & Tjälve 1979; Tjälve et al 1981; Stepien & Wilczok 1982).

We have reported the binding characteristics of fluoroquinolones to synthetic melanin in-vitro (Ono & Tanaka 2003). During the course of this study, we found that chloroquine bound to melanin was completely eluted by the washing with 1 M HCl, indicating that electrostatic interaction plays a critical role in the chloroquine–synthetic melanin complex formation in-vitro.

The aims of this study are to investigate differences in absorption, distribution and excretion, after a single oral dose of ^{14}C-chloroquine, between albino and pigmented rats and to assess the nature of the binding in-vivo between melanin and radioactivity representing ^{14}C-chloroquine or its metabolites.

Materials and Methods

Test materials

[Quinoline-3-^{14}C]chloroquine diphosphate (Figure 1) with specific activity of 340 MBq mmol^{-1} was radiosynthesized at Amersham Biosciences Corp. (NJ). A radiochemical purity of 98% was established by thin-layer chromatography. Non-radiolabelled chloroquine diphosphate was purchased from Sigma-Aldrich Fine Chemicals (MO). 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, 240–269 g, aged 7 weeks (n = 4 per time point), purchased from Japan SLC Inc. (Shizuoka, Japan) and male BN/Crj rats, 147–187 g, aged 7 weeks (n = 4 per time point) purchased from Sankyo Labo Service Corporation Inc. (Tokyo, Japan), respectively. The rats were adapted to laboratory conditions before the study at a temperature of 23 ± 2 °C and 55 ± 15% humidity. Rats 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 freely available 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).

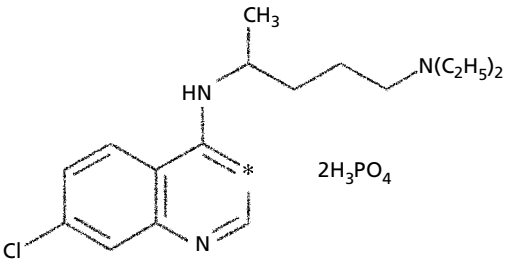

Figure 1 Chemical structure of ^{14}C-chloroquine diphosphate. Asterisk denotes ^{14}C-labelled position.

Dose formulation and administration

^{14}C-Chloroquine diphosphate was dissolved in distilled water, to achieve 20 mg/5 mL. In this study, the concentrations of drug were expressed as chloroquine diphosphate equivalents. The specific activity of the dosing solution was 320.4 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, 7 groups of 4 male albino or pigmented rats received single oral dose of ^{14}C-chloroquine at dose of 20 mg kg^{-1}. The rats were killed at 1 h, 24 h, 1, 4, 8, 12 and 24 weeks after oral dosing by ether inhalation and exsanguination 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 (including iris, ciliary body, choroids and retina) were collected. The urine samples were collected at 0–8, 8–24, 24–48, 48–72, 72–96 and 96–144 h after administration, and the faeces were collected at 0–24, 24–48, 48–72, 72–96 and 96–144 h after administration.

For whole-body autoradiography, 3 groups of 1 male albino or pigmented rats received a single oral dose of ^{14}C-chloroquine (20 mg kg^{-1}). No exsanguination of the rats was performed. Rats were killed by CO_2 inhalation at 4, 24 and 48 weeks after oral dosing, and immediately deep-frozen in dry ice–acetone bath. Sagittal whole-body sections of 30 μm in thickness were obtained with a cryomicrotome (LKB2250; Leica Inc., Wetzlar, Germany). The sections were freeze-dried and then exposed to a BAS 2000 bioimage analyser (Fuji Photo Film Co. Ltd, Tokyo, Japan).

Radioactivity analysis

The measurement of radioactivity in the blood, tissues and faeces was conducted by using a solubilization method. To each tissue sample and blood (about 100 mg or 100 μL), 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). Faeces 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 a sample of the homogenate (0.5 mL) was used for radioactivity analysis.

