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Tissue Distribution and Excretion of Amodiaquine in the Rat

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Abstract—^{14}C-Labelled amodiaquine ([^{14}C]AQ) has been administered to male Wistar rats by oral and intravenous routes (n = 6 for each route of administration). Excretion of total ^{14}C-activity was predominantly in the faeces after both oral and intravenous administration. After oral administration 86 ± 8·3% (mean ± s.d.) of the ^{14}C administered had been excreted (77 ± 9% in the faeces, 7 ± 1% in the urine and 2 ± 2% in cage washings) over 72 h. Of the ^{14}C administered, 4 ± 1% was recovered from the tissues, and this was widely distributed, with the main organs of accumulation being kidney, liver, red bone marrow and spleen. After intravenous administration, 102·6 ± 9·7% of the ^{14}C had been excreted (90·9 ± 9·6% in faeces, 10·9 ± 0·8% in urine and 0·5 ± 0·2% in cage washings) over 72 h. High-performance liquid chromatographic analysis of urine and faeces samples following oral administration of ^{14}C-AQ (8·6 mg kg^{-1}; base) revealed recoveries of 210 ± 70 μg amodiaquine (AQ) and 123 ± 32 μg desethylamodiaquine (AQm) in the faeces, and 2·4 ± 0·5 μg AQ and 18·5 ± 4·1 μg AQm in the urine. Female Wistar rats (n = 6) each received [^{14}C]AQ orally and were killed at the following times: 0·5, 1, 3, 6, 24 and 48 h. Autoradiographs were prepared from each animal and these revealed significant amounts of radioactivity in the tissues at 48 h. This was accumulated maximally by liver and kidney. Radioactivity was detected in bone marrow at 48 h. These data show that after oral administration of [^{14}C]AQ to rats, significant amounts of radiolabel were accumulated in the liver, and haemopoietic tissues, which are the sites of observed toxicity in man. Of the excreted radiolabel, only a small proportion was in the form of AQ or AQm.

Amodiaquine (AQ) has been used frequently over the last five years following the demonstration of its superiority to chloroquine in the treatment of chloroquine-resistant *Plasmodium falciparum* malaria (Watkins et al 1984). Unfortunately the prophylactic use of AQ has led to an unacceptably high prevalence of agranulocytosis, which has caused several deaths (Hatton et al 1986; Neftel et al 1986), and serious morbidity from hepatitis (Neftel et al 1986). Although now withdrawn for prophylaxis, the drug remains in widespread therapeutic use. Information is now needed regarding both its pharmacology and toxicology including the mass-fate of the drug, in order to understand the mechanism of toxicity.

Barrow (1974) examined the disposition of ^{14}C-labelled amodiaquine ([^{14}C]AQ) in small mammals and suggested that ⩽50% of the dose was excreted by day 9 after oral administration. He also demonstrated that the radiolabel was excreted predominantly in the faeces after both oral and intraperitoneal (i.p.) administration in rats, and examined the distribution of ^{14}C-radioactivity in several tissues, but bone marrow was not examined, and no mass-balance was obtained.

We have studied the excretion and tissue distribution of [^{14}C]AQ in the rat following oral and intravenous (i.v.) administration to determine the mass-fate of the drug, and to investigate accumulation of radiolabel in those tissues involved in the observed toxic manifestations, namely liver and sites of haemopoiesis. We have also determined the recoveries of the drug and desethylamodiaquine (AQm), the principal plasma metabolite in man, from urine and faeces by high performance liquid chromatography (HPLC) to determine the form(s) of excreted radiolabel. Autoradiographs have been prepared from animals killed at various times after oral administration of [^{14}C]AQ.

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Materials and Methods

Preparation of radiolabelled amodiaquine

[Quinoline-2-^{14}C]amodiaquine hydrochloride monohydrate ([^{14}C]AQ) was synthesized following the method of Burkhalter et al (1948) by Amersham International (UK). Radiochemical purity was tested by HPLC analysis, using the conditions set out below, and followed by liquid scintillation counting of eluted fractions. Of the ^{14}C injected 85% was seen to be associated with the AQ peak, much of the remainder eluting early with the solvent front. The radiolabel was therefore purified before use by thin layer chromatography (TLC), and radiochemical purity was tested before administration to animals on each occasion.

