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Sulphation and diglucuronidation as constraints to enterohepatic circulation of dichlorophen in rats

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Dichlorophen (2,2′-dihydroxy-5,5′-dichlorophenylmethane, Anthiphen) is an anthelminthic used for the treatment of infestations with *Taenia saginata, T. solium*, the dwarf tapeworm *Hymenolepus nana* and the fish tapeworm in man and domestic animals (Martindale 1977). Dichlorophen is excreted in rat as sulphate, mono- and di-glucuronide conjugates but the monoglucuronide appears to undergo extensive enterohepatic circulation (Dixon & Caldwell 1978).

In the present study the influence of sulphation and di-glucuronidation on the enterohepatic circulation of dichlorophen is investigated in rat.

Materials and methods

[Methylene-^{14}C]dichlorophen was synthesized from [^{14}C]formaldehyde and 4-chlorophenol, with a radiochemical purity of >98% (Dixon & Caldwell 1978). Female vom strain rats (bred in our laboratories) 220–280 g, were bile-duct cannulated (Abou-el-Makarem et al 1967) 1 h, after oral administration of [^{14}C]dichlorophen (5 μCi kg^{-1}, 50 mg kg^{-1} dissolved in propane-1,2-diol) and the bile and urine collected for 3 h. The bile was infused into the duodenum, through a cannula in another set of bile-duct cannulated rats, whose bile and urine were also collected. This process was repeated with a third set of rats. In some cases the second set of rats had their portal veins cannulated and 1·0 ml of blood was withdrawn 40 min after infusion of bile into the duodenum. Five rats were used for each set of experiments.

The metabolites in urine, bile and blood were analysed by thin-layer chromatography and reverse isotope dilution before and after treatment with β-glucuronidase and sulphatase (Dixon & Caldwell 1978). The [^{14}C] metabolites were quantified by radiochromatogram scanning and scintillation counting.

Results and discussion

The bile/urine ratio for excretion of ^{14}C in the animals dosed with dichlorophen was 8·25 and this fell to 0·63 and 0·5 respectively for 2nd and 3rd cycles in the animals receiving bile containing dichlorophen monoglucuronide. The total recoveries in 3 h of administered ^{14}C (urine and bile) in the three groups were 37 and 34% respectively. In all three cases the only biliary metabolite was the monoglucuronide, while in the urine, dichlorophen sulphate and diglucuronide were the major metabolites (Table 1). The hepatic portal venous blood of the rats receiving bile contained principally free dichlorophen and its monoglucuronide (Table 2), but the diglucuronide was not found.

Table 1. Urinary metabolites of [^{14}C]dichlorophen in bile-duct-cannulated rats. Figures are means ± s.e., n = 5.

	% ^{14}C present as:		
Group of rats:	1	2	3
Dichlorophen	6 ± 1·2	8 ± 1·7	6 ± 0·2
Dichlorophen sulphate	60 ± 5·5	70 ± 4·0	85 ± 3·0
Dichlorophen diglucuronide	30 ± 2·2	15 ± 2·3	8 ± 1·2
Dichlorophen monoglucuronide	4 ± 0·2	2 ± 0·7	1 ± 0·1
% Administered ^{14}C recovered in 3 h	4 ± 0·2	21 ± 1·1	23 ± 1·2

Table 2. Metabolites of [^{14}C]dichlorophen in hepatic portal blood. Figures are means ± s.e., n = 5.

	% ^{14}C present in that form:
Dichlorophen	64 ± 4·0
Dichlorophen sulphate	6 ± 0·2
Dichlorophen diglucuronide	0
Dichlorophen monoglucuronide	30 ± 2·6

These data are consistent with the following scheme for the disposition of dichlorophen administered orally. The drug is absorbed from the gut and is conjugated to the sulphate and monoglucuronide in the gut wall. These together with the drug then pass to the liver, where further formation of monoglucuronide and perhaps sulphate also occurs. The diglucuronide is formed in the liver or other organs perfused by the systematic circulation. The sulphate and diglucuronide are eliminated from the peripheral circulation via the kidneys. However, the bulk of the monoglucuronide formed undergoes biliary excretion and is thus eliminated presystemically. On subsequent passages through the enterohepatic cycle, the monoglucuronide is broken down (Martiala 1973) and converted to sulphate and diglucuronide, conjugates more suitable for renal excretion, thus restricting its further enterohepatic circulation. The diglucuronide (molecular weight 621) satisfies the molecular weight requirement for biliary excretion (Millburn et al 1967) but it appears to be too water-soluble to be excreted through this route (Smith 1973).