Serum and urine 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 and 900; Aloka Co. Ltd, Tokyo, Japan or LS600; Beckman Coulter Inc., CA) 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 hydrochloric acid (HCl)–ethanol (EtOH) (30:70, 1 mL) at 37 °C for 1 h. The supernatant concentrations of radioactivity eluted were determined by liquid scintillation counter after centrifugation at 11 000 rev min^{-1} (ca. 14 000 *g*) for 15 min. The radioactivity in the resulting tissue was measured by using the solubilization method described above. The sum of the radioactivity in the supernatants and the resulting tissue was taken as total radioactivity (100%) in the uveal tract. The percentage of radioactivity eluted from the tissue was calculated for each extraction.

Data analysis

Concentrations of radioactivity were expressed as microgram equivalents (μg equiv) of chloroquine diphosphate per mL of fluid or g of tissue. The net d min^{-1} were determined as the d min^{-1} minus the background d min^{-1}. Samples having a net d min^{-1} less than the background value were considered to contain an amount of radioactivity below the limit of quantification (LOQ). Radioactivity in urine and faeces was expressed as a percentage of the administered dose.

Radioactivity concentrations in uveal tract vs time data in pigmented rats were analysed for non-compartmental methods using 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 as $0.693/\lambda_z$. Maximum concentration (Cmax) and the time to reach Cmax (tmax) were obtained from the measured values. 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 concentration

The radioactivity concentrations in blood, serum and tissues after a single oral administration of ^{14}C-chloroquine diphosphate 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 similar (0.14 ± 0.01 and 0.13 ± 0.01 μg equiv mL^{-1}, respectively). The serum concentrations were still measurable (0.02 ± 0.00 μg equiv mL^{-1}) even at 1 week after dosing and were below LOQ thereafter. Most of the tissues, except for liver, showed much higher concentrations at 24 h than at 1 h after dosing. The mean peak tissue concentrations were ranked as follows: liver > Harderian gland > lung > kidney > uveal tract > heart > skin > hair > corpus vitreum > brain > blood > serum. Corpus vitreum and uveal tract radioactivity concentrations were ca. 18 times and 62 times those in serum.

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

Tissue	Time after administration						
	1 h	24 h	1 Week	4 Weeks	8 Weeks	12 Weeks	24 Weeks
Whole blood	0.43 ± 0.03	0.37 ± 0.02	0.13 ± 0.01	ND	0.04 ± 0.00	ND	ND
Serum	0.14 ± 0.01	0.13 ± 0.01	0.02 ± 0.00	ND	ND	ND	ND
Liver	78.41 ± 13.30	35.96 ± 1.60	5.47 ± 0.75	0.08 ± 0.01	0.02 ± 0.00	0.01 ± 0.01	0.02 ± 0.02
Kidney	7.50 ± 0.72	16.01 ± 0.84	0.75 ± 0.06	0.01 ± 0.01	0.01 ± 0.00	ND	ND
Heart	2.77 ± 0.25	5.21 ± 0.42	0.51 ± 0.02	ND	ND	ND	0.02 ± 0.01
Lung	10.75 ± 0.88	30.76 ± 0.85	1.38 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	ND	0.01 ± 0.01
Brain	0.08 ± 0.00	1.59 ± 0.11	0.23 ± 0.02	ND	ND	ND	ND
Skin	0.48 ± 0.04	4.57 ± 0.06	0.44 ± 0.03	ND	ND	ND	ND
Harderian gland	0.85 ± 0.09	38.51 ± 2.64	2.92 ± 0.39	0.01 ± 0.01	ND	ND	ND
Hair	0.16 ± 0.12	4.16 ± 0.27	0.58 ± 0.25	0.21 ± 0.04	0.17 ± 0.07	0.07 ± 0.01	0.01 ± 0.01
Corpus vitreum	0.17 ± 0.04	2.36 ± 0.03	0.47 ± 0.02	0.04 ± 0.01	0.01 ± 0.00	ND	ND
Uveal tract	0.40 ± 0.14	8.03 ± 0.52	1.48 ± 0.42	ND	ND	ND	ND

Concentration is expressed as μg equiv g^{-1} or μg equiv mL^{-1} of chloroquine diphosphate. ND, Not detectable (below LOQ, lower limit of quantification). Each value represents the mean ± s.e. for 4 rats.