Purification of radiolabelled amodiaquine

[^{14}C]AQ was dissolved in ethanol (2 mL) and applied as streaks to TLC plates (Kieselgel 60-ART 5748; Merck) which were developed in triethylamine–methanol (50:50 v/v). The [^{14}C]AQ was located by autoradiography and eluted with ethanol. After centrifugation to remove silica gel, HCl was added (42·36 μmol/10 mg AQ) to convert the drug to its hydrochloride form. The ethanolic [^{14}C]AQ stock solution was stored at −20°C protected from light by aluminium foil.

Recovery of [^{14}C]AQ was determined by comparison of the peak heights obtained from aliquots of the [^{14}C]AQ stock with those from known quantities of AQ injected onto the HPLC system. The specific activity of the purified [^{14}C]AQ

was determined by liquid scintillation counting and was found to be 8·57 μCi mg^{-1} (base). Radiochemical purity was determined by HPLC as before and was found to be 98%.

Other materials
Amodiaquine dihydrochloride dihydrate was supplied by Parke-Davis (Pontypool UK). Desethylamodiaquine was obtained from Warner-Lambert (Ann Arbor, Michigan USA). The internal standard for HPLC analysis was 6-methoxy-8-aminoquinoline, and was obtained from Aldrich (Gillingham UK). Ammonia solution (sp.gr. 0·88) and hydrogen peroxide solution (30%) were from BDH Chemicals (Poole UK). Diethyl ether, dichloromethane, methanol, and acetonitrile (all HPLC grade), and triethylamine, orthophosphoric acid and glacial acetic acid (all AR grade) were supplied by Fisons (Loughborough UK). NCS tissue solubilizer was obtained from Amersham International (UK). Glucuronidase/sulphatase (4260 units mL^{-1}) was obtained from Sigma (Poole UK).

Oral administration of [^{14}C]amodiaquine
Male Wistar rats (220–250 g; n = 6) were given [^{14}C]AQ (8·6 mg kg^{-1} base; 8 μCi) in aqueous solution (2 mL) by orogastric intubation. Animals were housed separately in plastic metabolism cages (Techniplast, Scanbur, Denmark) and were allowed free access to food and water. Urine and faeces samples were collected twelve-hourly up to 72 h. At 72 h animals were killed and the kidneys, liver, spleen, testes, heart, lungs, brain (and eyes), and gut (including content) removed. Red bone marrow ($\sim$100 mg from each animal) was expressed from the tibiae and femora. The carcass was retained.

Intravenous administration of [^{14}C]amodiaquine
Male Wistar rats (230–250 g; n = 6) under ether anaesthesia were given [^{14}C]AQ (3·8 mg kg^{-1} base; 2 μCi) in saline (0·9%; 0·4 mL) by the tail vein. Satisfactory i.v. administration was determined by observation of venous filling, and lack of tissue induration at the end of injection. After recovery, animals were housed separately in metabolism cages, and were allowed free access to food and water. Collections (12 h) or urine and faeces were made up to death at 72 h.

Cage washes
At 72 h after both oral and i.v. administration, cages were dismantled. Methanol (40 mL) was then used to wash all inner surfaces, and excreta containers, and retained for scintillation counting.

Autoradiography
Female Wistar rats (35 g; n = 6) received [^{14}C]AQ (9·9 mg kg^{-1} base; 2·48 μCi) in aqueous solution (0·1 mL) by orogastric intubation. Animals were killed 0·5, 1, 3, 6, 24, and 48 h after dosing. Autoradiographs were prepared by the method of Powell et al (1967); animal surfaces were exposed to X-ray film in contact with solid CO_2 for 3 weeks.

Sample handling
Urine was stored in plastic containers protected from light by aluminium foil at -20°C until assay. After removal of contaminating food pellets, faeces were homogenized in phosphate buffer (pH 7·4; four times the weight of faeces) using an Ultra-Turrax blender. All tissues (except red bone marrow) were homogenized in phosphate buffer (pH 7·5; three times the weight of tissue) using glass mortars and Teflon pestles. Red bone marrow was freeze-dried and reconstituted in phosphate buffer (pH 7·5; 500 μL). The residual carcass was dissected and homogenized in water using a Waring blender. Aliquots of faecal and tissue homogenates were stored at -20°C in plastic containers protected from light by aluminium foil.

