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Disposition and metabolism of [14-^{14}C] 4-demethoxydaunorubicin HCl (idarubicin) and [14-^{14}C]daunorubicin HCl in the rat

A comparative study

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Summary. The disposition of [14-^{14}C]4-demethoxydaunorubicin HCl ([14-^{14}C]idarubicin HCl, [^{14}C]IDR) and of [14-^{14}C]daunorubicin HCl ([^{14}C]DNR) was studied in male Sprague Dawley rats. [^{14}C]IDR was administered either IV at 0.25 mg/kg body weight or PO at 1 mg/kg body weight, whereas [^{14}C]DNR was dosed IV at 1 mg/kg body weight. The main elimination route for both compounds was the bile, fecal excretion representing 0.75–0.8 times the total dose at 72 h. Radioactivity due to [^{14}C]IDR-derived species is released by the tissues at a slower rate than activity derived from [^{14}C]DNR. After IV treatment comparable plasma levels are obtained, but tissue radioactivity is markedly lower with [^{14}C]IDR, in keeping with the lower dosage. The ratio of plasma to tissue radioactivity is even higher in animals treated PO with [^{14}C]IDR, because of the more extensive metabolism after this route of administration. The 13-dihydro derivatives of both [^{14}C]IDR and [^{14}C]DNR are the main metabolites in tissues, but in the case of the former, products of phase II reactions become more important at later times in liver and kidney and in excreta.

Introduction

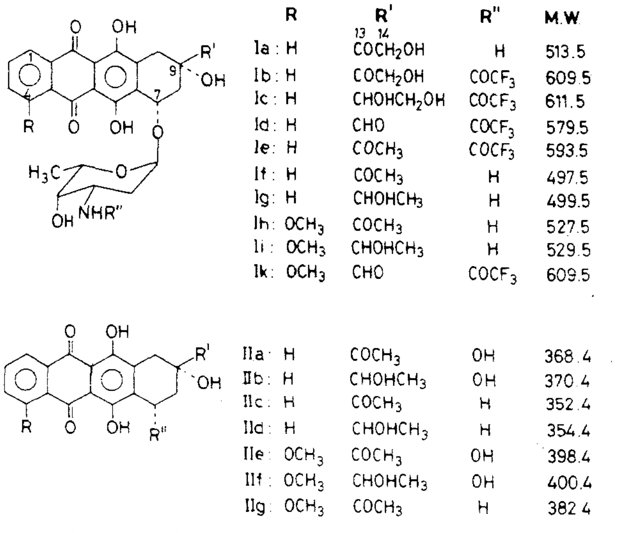
4-Demethoxydaunorubicin (idarubicin) is a new daunorubicin analogue endowed with outstanding antileukemic activity in different murine tumor models and is also active when administered by the oral route. The therapeutic index of idarubicin is more favorable than that of daunorubicin, particularly as far as cardiotoxicity in laboratory animals is concerned [2, 3, 14, 16]. Preliminary clinical results confirm the high therapeutic potency of idarubicin [9, 11, 20, 21]. We wish to report the synthesis of ^{14}C-labelled idarubicin and its disposition and metabolism in the rat in comparison with [^{14}C]daunorubicin.

The synthesis of [14-^{14}C]4-demethoxydaunorubicin HCl ([^{14}C]IDR) has been carried out by a modification of the procedure used for the synthesis of [14-^{14}C]daunorubicin HCl ([^{14}C]DNR) [23]. The rat has been used for the study of daunorubicin metabolism by various authors [1, 6, 7, 13, 15, 17, 22, 24, 25]. Idarubicin distribution in tumor-bearing mice has been investigated. Formelli et al. found that after IV administration of equal doses of DNR and IDR, levels of total drug-derived fluorescence of IDR were higher than those of DNR in all tissues analyzed, and that the rate of fluorescence disappearance from organs was slower in the animals treated with IDR than in those treated with DNR [18]. Broggini et al. suggested that IDR might show a more favorable tumor-to-tissue distribution ratio than the parent DNR, expecially after oral administration, in agreement with previous observations [12].