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

Tissue	Time after administration						
	1 h	24 h	1 Week	4 Weeks	8 Weeks	12 Weeks	24 Weeks
Whole blood	0.48 ± 0.06	0.35 ± 0.01	0.16 ± 0.03	ND	0.03 ± 0.00	0.01 ± 0.01	ND
Serum	0.16 ± 0.02	0.10 ± 0.00	ND	ND	ND	ND	ND
Liver	67.93 ± 15.14	34.07 ± 1.24	4.28 ± 0.25	0.10 ± 0.02	0.03 ± 0.00	0.03 ± 0.01	0.01 ± 0.00
Kidney	10.94 ± 2.54	16.15 ± 1.69	0.67 ± 0.05	0.03 ± 0.00	0.01 ± 0.00	ND	0.01 ± 0.01
Heart	3.87 ± 0.80	5.48 ± 0.18	0.80 ± 0.06	0.01 ± 0.01	ND	ND	ND
Lung	15.95 ± 3.47	34.76 ± 1.56	2.06 ± 0.08	0.05 ± 0.01	0.01 ± 0.00	ND	ND
Brain	0.13 ± 0.03	1.69 ± 0.03	0.14 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	ND	0.01 ± 0.01
Skin	0.57 ± 0.16	4.57 ± 0.17	0.53 ± 0.03	ND	ND	ND	ND
Harderian gland	2.00 ± 0.47	57.40 ± 1.59	5.20 ± 0.21	0.29 ± 0.04	0.11 ± 0.01	0.05 ± 0.00	0.03 ± 0.01
Hair	0.06 ± 0.02	3.99 ± 0.28	0.31 ± 0.07	0.63 ± 0.35	0.26 ± 0.04	0.09 ± 0.02	0.01 ± 0.01
Corpus vitreum	0.15 ± 0.07	12.78 ± 1.77	9.55 ± 1.16	7.89 ± 0.54	5.86 ± 0.65	2.73 ± 0.37	1.17 ± 0.13
Uveal tract	2.65 ± 0.69	153.98 ± 13.27	158.42 ± 7.86	148.11 ± 14.75	112.61 ± 9.05	100.88 ± 5.42	67.75 ± 6.19

Concentration is expressed as μg equiv g^{-1} or μg equiv mL^{-1} of chloroquine diphosphate. ND, Not detectable (below LOQ, lower limit of quantification). Each value represents the mean ± s.e. for 4 rats.

In pigmented rats, the serum concentrations at 1 and 24 h after dosing were similar (0.16 ± 0.02 and 0.10 ± 0.00 μg equiv mL^{-1}, respectively). The serum concentrations were less than LOQ at 1 week and thereafter. Most of the tissues, except for liver, showed much higher concentrations at 24 h than at 1 h after dosing. The uveal tract showed maximum concentration at 1 week after dosing. The mean peak tissue concentrations were ranked as follows: uveal tract > liver > Harderian gland > lung > kidney > corpus vitreum > heart > skin > hair > brain > blood > serum. The corpus vitreum and uveal tract radioactivity concentrations were ca. 128 times and 1540 times those in serum at 24 h after dosing.

After oral administration to pigmented rats, tissue distribution patterns were similar to those seen in albino rats except for ocular tissues. Especially, ^{14}C-chloroquine showed a very strong affinity for melanin-containing ocular tissues. The radioactivity concentrations in the uveal tract vs time profiles in albino and pigmented rats are shown in Figure 2. In pigmented rats, the uveal tract concentrations at 1 and 24 h after oral dosing were 2.65 ± 0.69 and 153.98 ± 13.27 μg equiv g^{-1}, respectively, and reached a maximum value (Cmax) of 158.42 ± 7.86 μg equiv g^{-1} at 1 week. After the Cmax was reached, the concentrations declined very slowly with a terminal half life of 4476 h (187 day). The AUC for uveal tract was 842.3 mg·h g^{-1}. In albino rats, the uveal tract concentrations were much lower than those in pigmented rats and reached a maximum value of 8.03 ± 0.52 μg equiv g^{-1} at 24 h after dosing. The concentrations were below LOQ at 4 weeks and 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 chloroquine-related radioactivity are shown in Table 3.