Liquid scintillation counting
Radioactivity was determined by liquid scintillation counting using a Beckman LS/1801 counter (Beckman instruments inc., California USA) programmed for automatic quench correction. To duplicate samples of urine (100 μL) was added scintillation cocktail (5 mL) followed by vortex mixing. To triplicate samples of faecal and tissue homogenates (100 μL) was added NCS tissue solubilizer (500 μL). The mixtures were incubated overnight at 50°C. While still hot, the digest was decolourized using H_2O_2 (30% w/v; 500 μL), vortex mixed and incubated at 50°C for a further 0·5 h. To the decolourized digest was added glacial acetic acid (50 μL), to reduce chemiluminescence, followed by scintillation cocktail (15 mL). After vortex mixing of urine or decolourized digests with scintillation cocktail, specimens were incubated at room temperature in the dark for 18 h before counting to reduce chemiluminescence further. In all cases four control specimens of urine or digest were treated identically to the test samples. The mean disintegration per minute (d min^{-1}) value from the control specimens was subtracted from those of the test samples.

To test the adequacy of quench correction for faecal or tissue digests, a known weight of ^{14}C-labelled hexadecane was added to samples of radiation-free faecal homogenate (25, 50, 75, 100, 125 and 150 μL). These were then treated as above. After subtraction of background counts, the activities of the hexadecane-spiked faeces were 98 ± 3% (mean $\pm$ s.d.) of the calculated value demonstrating that the automatic quench correction curve was accurate for treated digests of whole faeces or tissue.

High performance liquid chromatography urine samples. To urine (0·5 mL) containing internal standard (1 μg) was added distilled H_2O (0·5 mL) followed by NH_3 solution (2·0 mL; sp. gr. 0·88). This was then extracted with diethyl ether (2 × 5·0 mL) by mechanical tumbling (15 min). After centrifugation (2000 *g*; 10 min) and separation, the combined organic phases were evaporated to dryness under a gentle stream of nitrogen at $\leqslant$25°C.

Faecal samples. To faecal homogenates (1·0 mL) containing internal standard (5 μg) was added NH_3 solution (2·0 mL; sp.gr. 0·88). They were then extracted with dichloromethane (1 × 7·0 mL) by vortex mixing (15 s) followed by mechanical tumbling (15 min). After centrifugation (2000 *g*; 10 min) samples were seen to be in 3 phases: aqueous, solid faecal pellet, and organic. The aqueous phase (uppermost) was discarded, and the faecal pellet carefully removed. To the organic phase was added distilled H_2O–NH_3 mixture (25:1 v/v; 4·0 mL). After vortex mixing (15 s) and centrifugation

(2000 *g*; 10 min) the aqueous phase was discarded and the organic phase evaporated to dryness under a stream of nitrogen at ⩽25°C. This resulted in a yellow coloured residue soluble in methanol.

Chromatography. HPLC was performed by the method of Winstanley et al (1987). Mobile phase for urine assay was modified to (1%) triethylamine–methanol (4:1 v/v) buffered to pH 2·73 with orthophosphoric acid, and flowing at 4·5 mL min^{-1}. For adequate chromatography of faecal homogenates the mobile phase was adjusted to (1%) triethylamine–methanol (8·5:1·5 v/v) buffered to pH 2·9 with orthophosphoric acid. Flow rate was 6·0 mL min^{-1}. A reversed-phase prepacked plastic column (μBondapak phenyl: 10 μm particles; 10 cm × 8 mm i.d.; Millipore-Waters Harrow, Middlesex, UK) was used, housed in a radial compression module (Z-module; Millipore-Waters).

Calibration curves were constructed using drug-free biological fluid containing known concentrations of AQ and AQm. One control sample was included in each run together with the calibration samples and the tests. Calculation of AQ or AQm concentration in the unknowns was made by comparison of the peak height ratios (AQ or AQm: internal standard) with those of the calibration samples.

Analytical recovery. Analytical recovery from urine and faeces was determined by comparison of peak height produced from extracted samples with peak height from stock solutions. Assay reproducibility was assessed intra-assay and inter-assay (over 2 months) at two concentrations for each compound, in each biological fluid.

Determination of radioactivity extractable into organic solvent from faecal homogenates

Faecal homogenates (1·0 mL) were extracted into dichloromethane by the method described above. After evaporation under a stream of nitrogen, samples were reconstituted in methanol (500 μL). Radioactivity was then determined by liquid scintillation counting. Control samples were carried through the extraction and were counted with the tests.