Materials and methods

Labelled compounds. [^{14}C]DNR was prepared starting from [^{14}C]diazomethane via 9-deacetyl-9-formyl-*N*-trifluoroacetylDNR (Ik, Fig. 1). The radiochemical purity was ≥98% as determined by TLC (silicagel Merck F 254 plates; system A, Table 1), and different batches of the labelled drug with specific activity between 152 MBq/mmol or 4.13 mCi/mmol (271 KBq/mg or 7.32 µCi/mg) and 996 MBq/mmol or 26.9 mCi/mmol (1766 KBq or 47.73 µCi/mg) were prepared.

Isotopically labelled IDR was prepared starting from 4-demethoxydoxorubicin (Ia) by the following synthetic procedure. *N*-Trifluoroacetyl-4-demethoxydoxorubicin (Ib), obtained from 1a (371 µmol as the hydrochloride) by



	R	R'	R''	M.W
Ia:	H	$COCH_2OH$	H	513.5
Ib:	H	$COCH_2OH$	$COCF_3$	609.5
Ic:	H	$CHOHCH_2OH$	$COCF_3$	611.5
Id:	H	CHO	$COCF_3$	579.5
Ie:	H	$COCH_3$	$COCF_3$	593.5
If:	H	$COCH_3$	H	497.5
Ig:	H	$CHOHCH_3$	H	499.5
Ih:	OCH_3	$COCH_3$	H	527.5
Ii:	OCH_3	$CHOHCH_3$	H	529.5
Ik:	OCH_3	CHO	$COCF_3$	609.5
IIa:	H	$COCH_3$	OH	368.4
IIb:	H	$CHOHCH_3$	OH	370.4
IIc:	H	$COCH_3$	H	352.4
IId:	H	$CHOHCH_3$	H	354.4
IIe:	OCH_3	$COCH_3$	OH	398.4
IIf:	OCH_3	$CHOHCH_3$	OH	400.4
IIg:	OCH_3	$COCH_3$	H	382.4

Fig. 1. Structural formulas and molecular weights of intermediates in the synthesis of [^{14}C]DNR. Identified metabolites of the two drugs are also shown

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reaction with trifluoroacetic anhydride in methylene chloride at 0 °C, was submitted to reduction with 100 mg $NaBH_3CN$ in methanol at pH 4 and room temperature (rt) with stirring. The reduction of the 13-keto group was completed in 48 h to give crude compound Ic, which was extracted from the reaction mixture with 1-butanol and then purified by preparative TLC (silicagel Merck plates 2 mm thick; $CHCl_3$/MeOH 8 :2 by volume). Field Desorption Mass Spectrometry (FDMS), NMR, and TLC analysis of Ic confirmed the expected structure and that its degree of purity was adequate for the next step.

Compound Ic (222 µmol) dissolved in a mixture of 2-methyl-2-propanol: water (1 :1 by volume) was submitted to oxidation with sodium periodate (243 µmol) at rt to give compound Id (reaction completed in about 90 min). The resulting crude product was extracted from the reaction mixture with chloroform and then purified by TLC (silicagel Merck plates 2 mm thick; $CHCl_3$:MeOH 8 :2 by volume) to give 155 µmol authentic Id (identified by FDMS: main peak with m/z = 579) but not sufficiently chromatographically pure. This sample was mixed with another one obtained from a different preparation carried out starting from 632 µmol Ib. The resulting amount of impure Id (about 381 µmol) was submitted to further purification by column chromatography (silicagel Merck 60; 70–230 mesh; 10 mm ID × 35 cm high). Pure Id (286 µmol) was obtained after elution with chloroform containing increasing amounts of acetone (up to 30% by volume). [^{14}C]Diazomethane (prepared from 370 MBq/10 mCi, about 400 µmol, [^{14}C]methylamine HCl purchased from Amersham Int. UK [10], was added with the aid of a vacuum manifold to 280 µmol Id in 10 ml of a mixture of CH_2Cl_2: ethyl ether 1 :1 by volume (for experimental details see [27]. After 60 min stirring at rt in vacuo, the crude product was purified by preparative TLC (silicagel Merck plates 2 mm thick chloroform: acetone 7 :3 by volume) giving 24 µmol 97% radiochemically pure [14-^{14}C]4-demethoxy-*N*-trifluoroacetyldaunorubicin (Ie). This compound was submitted to a mild alkaline hydrolysis at 0°C for 60 min in 0.1 *N* NaOH (10 ml) and gave, after extraction with chloroform and salification with 0.1 *N* methanolic HCl, 21 µmol [^{14}C]IDR (If), which was 96% pure with a specific activity of 793 MBq/mmol (21.4 mCi/mmol) (4.5% radiochemical yield based upon [^{14}C]methylamine HCl).