In albino rats, ca. 75%, 96% and 100% of the radioactivity bound to uveal tract was released when the tissues were extracted successively with neutral phosphate buffer and 1 M HCl–EtOH (30:70) at 1 h, 24 h and 1 week, respectively. Most of the radioactivity was released by extracting with neutral phosphate buffer.

In pigmented rats, the uveal tract radioactivity concentrations were markedly higher than those in the albino rats and were still 67.75 ± 6.19 μg equiv g^{-1} even at 24 week after dosing. Although less than 2% of the radioactivity was released by extracting with neutral phosphate buffer, more than 93.6% was released by extracting with 1 M

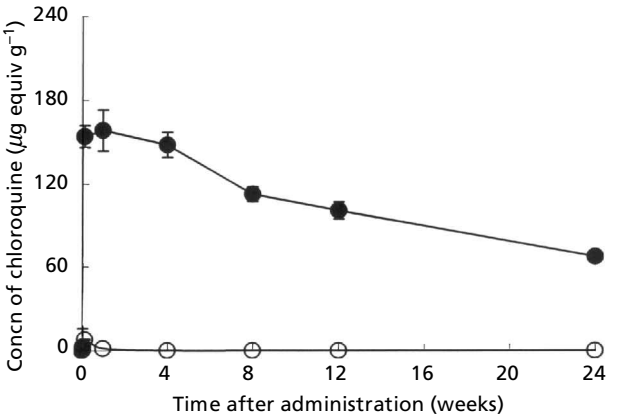

Figure 2 Radioactivity concentrations in uveal tract after a single oral administration of ^{14}C-chloroquine (20 mg kg^{-1}) to pigmented and albino rats. Each value represents mean ± standard error for four rats. Filled circle, pigmented rats; open circle, albino rats.

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–ethanol.

Strain	Wash no.	Time after administration of ^{14}C-chloroquine						
		1 h	24 h	1 Week	4 Weeks	8 Weeks	12 Weeks	24 Weeks
SD	1	75.00 ± 25.00	47.97 ± 2.95	60.86 ± 5.06	ND	ND	ND	ND
	2	ND	20.13 ± 1.05	29.57 ± 4.63	ND	ND	ND	ND
	3	ND	13.36 ± 2.37	4.06 ± 4.06	ND	ND	ND	ND
	4	ND	14.66 ± 1.40	5.51 ± 5.51	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND	ND
	Total	75.00 ± 25.00	96.12 ± 0.29	100.00 ± 0.00	0.00	0.00	0.00	0.00
BN	1	ND	0.49 ± 0.17	0.61 ± 0.11	1.16 ± 0.28	0.80 ± 0.35	0.54 ± 0.14	0.66 ± 0.22
	2	ND	0.25 ± 0.09	0.34 ± 0.03	0.49 ± 0.13	0.36 ± 0.11	0.48 ± 0.10	0.36 ± 0.15
	3	ND	0.69 ± 0.03	0.57 ± 0.03	0.59 ± 0.08	0.44 ± 0.08	0.62 ± 0.04	0.82 ± 0.03
	4	86.65 ± 4.66	86.61 ± 4.17	84.61 ± 1.44	84.25 ± 1.19	78.32 ± 5.08	75.22 ± 2.36	79.61 ± 1.42
	5	10.16 ± 3.53	9.38 ± 3.81	11.32 ± 1.08	10.59 ± 0.97	16.73 ± 5.03	19.62 ± 2.07	13.98 ± 1.11
	Total	96.81 ± 1.14	97.42 ± 0.61	97.44 ± 0.25	97.08 ± 0.29	96.65 ± 0.43	96.47 ± 0.49	95.43 ± 0.51

Data are expressed as % of total radioactivity. Washing solution; no. 1–3, 0.067 M phosphate buffer (pH 7.4), no. 4 and 5, 1 M HCl–EtOH (30:70). ND, Not detectable (below LOQ, lower limit of quantification). Each value represents the mean ± s.e. for 4 rats.