Aliquots of the same faecal samples (1·0 mL) were then incubated at 37°C for 24 h with an excess of sulphatase/glucuronidase (10 u/100 μL homogenate). Samples were then extracted and counted as before.

Calculations

Radioactive dose. The amount of radioactivity in each dose was calculated by liquid scintillation counting of 3 × 50–100 μL aliquots of the dosing solution; background counts were deducted from results. Syringes were weighed before and after administration.

Pharmacokinetics. Apparent elimination rate constants (λ_z) were calculated from the terminal phases of urinary and faecal excretion rate vs time curves by log-linear least-squares regression analysis. The apparent terminal half-life ($t_{\frac{1}{2}}$) of AQ was calculated from $0{\cdot}693/\lambda_z$.

Statistics. Urinary and faecal recovery of radioactivity after oral and i.v. administration were compared by Student's *t*-test, accepting $P \leqslant 0{\cdot}05$ as significant.

Results

HPLC assay sensitivity and reproducibility

AQ, AQm and internal standard were resolved to baseline with retention times of 3·0, 4·0 and 5·2 min, respectively. The minimum detectable concentration of AQ and AQm in rat urine and faecal homogenates was 10 ng mL^{-1}. Calibration curves were linear (r⩾0·99) in the ranges 0–1000 ng mL^{-1} and 0–2000 ng mL^{-1} for AQ and AQm, respectively, in urine, and 0–1000 ng mL^{-1} for both compounds in faecal homogenates. Coefficients of variation both intra-assay and inter-assay were ⩽11·0%.

Oral study

Following oral administration of [^{14}C]AQ 86±8·3% of the dose had been excreted by 72 h; 77±9% in faeces, 7±1% in urine, and 2±2% in cage washes. Fig. 1 shows the tissue distribution of the remaining detectable ^{14}C at 72 h; in all 90±9% of the administered dose was accounted for. Although the ^{14}C content of the red bone marrow could be measured, the total mass of marrow in each animal was unknown. However, Baker et al (1979) estimate bone marrow to account for 3% body weight, which allows an estimate to be made of the percentage of the ^{14}C-activity to be found in the marrow.

Radioactivity in the residual carcass was calculated for the first three animals and was found to be ~2%; because of difficulties in obtaining true homogeneity, this can be no more than an approximate value, and is therefore not included in Fig. 1.

The distribution of ^{14}C activity in the tissues expressed as percentage dose per μg tissue is shown in Fig. 2. The greatest concentration of ^{14}C was found in the kidneys and liver. The two haemopoietic tissues, marrow and spleen, were the next highest sites of accumulation.

Fig. 3 shows the percentage of orally administered ^{14}C-activity excreted per h, vs time for both faeces and urine (mean±s.d.). Urinary recovery of ^{14}C activity was low

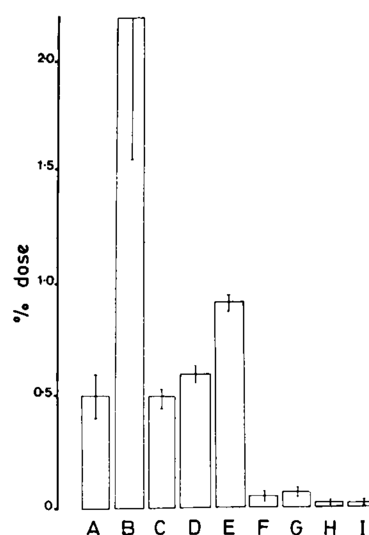

FIG. 1. Tissue distribution of radioactivity (% dose) 72 h after oral administration of [^{14}C]amodiaquine (mean±s.d.). Key: A kidneys, B liver, C marrow, D spleen, E gut, F lungs, G testes, H heart, I brain and eyes.

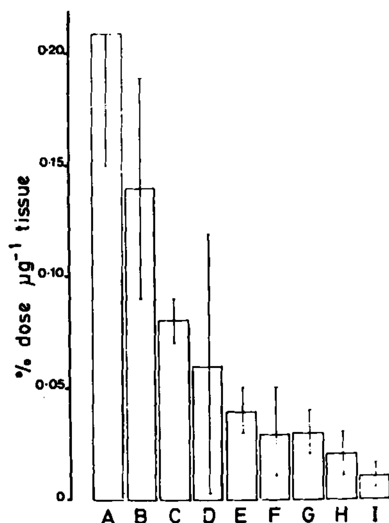

FIG 2. Tissue distribution of radioactivity (% dose μg^{-1} tissue) 72 h after oral administration of [^{14}C]amodiaquine (mean ± s.d.). Key as Fig. 1.