Formulation of the radiolabelled drugs for IV injection was performed by dissolving [^{14}C]IDR or [^{14}C]DNR in aqueous NaCl solution (0.9% w/v) to give final drug concentrations of 0.13 and 0.5 mg/ml, respectively. Amounts of the said solutions corresponding, respectively to doses of 0.25 and 1.0 mg/kg were injected via the tail vein. For oral experiments an aqueous solution of [^{14}C]IDR (0.5 mg/ml) was administered at a dose of 1 mg/kg. Dosages were chosen as equal fractions of the acute LD_{50} in the rat and were within pharmacologically effective ones [9, 11, 14, 16, 20, 21].

Animals. Male Sprague-Dawley CD outbred rats (Charles River Italia) weighing 190–230 g were used. The animals were kept in all-glass Jencons Mark III metabolic cages starting 5–10 days before treatment and were maintained on a normal laboratory diet (Charles River type 4RF21). The animals were sacrificed by bleeding of the abdominal aorta under ether anesthesia at 40 min, 4 h, 24 h, and 72 h after drug administration. In the biliary excretion experiments, rats, after biliary duct cannulation with a polyethylene tube (PE-Clay Adams USA) under halothane anesthesia, were housed in single cages for at least 1 h before IV drug administration. The animals were allowed free movement in the cage and were given drinking water ad libitum throughout the period of bile collection.

Determination of radioactivity. Expired air was bubbled through a 20% (by volume) ethanolamine-methyl alcohol mixture by means of a stream of decarbonated air. Samples of ethanolamine and of collected biological fluids were counted by liquid scintillation counting. Radioactivity levels in lyophilized tissues and feces were determined by the combustion technique (Packard Tricarb oxidizer model 306) [5].

Identification of drug and metabolites. Urine. Urine samples were extracted by absorption on Amberlite XAD-2 columns according to Fujimoto and Haarstad [19] and elution of the adsorbed radioactivity with methanol containing 3% acetic acid. This procedure gave a methanolic eluate containing from 66% up to nearly 100% of urinary radioactivity. Methanolic eluates were concentrated to 0.5–1 ml under reduced pressure and analyzed by TLC with solvent system A in comparison with standard compounds (Table 1). Radiochromatographic patterns were obtained with a Radiochromatoscanner Packard 7201. The presence of conjugates was checked by enzymic hydrolysis of samples containing at least 0.5 KBq (13.5 nCi) in pH 5.4 0.1 *M* acetate buffer with 50 µl glucuronidase and arylsulfatase solution (12 and 60 units/ml, respectively; Merck) at 42 °C for 16 h. The hydrolysates were extracted and analyzed as already described for nonhydrolyzed samples.