HCl–EtOH (30:70). Surprisingly, more than 95% of the radioactivity was released by the extraction procedure at all time points tested.

Whole-body autoradiography

At 4 and 24 weeks after oral dosing to albino rats all tissues except for the testis showed negligible concentrations of radioactivity, and at 48 weeks after dosing almost no radioactivity was detected in any tissues.

At 4 weeks after oral administration to pigmented rats, relatively high concentrations were determined in the uveal tract, skin and testis, whereas almost none was found in other tissues (Figure 3). Relatively high radioactivity was still determined in the uveal tract and lower radioactivity in the testis even at 48 weeks after dosing. There was a very strong affinity for melanin-containing structures located in the wall of the eye.

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, 38.3 ± 1.3% of the dose was excreted in the urine and 31.1 ± 1.2 % in the faeces with the total recovery being 69.5 ± 2.3%. The excretion pattern in pigmented rats was similar to that seen in albino rats with the urinary, faecal and total excretion being 32.6 ± 1.3, 40.4 ± 1.5 and 73.0 ± 1.2% of the dose, respectively.

Discussion

Melanin in animals and man is synthesized entirely within melanocytes as melanosomes, which are found in both external and internal tissues (eye, skin, ear, brain and hair) (Ings 1984). The remarkable affinity and capacity of melanin to bind various chemicals, including drugs, are proposed as one of the strongest retention mechanisms of the body. However, the physiological function and binding mechanisms are not fully understood. 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 reaction, would also contribute to the binding for such drugs as chlorpromazine and chloroquine (Larsson & Tjälve 1979; Tjalve et al 1981; Stepien & Wilczok 1982). Covalent binding has been suggested as being responsible for the strong and partly irreversible binding of chlorpromazine and chloroquine to melanin (Larsson & Tjälve 1979; Ings 1984).

We have previously reported the in-vitro binding characteristics of fluoroquinolones to synthetic melanin (Ono & Tanaka 2003). During the course of this study, we found that chloroquine bound to melanin was almost completely released by extracting with 1 M HCl, indicating that electrostatic interaction between the protonated amino group of chloroquine and the carboxyl groups of melanin would play a critical role in the drug–melanin complex formation in-vitro.

It was reported that after intravenous administration of ^{14}C-chloroquine, radioactivity was rapidly localized in the uveal tract of pigmented mice (Lindquist & Ullberg 1972; Tjalve et al 1981) and rats (Kasuya et at 1976). The concentration in the uveal tract, semiquantitated by using whole-body autoradiography, was still high 1 year after administration (Lindquist & Ullberg 1972). However, the nature of the interaction between melanin and chloro-