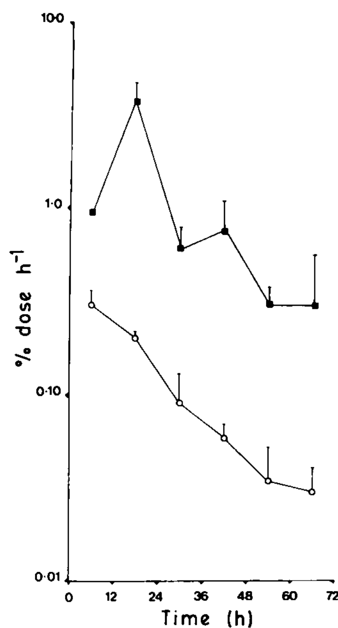

FIG. 3. Excretion rates of radioactivity (% dose h^{-1}) vs time (h) in faeces (■) and urine (○) following oral administration of [^{14}C]amodiaquine (mean ± s.d.)

(7 ± 1% dose) and declined in a log-linear fashion after the first 12 h collection. In contrast, faecal recovery of ^{14}C activity was high (77 ± 9% dose). In the first 12 h collection excretion rates varied widely. The peak excretion rate of ^{14}C was in the 12–24 h collection in the case of all 6 animals.

Figs 4 and 5 illustrate the excretion rates in urine and faeces of AQ and AQm vs time. Urinary recovery of AQ was 2·4 ± 0·5 µg (~0·1% of the dose) and of AQm was 18·5 ± 4·1 µg. The peak urinary excretion rate of AQ was see in the first 12 h collection of each animal, and AQ was not detectable in the urine after 24 h. In contrast AQm was detectable in the urine to 72 h; λ_z for AQm was 0·041 ± 0·005 h^{-1} (n = 6). Faecal recoveries of AQ and AQm were 210 ± 79 µg (10% of the dose) and 123 ± 32 µg, respectively. The peak excretion rate of AQ in the faeces was seen in the 12–24 h collections of all animals and fell rapidly; thereafter there was a slower elimination phase with $t_{\frac{1}{2}}$ = 10 ± 4 h (n = 4). AQ was detectable in the faeces up to 72 h. The peak excretion rate of the AQm in the faeces was also seen in the 12–24 h collections, and fell slowly thereafter; λ_z for AQm was 0·047 ± 0·005 h^{-1} (n = 3) from faeces.

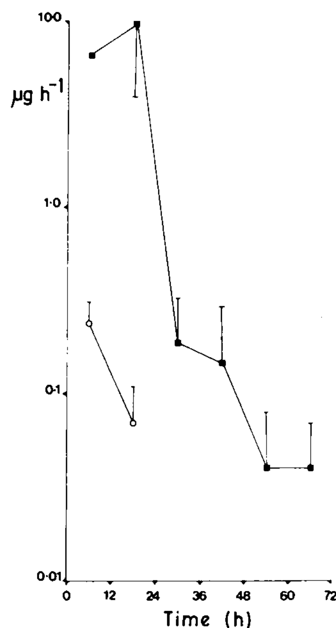

FIG. 4. Excretion rates of amodiaquine (µg h^{-1}) vs time (h) in faeces (■) and urine (○) following oral administration of [^{14}C]amodiaquine (mean ± s.d.).

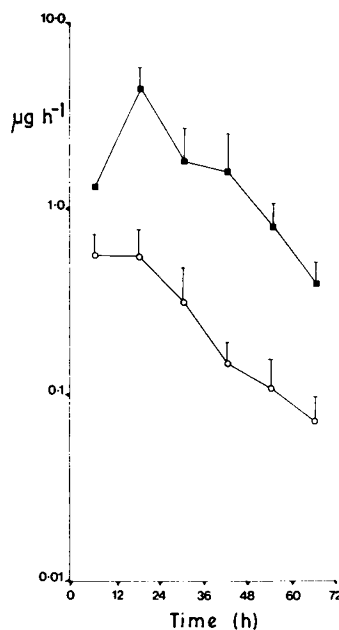

FIG. 5. Excretion rates of desethylamodiaquine (µg h^{-1}) vs time (h) in faeces (■) and urine (○) following oral administration of [^{14}C]amodiaquine (mean ± s.d.).