Tissues. Samples of tissues homogenates were mixed with 50 µg unchanged drug and its 13-dihydro derivatives in a 1 :1 ratio as nonradioactive carriers. Four extractions with 8–10 ml methanol :acetic acid (97 :3 by volume) gave a hydroalcoholic solution, which was centrifuged (14 000 rpm for 2 h at −5 °C) and analyzed by radio-

Table 1. R_f values of idarubicin (4-demethoxydaunorubicin IDR), daunorubicin (DNR), idarubicin aglycone (4-demethoxydaunomycinone IDRA), daunorubicin aglycone (daunomycinone DNRA), and related compounds in thin-layer chromathography [a]

Compound	A [c]	B [c]	C [c]
IDR (If) [b]	0.86	0.82	0.00
13-DihydroIDR (Ig) [b]	0.76	0.67	0.00
IDRA (IIa)	0.99	0.97	0.32
13-DihydroIDRA (IIb)	0.96	0.94	0.14
7-DeoxyIDRA (IIc)	0.99	0.99	0.49
7-Deoxy-13-dihydroIDRA (IId)	0.97	0.99	0.18
DNR (Ih) [b]	0.70	0.63	0.00
13-DihydroDNR (Ii) [b]	0.57	0.45	0.00
DNRA (IIe)	0.97	0.96	0.36
13-DihydroDNRA (IIf)	0.94	0.93	0.16
7-DeoxyDNRA (IIg)	0.98	0.97	0.51

[a] Silicagel F 254 Merck plates-thickness 0.5 mm

[b] As the hydrochloride

[c] The following solvent systems were used: A, chloroform/methanol/acetic acid/water (60:20:14:6 by volume); B, chloroform/methanol/acetic acid/water (80:20:14:6 by volume); C, chloroform/methanol/acetic acid (95:3:2 by volume)

chromatography (TLC, system A). The radioactivity of the remaining solids was determined by the combustion technique.

Bile. Samples were collected in aluminium foil-covered, ice-cooled glass ampoules at 8 and 20 h after IV administration of the drugs, and were stored at −20 °C. Volumes ranging from 50 to 200 µl of bile were applied as a band onto the concentrating zone of 5 × 20 TLC silica gel 60 *F* 254 plates 0.5 mm thick and developed in solvent system A. The radiochromatographic pattern was determined in a Packard model 7201 Radiochromatoscanner. In addition to aglycone, unchanged drug and its 13-dihydro derivative, three polar fractions were obtained, the first (fraction A) representing the radioactivity adhered to the concentrating zone, and the two others (fractions B and C) corresponding to the radioactivity in the R_f ranges 0–0.26 (0.0–0.42) and 0.26–0.55 (0.41–0.56), respectively (data in parenthesis refer to DNR fractions). Polar fractions A, B, and C from IDR samples were separated by scraping and extracted first with a mixture of methanol, water, and acetic acid (5 : 4 : 1 by volume) and then twice with 10% acetic acid in methanol. Pooled extracts were evaporated under vacuum, lyophilized, dissolved in water, and incubated for 4 h at 40 °C in 0.2 *M*, pH 5.2 acetate buffer with 50 µl enzyme solution (see above). Hydrolysates were directly applied onto TLC semipreparative plates (0.5-mm-thick silicagel F 254, Merck) and developed first in solvent system C and subsequently in solvent system B, chromatograms being scanned as above.

Statistical evaluation of data. Experimental data regarding levels of tissue radioactivity have been transformed into logarithmic ones according to an exponential decay model. After the homogeneity of variance had been tested by means of the Bartlett test [8], a two-way analysis of variance was performed, the parameters evaluated being concentration and time. Regression, curvature, and parallelism were evaluated, and on this basis different groups of organs and tissues with similar behavior were identified.

Results

Excretion

Recovery of radioactivity in excreta and in tissues of animals at different times after IV administration of the labelled drugs is presented in Table 2. Radioactivity detectable in expired air was measured in the 24-h animals and found to be 0.4% (SD = ±0.2, $n = 3$) of the dose for $[^{14}C]$IDR and 0.3% (SD = ±0.2, $n = 3$) for $[^{14}C]$DNR. As already observed for other radioactive anthracyclines [5, 19], most of the radioactivity is recovered in the feces, indicating that biliary excretion is the main route of elimination for this class of compounds. The tissue disposition of DNR is clearly faster than that of IDR. As a consequence, tissues contain a higher proportion of administered radioactivity in the rats treated with $[^{14}C]$IDR than in those treated with $[^{14}C]$DNR, the difference being statistically significant ($P \leq 0.05$). Biliary excretion experiments confirmed the lower rate of elimination of IDR than of DNR (Table 3). The difference between the two drugs in the radioactivity measured in the bile at 20 h was statistically significant ($P \leq 0.05$).