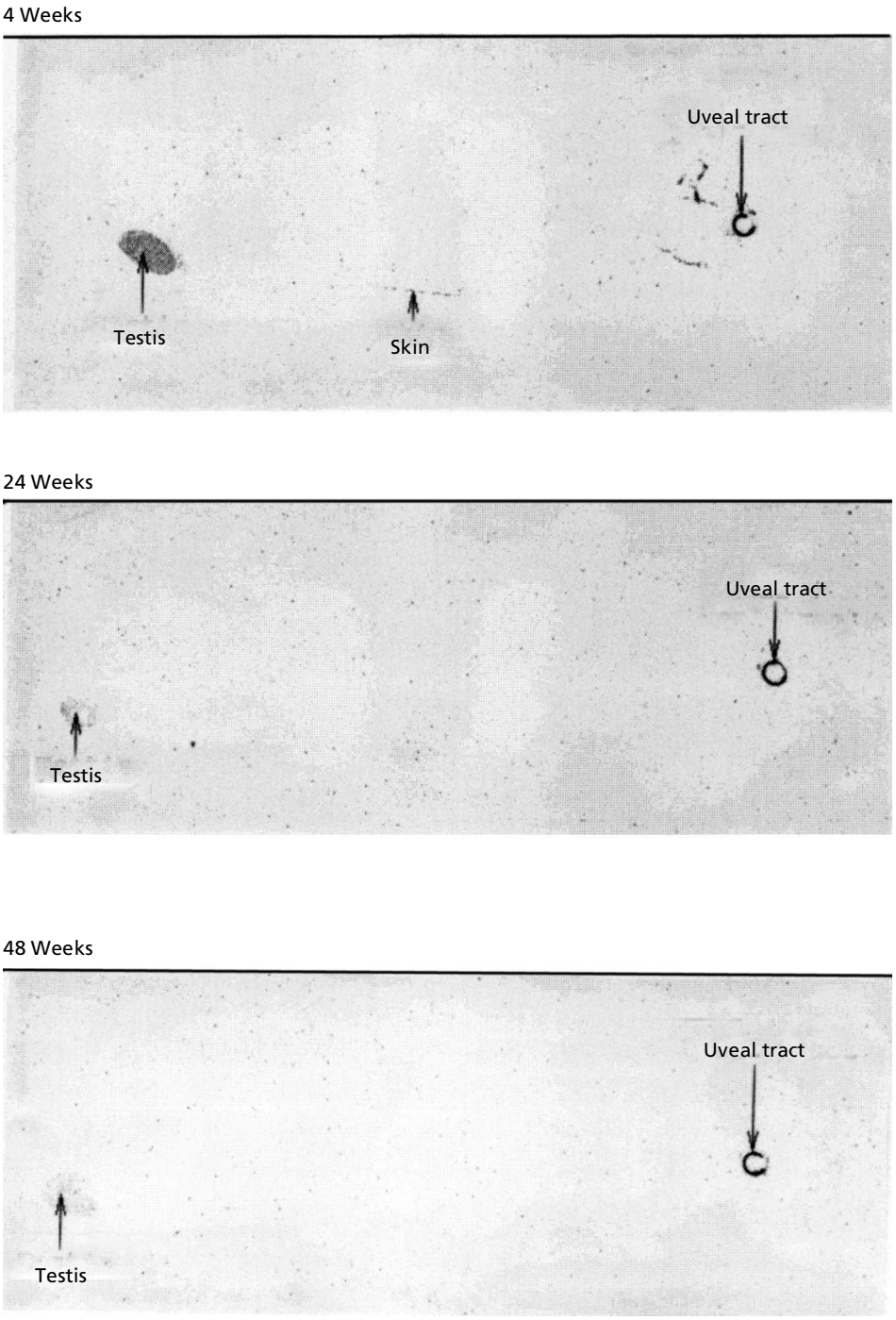

Figure 3 Whole-body autoradiographs of pigmented rats 4, 24 and 48 weeks after a single oral administration of ^{14}C-chloroquine ($20\,mg\,kg^{-1}$).

quine in-vivo still has not been fully characterized, especially at long time after dosing.

Extensive uptake of ^{14}C-chloroquine-related radioactivity into tissues is indicated by higher concentrations in most tissues compared with serum levels both in albino and pigmented rats. At 24 h after dosing, the radioactivity concentrations in the brain were higher than in serum, indicating that radioactivity crosses the blood–brain barrier. No quantitative differences in the distribution of radioactivity were found between albino and pigmented rats, except for eyes. The concentrations in the uveal tract of pigmented rats were much higher than those of albino

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

Time (h)	Albino rats			Pigmented rats		
	Urine	Faeces	Total	Urine	Faeces	Total
0–24	26.0 ± 0.7	14.6 ± 0.9	40.6 ± 1.2	20.8 ± 1.0	17.5 ± 1.6	38.3 ± 1.2
−48	33.5 ± 1.1	25.2 ± 0.9	58.7 ± 1.9	27.6 ± 1.0	31.5 ± 1.4	59.1 ± 1.1
−72	35.7 ± 1.3	28.3 ± 1.1	64.0 ± 2.2	29.9 ± 1.2	36.2 ± 1.4	66.1 ± 1.1
−96	36.8 ± 1.3	29.7 ± 1.2	66.6 ± 2.3	31.0 ± 1.2	38.4 ± 1.5	69.5 ± 1.1
−144	38.3 ± 1.3	31.1 ± 1.2	69.5 ± 2.3	32.6 ± 1.3	40.4 ± 1.5	73.0 ± 1.2

Data are expressed as % of dose. Each value represents the mean ± s.e. for 4 rats.

rats and declined very slowly (Figure 2). This difference in the distribution pattern was interpreted to reflect distinct affinity of ^{14}C-chloroquine and its metabolites to melanin.