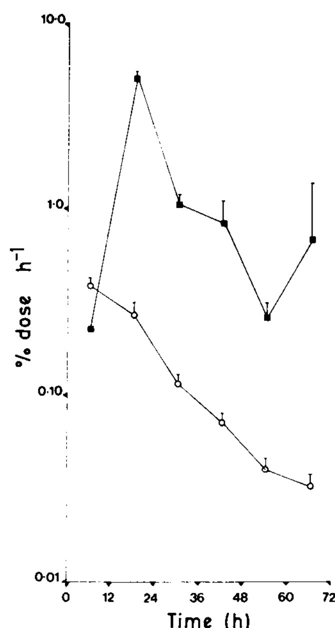

FIG. 6. Excretion rates of radioactivity (% dose h^{-1}) vs time (h) in faeces (■) and urine (○) following intravenous administration of [^{14}C]amodiaquine (mean ± s.d.).

Radioactivity extracted from faecal homogenates into organic solvent

Of the radioactivity present in each of the faecal homogenates examined, 10±5% could be extracted into organic solvent. Incubation of aliquots of the same homogenates with sulphatase/glucuronidase increased the amount of extractable radioactivity to 12·5±5%.

Intravenous study

Fig. 6 shows the percentage of intravenously administered ^{14}C excreted per h vs time for both faeces and urine. There were no significant differences in the rate or extent of urinary or faecal excretion of ^{14}C after i.v. administration compared with oral administration. Of the dose 102·6±9·7% was excreted by 72 h; 90·9±9·6% in faeces, 10·9±0·8% in urine and 0·5±0·2% in cage washes.

Autoradiography

Fig. 7 shows autoradiographs obtained from animals killed 0·5, 1, 3, 6, 24 and 48 h after oral administration of ^{14}C-activity. At 0·5 h, most of the radioactivity was associated with the gastrointestinal tract and contents, but radioactivity was also present in both liver and kidney. From 1–48 h autoradiographs showed progressively less radioactivity in the gastrointestinal tract, and greater amounts in the tissues. At 48 h significant amounts of radioactivity remained in the tissues. This was predominantly in liver and kidneys, but bone-marrow, brain, eye and intestinal walls could be visualized. Radioactivity was not detected in the blood.

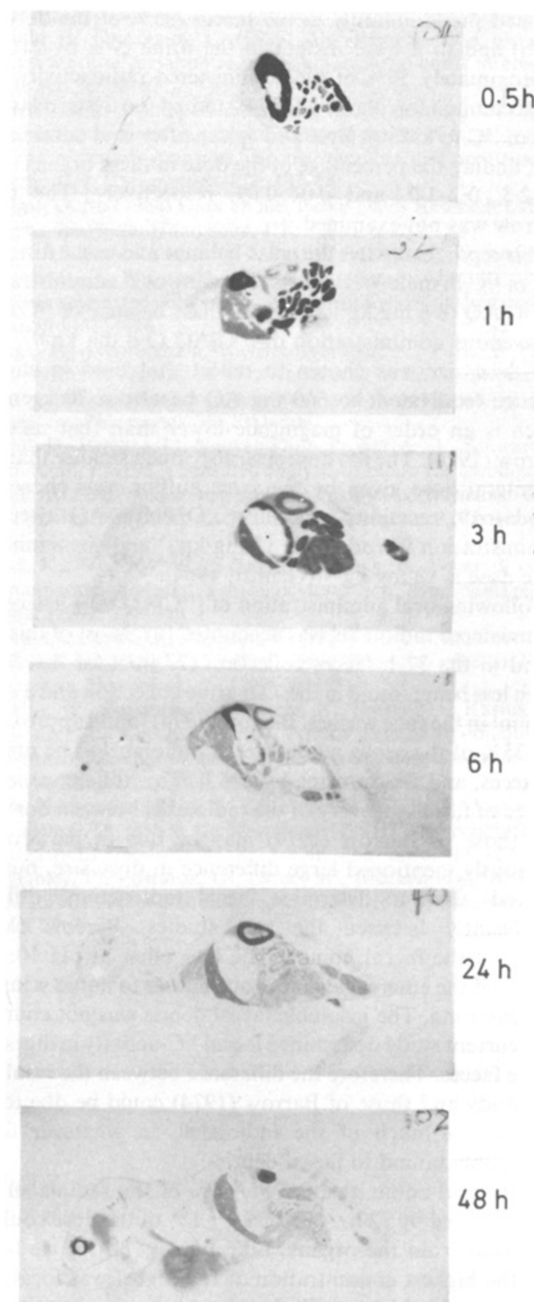

FIG. 7. Autoradiographs obtained from rats killed 0·5, 1, 3, 6, 24, 48 h after oral administration of [^{14}C]amodiaquine.