After oral administration (Table 2) urinary excretion of radiolabel (expressed as a percentage of the dose) is approximately 50% of the value obtained after IV administration. It is noteworthy that tissue radioactivity at 24 h is less than 20% of the amount found in the corresponding IV-treated rats. This observation might indicate a different degree of biotransformation of the drug when administered PO (first-pass effect) than when administered IV.

Tissue distribution

Levels of radioactivity in tissues of rats treated IV are shown in Tables 4 and 5. After a 40-min interval, radioactivity of tissues such as liver, kidney, lung, and spleen decreased, whereas bone marrow, thymus, hypophysis, and lymph nodes maintained sustained levels of radioactivity up to 24 h, the thymus, hypophysis, and lymph nodes appearing, together with liver, the most radioactive tissues at

Table 2. Recovery of radioactivity[a] in excreta and tissues of Sprague-Dawley rats of different times after IV administration of $[^{14}C]$DNR (Ih) and IV and oral administration of $[^{14}C]$IDR (If)

Drug (route)	Time (h)	Dose (mg/kg)	Urine	Feces	GI content	Tissues	Total
DNR	40[b]	0.99 ±0.01	n.d.	3.09 ±0.10	19.17 ±2.39	66.20 ±1.16	88.46 ±1.87
(IV)	4	0.88 ±0.01	5.04 ±2.47	0.62 ±0.54	26.07 ±6.34	51.50 ±5.51	83.23 ±3.49
	24	1.05 ±0.05	15.08 ±1.00	56.98 ±8.60	10.36 ±3.27	16.09 ±3.93	98.50 ±2.71
	72	0.95 ±0.01	14.52 ±0.78	69.80 ±1.94	1.07 ±0.15	3.28 ±0.71	88.67 ±0.89
IDR	40[b]	0.25 ±0.02	n.d.	0.88 ±0.49	8.14 ±2.06	95.33 ±3.09	104.34 ±1.71
(IV)	4	0.28 ±0.01	2.43 ±0.44	0.18 ±0.13	25.51 ±0.65	65.78 ±1.90	93.90 ±1.83
	24	0.25 ±0.02	12.04 ±1.89	36.26 ±4.79	15.34 ±3.67	26.20 ±1.61	90.26 ±5.31
	72	0.24 ±0.02	16.09 ±0.77	81.43 ±0.34	1.04 ±0.25	4.41 ±0.61	104.97 ±1.14
IDR	4	0.95 ±0.18	1.51 ±1.22	0.03 ±0.02	62.12 ±8.49	35.11 ±12.68	99.41 ±3.96
(PO)	24	1.01 ±0.40[c]	5.33 ±0.66	75.71 ±5.82	8.65 ±3.79	4.90 ±0.88	94.73 ±3.20

[a] Data are expressed as mean (± SD, $n = 3$) percentages of dose

[b] min

[c] $n = 4$

Table 3. Cumulative biliary excretion of radioactivity after IV administration of [^{14}C]IDR (If) (0.23) mg/kg b.w.) or [^{14}C]DNR (Ih) (0.96 mg/kg b.w.) to male Sprague-Dawley rats[a]

Compound	Time after administration (h)	
	8	20
4-Demethoxydaunorubicin	12.5 ± 3.2	27.3[b] ± 8.0
Daunorubicin	42.7 ±12.8	59.1[b] ±12.6

[a] Data are expressed as percentages of dose (mean ± SD, $n = 3$)
[b] $P \leq 0.05$

72 h. In heart tissues and in muscles the specific radioactivity at 24 h is approximately 10 times lower than the corresponding value at 40 min after treatment. Within the different parts of the heart, the auricles, especially the left ones, show a lower rate of elimination of radioactivity than the other heart tissues.