Whole-body autoradiographic results obtained at 4, 24 and 48 weeks after dosing to pigmented rats also showed very high affinity of ^{14}C-chloroquine-related radioactivity to the melanin-containing ocular tissues or uveal tract and lower affinity also to testis (Figure 3). Especially in uveal tract, a high concentration of radioactivity was still observed even at 48 weeks after dosing. Thus, once ^{14}C-chloroquine-related radioactivity binds to the melanin containing tissues, it is retained for a very long time. A similar result was reported in pigmented mice (Lindquist & Ullberg 1972). This would suggest a very low turnover of ocular melanin and also irreversible binding (Ings 1984). It was reported that electrostatic, hydrophobic and van der Waals' interactions participate in the formation of chloroquine–synthetic melanin complex in-vitro (Larson & Tjälve 1979; Stepien & Wilczok 1982). It was also reported that the binding mechanisms described above for the in-vitro affinity were valid also for the situation in-vivo after a single injection of ^{14}C-chloroquine to pigmented mice (Tjälve et al 1981). However, this experiment had been performed only at short post-injection intervals (1 h and 1 day). There has been no report on the mechanisms of chloroquine binding to melanin-containing tissues in-vivo at longer post dosing-intervals. Thus, we investigated the binding nature of ^{14}C-chloroquine-related radioactivity to the melanin-containing uveal tracts obtained at 1 h to 24 weeks after dosing by extracting them with neutral phosphate buffer and 1 M HCl–EtOH (30:70), successively.

Extraction of the melanin–drug complexes with various solutions makes it possible to explain the nature of the binding between the drugs and melanin. The elution of the drug by neutral buffer solution would suggest participation of van der Waals' forces. Recovery of the drug by strongly acidic 1 M HCl–EtOH (30:70) would indicate that electrostatic or hydrophobic forces can play an important role in the complex formation.

In albino rats, most radioactivity was released with neutral phosphate buffer, suggesting that the binding is weak and reversible, and that van der Waals' interactions would mainly contribute to the binding. In pigmented rats, almost all radioactivity at all time points tested was released with 1 M HCl–EtOH (30:70), indicating that the binding is not covalent but is reversible even at 24 weeks after dosing and that hydrophobic or electrostatic interaction plays a critical role in the binding of chloroquine and its metabolites with the melanin-containing ocular tissues. We previously reported that chloroquine bound to melanin was completely eluted by washing with 1 M HCl, indicating that electrostatic interaction plays a major role in the chloroquine–synthetic melanin complex formation in-vitro (Ono & Tanaka 2003). It was suggested that the binding mechanisms in-vitro and in-vivo were similar even at long post dose-intervals.

Approximately 70% of the radioactivity given orally was recovered in urine and faeces up to 144 h after dosing in both pigmented and albino rats. Excretion was not completed even at 144 h after dosing. This could be attributable to extensive tissue distribution and retention of chloroquine and its metabolites in the body. The excretion pattern in pigmented rats was similar to that seen in albino rats.

In summary, we have demonstrated the disposition of ^{14}C-chloroquine after single oral administration in albino and pigmented rats. ^{14}C-chloroquine is extensively distributed in most tissues. There were no marked differences in the disposition pattern except for the ocular pharmacokinetics. In pigmented rats, ^{14}C-chloroquine showed very high affinity to melanin-containing ocular tissues. High radioactivity concentrations in the uveal tract were measured by liquid scintillation counting up to 24 weeks after dosing and radioactivity was still detected, by whole-body autoradiography, even at 48 weeks after dosing. However, almost all radioactivity was released from the tissue with 1 M HCl–EtOH (30:70), indicating that the strong binding is reversible and that hydrophobic or electrostatic interaction plays a critical role in the binding of chloroquine and its metabolites to the melanin-containing ocular tissues.

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