Discussion

The high prevalence of adverse effects with the prophylactic use of AQ is a cause of current concern, particularly since the drug remains in use for the treatment of acute infections of *P. falciparum* in some parts of the world. The clinical pharmacology of AQ is now better understood (Churchill et al 1985, 1986; Mount et al 1987; Winstanley et al 1987; White et al 1987) but information is needed concerning the absorption and excretion of AQ after oral administration, which is the route most commonly used, and its distribution to the tissues, in particular the red bone marrow.

Barrow (1974) administered [^{14}C]AQ to Wistar and Sprague-Dawley rats by oral and intraperitoneal (i.p.) routes (in doses of 100 and 30 mg kg^{-1}, respectively) collecting urine and faeces up to 240 h. The results of these excretion studies were similar for both oral and i.p. routes: the radiolabel was

excreted predominantly in the faeces (35% of the dose by 216 h) and to a lesser extent in the urine (9% by 216 h). Approximately 50% of the administered radioactivity was not accounted for. Barrow (1974) studied the tissue distribution of ^{14}C to kidney, liver and spleen after oral administration, finding the percentage of the dose in these organs to be 0·3–2·3, 0·2–1·7, and 0·04–0·09, respectively. The bone marrow was not examined.

This report estimates the mass-balance and tissue distribution of ^{14}C in male Wistar rats following oral administration of [^{14}C]AQ (8·6 mg kg^{-1}), and the mass balance of ^{14}C after intravenous administration of [^{14}C]AQ (3·8 mg kg^{-1}). The oral dose size was chosen to reflect that used in clinical practice (equivalent to 600 mg AQ base to a 70 kg man) which is an order of magnitude lower than that used by Barrow (1974). The i.v. dose size, also much smaller than the parenteral dose given by the same author, was chosen to avoid toxic reactions, since the LD50 for AQ after i.v. administration to rodents is 17 mg kg^{-1} and the minimum toxic dose is 10 mg kg^{-1} (Gruhzit 1964).

Following oral administration of [^{14}C]AQ 90 ± 9% of the administered radiolabel was accounted for. Most of this was found in the 72 h faeces collection (77 ± 9% of the dose), much less being found in the 72 h urine collection and a small amount in the cage washes. Barrow (1974) found approximately 35% of the orally administered radiolabel to be present in faeces, and 7% in urine by 216 h. This difference in the degree of faecal recovery of the radiolabel between our data and those of Barrow (1974) may be due in part to the previously mentioned large difference in dose size, but the methods used to determine faecal radioactivity differed significantly between the two studies. Barrow (1974) extracted the faecal homogenate into ether at pH 10, and subjected the ethereal and aqueous phases to liquid scintillation counting. The insoluble faecal debris was not counted. This current study determined faecal ^{14}C-activity in digests of whole faeces. Therefore the difference between the results of this study and those of Barrow (1974) could be due to the presence of much of the radiolabel, in whatever form, irreversibly bound to faecal debris.

After oral administration 86 ± 8% of the radiolabel had been excreted by 72 h. A further 4 ± 1% of the dose could be recovered from the organs. Like Barrow (1974) we found that the highest concentration of radiolabel was located in the kidneys and liver, with the next most concentrated sites being red bone marrow and spleen. This is in agreement with the observed toxic effects of AQ in man where agranulocytosis (Hatton et al 1986) and hepatitis (Neftel et al 1986) are the most serious manifestations. Powell & Curtis (unpublished observations) have demonstrated that in the rat isolated perfused liver system 29% of the administered dose of ^{14}C activity was associated with the liver at the end of perfusion, and of this only 0·9% could be dialysed. This suggests that tissue binding of AQ or its derivatives is irreversible. These findings are supported by work from Maggs et al (1987) using human liver microsomes in-vitro.