Statistical analysis of the experimental data reported in Tables 4 and 5 showed that the rate of elimination of radioactivity for the two drugs is substantially similar in heart tissues (with the single exception of left auricle), kidney, diaphragm, intestinal tissue, thymus, testes, and hypophysis. On the other hand, other organs showed a signi-

Table 4. Specific tissue radioactivity[a] at various times after IV administration of 0.25±0.02 ($n = 12$, ±SD) mg/kg [^{14}C]IDR (If) to male Sprague-Dawley rats

Tissue	40 min	4 h	24 h	72 h
Blood[b]	25.5 ± 2.18	23.7 ± 3.07	8.57± 0.737	2.83± 0.557
Plasma[b]	12.9 ± 2.84	20.4 ± 3.99	8.66± 1.10*	3.20± 0.577
Liver	786 ± 76.4	504 ± 51.0	214 ±117	129 ±35.8
Kidney	1439 ± 68.9	851 ± 72.9	205 ± 40.8	30.8 ± 6.66
Lung	2091 ±273	1423 ±132	255 ± 44.1	37.5 ± 3.27
Spleen	2025 ±527	1549 ±108	479 ± 87.1	64.0 ±10.2
Brain	9.18± 0.367	7.96± 1.00	4.19± 0.552	2.14± 0.380
Bone marrow	432 ± 44.3	562 ±169	641 ±129	59.9 ± 5.21
Testes	17.9 ± 1.45	22.1 ± 5.88	20.3 ± 0.252	17.5 ± 1.95
Thymus	195 ± 39.3	252 ± 13.1	390 ± 84.0**	302 ±23.4
Hypophysis	701 ±233	959 ± 71.7	461 ± 58.7***	217 ±39.0
Lymph nodes (neck)	604 ± 89.4	836 ± 55.5	504 ±165	99.1 ±15.4
Suprarenal glands	1467 ±332	921 ± 33.9	216 ± 40.4	32.9 ± 5.18
Intestine (tissue)	473 ± 46.4	458 ± 24.1	183 ± 27.1	14.8 ± 1.50
Heart (in toto)	615 ± 91.6	263 ± 18.7	64.0 ± 14.4	8.14± 0.178
Left auricle	691 ± 80.2	329 ± 29.2	104 ± 22.9	19.7 ± 2.83
Muscle (striatum)	226 ± 25.0	152 ± 12.5	29.8 ± 4.86	3.44± 0.243

[a] Data are expressed as mean ($n = 3$, ±SD) nanogram equivalents of drug per gram of fresh tissue
[b] Read ng/ml
* $P \leqslant 0.01$; ** $P \leqslant 0.05$; *** $P < 0.10$, i.e., statistically significant differences from values recorded after administration PO

Table 5. Specific tissue radioactivity[a] at various times after IV administration of 0.97±0.07 ($n = 12$, ±SD) mg/kg [^{14}C]DNR (Ih) to male Sprague-Dawley rats