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Experimental evidence of characteristic tissue distribution of adriamycin. Tissue DNA concentration as a determinant

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The characteristic tissue distribution of anthracycline antitumor agents, e.g. adriamycin (ADR) and daunorubicin, has been examined in man and animals (Alberts et al 1971; Yesair et al 1972; Bachur et al 1974; Tavoloni & Guarino 1980), and has shown the antibiotics to be well-distributed in tissues and to have clear differences in apparent tissue-to-plasma partition coefficients ($K_{p,app}$) among tissues. It has been reported that these antibiotics interact with DNA and chromatin (Zunino et al 1972; Sabeur et al 1979) to produce their activity (Di Marco et al 1975). The rapid tissue distribution and the nuclear localization of both agents, have also been demonstrated by fluorescence microscopy in normal hamsters (Egorin et al 1974). The nature of tissue specificity is usually an important determinant in drug distribution, so tissue distribution of ADR could depend on probable determinants such as tissue DNA concentration, affinity to DNA and/or a specific mechanism of plasma-membrane transport. To elucidate the mechanism of tissue distribution of ADR, we have demonstrated a preliminary approach to the relation between the in vivo tissue distribution of ADR and the amount of DNA in tissue (wet wt).

Adriamycin hydrochloride (ADR) was generously supplied by Kyowa Hakko Kogyo Co, Ltd (Tokyo). All other reagents are commercially available and of analytical grade. Adult male Wistar rats (Nihon Ikagaku Dobutsu, Tokyo), 245–255 g, and male albino rabbits (Ichikawaya, Tokyo), 2·6–2·8 kg, were used after overnight fasting.

For rats, ADR was dissolved in 0·9% NaCl (saline) and administered via a femoral vein at 10 mg kg^{-1} under light ether anaesthesia. After recovery from anaesthesia, animals had free access to food and water. At 6, 12, 24 and 48 h after the administration of antibiotic, blood samples were collected via a jugular artery and then the rat was killed by bleeding. Each tissue was immediately excised, rinsed with saline and stored at −40 °C. The urinary excretion of ADR was determined from the total amount excreted through a urinary bladder. ADR in plasma and tissues were determined by a t.l.c. scanning method according to Watson & Chen (1976), after extraction and development on t.l.c. plates by the method of Cradock et al (1973), in a Hitachi MPF-4 fluorospectrometer.

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Tissue DNA was extracted by the method of Schmidt-Thannhauser-Schneider (Schneider 1946) and was determined by the method of Burton (1956). The tissue was prepared in the same manner as for the in vivo ADR distribution study. The blood-to-plasma distribution ratio of ADR was estimated after injection of heparin at a dose of 0·1 ml/100 g (100 units), and the whole blood collected via a jugular artery at 30 min. Small samples (10 µl) of isotonic solutions containing various amounts of ADR ($2{\cdot}16 \times 10^{-5}$–$6{\cdot}47 \times 10^{-4}$ M) were then added in the test tubes containing 1·5 ml of blood for the high concentration and 5 ml for the low concentration of ADR, that had been preincubated for 3 min at 37 °C. The tubes were then incubated with shaking (2 Hz) for 5 min at 37 °C. Then, the plasma was separated by centrifugation at 3000 rev min^{-1}

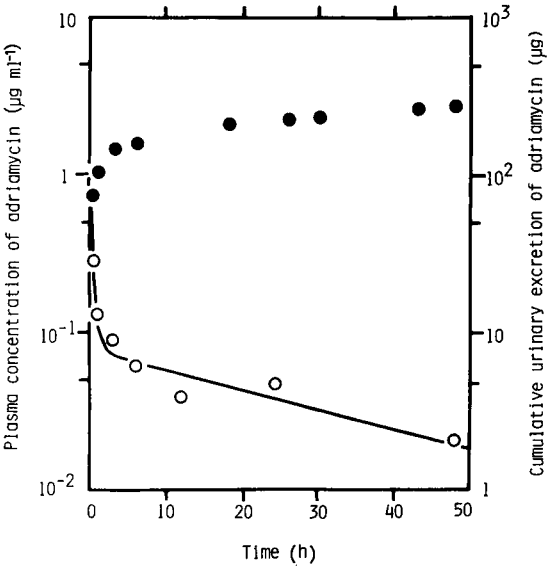

FIG. 1. Plasma disappearance and cumulative urinary excretion time course after intravenous administration of 10 mg kg^{-1} of adriamycin (ADR) in rats. Each point in urinary excretion represents the mean of 3 rats, while that in plasma concentration was obtained from the individual rat. The plasma disappearance curve was calculated by an iterative least squares method using a digital computer. Key: (○) plasma concentration; and (●) cumulative urinary excreted amount.