The presence in the faeces of 86% of an orally administered dose of radiolabelled drug could be due to its in complete absorption, or absorption followed by excretion into the gut via the bile. Poor absorption of AQ seems unlikely since clinical use of the drug over the last 40 years has demonstrated its therapeutic, and more recently toxic, effects. When [^{14}C]AQ was administered i.v. the faecal excretion of radiolabel by (0–72 h) was 91% of the dose. It would appear that the faecal excretion of radiolabel following oral administration of [^{14}C]AQ is largely the result of excretion into the gut of previously absorbed radiolabel. This is likely to represent biliary excretion, which is supported by the findings of Powell & Curtis (unpublished observations) who have administered [^{14}C]AQ into the duodenal lumen of wholly anaesthetized rats (n = 2). Biliary excretion of [^{14}C]AQ in this study accounted for 19 and 9% of the administered dose over 6 h, urinary excretion accounting for 1·4 and 2·3% of the dose over the same period.

AQ was detectable in the urine at low concentration in only the first two 12 h collections (Fig. 2). The faecal concentration of AQ could be determined to the end of collection; the peak excretion rate was seen in the 12–24 h samples and then fell steeply. Thereafter the excretion rate fell more slowly. It was possible to calculate $t_{\frac{1}{2}}$ for AQ from the faecal data of four animals and this value of 10 ± 4 h is of the same order of magnitude as previously reported in man (Winstanley et al 1987). AQm was detectable in urine and faeces to the end of sampling, the peak excretion rate was seen in the 12–24 h collections. The elimination rate constant (λ_z) for AQm could be calculated from both faeces and urine data, and was the same for both excreta.

Recovery of AQ and AQm in the faeces by 72 h after an oral dose of ~2·5 mg was 210 ± 79 and 123 ± 32 μg, respectively. However, 86 ± 8% of the administered radiolabel was present in these collections, and the sum of the two identified compounds would account for no more than 15%, suggesting that most of the radiolabel excreted in the faeces is not in the form of free AQ or AQm. This is in agreement with Barrow (1974) who found that only 3–20% of the ^{14}C voided in the faeces, after oral administration, could be recovered by ethereal extraction at pH 10. Thin layer chromatographic analysis of urine by Barrow (1974) revealed at least six radioactive compounds of which AQ accounted for $\leqslant 5\%$ of the radioactivity present on the plate. Churchill et al (1985, 1986) have identified three metabolites of AQ in man, and postulated the existence of a further two. HPLC traces of extracts of faecal homogenates obtained from rats after oral AQ in this study showed two peaks not present in the blanks. The smaller peak had a retention time of 2·1 min and was poorly resolved from the larger peak which eluted with a retention time of 2·4 min; both peaks preceded AQm.

In view of the low faecal recoveries of AQ and AQm, faecal homogenates were extracted with organic solvent, under the conditions used for HPLC analysis, before and after incubation with a deconjugating enzyme preparation. The resulting organic extracts were then subjected to liquid scintillation counting. Recovery of radiolabel was poor despite extraction efficiencies of 60 and 50% for AQ and AQm, respectively, from spiked samples of faecal homogenate. Incubation with deconjugating enzyme increased the extractable radiolabel from 10 ± 5 to 12·5 ± 5% indicating that glucuronide and sulphate conjugates of AQ or its metabolites are not major excretion products in the bile of Wistar rats. Few data are available on the protein binding of AQ, but Maggs et al (1987) have demonstrated non-enzymic autoxidation of AQ to a quinoneimine, leading to irreversible binding to human

liver microsomes in-vitro and have suggested a role for this compound in the observed toxicity. AQ is a potentially unstable molecule, and it is possible that much of the radiolabel excreted in the faeces of rats given [^{14}C]AQ orally may be in the form of breakdown products of AQ irreversibly bound to large molecules and thus unavailable for solvent extraction.

In summary, we have shown that after oral administration of [^{14}C]AQ to rats radiolabel is extensively absorbed, and subsequently excreted back into the gut. The principal route of excretion is faecal. Most of the radiolabel is excreted by 72 h but that which remains in the tissues is widely distributed and concentrates in liver and haemopoietic tissues, which are the sites of AQ toxicity in man. Little of the excreted radiolabel is in the form of AQ or AQm, either free or conjugated.

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