Tissue	40 min	4 h	24 h	72 h
Blood[b]	67.9± 9.46	29.6± 2.84	20.1± 2.69	4.37± 1.39
Plasma[b]	30.5± 2.13	16.6± 3.21	13.6± 3.91	3.25± 1.08
Liver	1846 ±144	585 ±114	197 ± 27.1	40.4 ± 13.1
Kidney	3872 ±314	1400 ±310	414 ±110	47.9 ± 5.06
Lung	4337 ±211	1661 ±515	585 ±109	96.8 ± 6.73
Spleen	3948 ±483	2791 ±563	1747 ±248	228 ± 3.00
Brain	24.6± 1.00	15.6± 4.64	10.9± 3.51	2.84± 0.23
Bone marrow	1443 ± 27.5	1406 ±278	1578 ±246	293 ± 39.8
Testes	56.6± 11.5	40.0± 18.9	66.8± 14.1	46.5 ± 6.67
Thymus	386 ± 31.9	341 ± 97.9	811 ±132	358 ±276
Hypophysis	2782 ±539	2083 ±351	1576 ±405	497 ±155
Lymph nodes (neck)	1282 ±115	1090 ±203	1448 ±409	340 ± 34.2
Suprarenal glands	2971 ±963	2379 ±365	1023 ±276	120 ± 48.6
Intestine (tissue)	1512 ±174	993 ±275	388 ± 57.7	47.9 ± 14.3
Heart (in toto)	2242 ±204	774 ±213	241 ± 33.2	21.7 ± 0.945
Left auricle	2380 ±198	1144 ±374	356 ±104	25.6 ± 12.4
Muscle (striatum)	650 ±103	339 ± 81.0	120 ± 15.0	10.5 ± 1.67

[a] Data are expressed as mean ($n = 3$, ±SD) nanogram equivalents of drugs per gram of fresh tissue
[b] Read ng/ml

Table 6. Specific tissue radioactivity[a] at various times after oral administration of [^{14}C]IDR (If) to male Sprague-Dawley rats

Time (h)	4	24
Number of animals	3	4
Dose (mg/kg) (mean ± SD)	0.95 ± 0.18	1.01 ± 0.04
Tissue	ng/g	ng/g
Blood[b]	189 ± 34.1	16.1 ± 3.39
Plasma[b]	255 ± 61.1	18.5 ± 3.73*
Liver	1217 ± 322	202 ± 35.3
Kidney	1217 ± 139	152 ± 82.5
Lung	1101 ± 611	206 ± 97.2
Spleen	726 ± 328	324 ± 101
Brain	31.8 ± 9.46	5.25 ± 1.84
Bone marrow	251 ± 93.5	367 ± 40.4
Testes	53.4 ± 20.0	15.3 ± 2.74
Thymus	105 ± 26.0	203 ± 39.7**
Hypophysis	336 ± 155	339 ± 54.1[c,]***
Lymph nodes (neck)	302 ± 174	361 ± 33.7[c]
Suprarenal glands	314 ± 251	179 ± 47.6
Stomach (tissue)	3414 ± 2688	76.6 ± 35.1[c]
Duodenum (tissue)	2404 ± 814	154 ± 63.6
Intestine (tissue)	2806 ± 2584	250 ± 106
Heart (in toto)	225 ± 80.7	42.3 ± 10.1
Left auricle	287 ± 112	66.1 ± 17.1
Muscle (striatum)	84.1 ± 25.9	18.2 ± 6.02

[a] Data are expressed as mean (± SD) nanogram equivalents of drug per gram of fresh tissue

[b] Read ng/ml

[c] $n = 3$

* $P \leqslant 0.01$; ** $P \leqslant 0.05$; *** $P \leqslant 0.10$, i.e. statistically significant differences from values recorded after IV administration

ficantly or highly significantly different behavior in the variation of tissue levels of ^{14}C over time. Such tissues are liver, spleen, left auricle, lung, lymph nodes, bone marrow, and brain.

The distribution of radioactivity in different tissues after oral administration of [^{14}C]IDR is shown in Table 6. At 4 h after administration the highest levels of ^{14}C were found in the tissues of the gastrointestinal tract. A high level of radioactivity was also found in plasma (more than 10 times the value after IV administration), but lower levels were found in slow-eliminating tissues such as bone marrow, thymus, hypophysis, and lymph nodes. In the 24-h animals the picture is more similar to that observed after IV treatment, although levels of ^{14}C in the slow-eliminating tissues are still lower. Notwithstanding the significantly higher ($P \leq 0.01$) plasma levels recorded after oral treatment, lower values of radioactivity were found in heart tissues than after administration by the IV route (Tables 4 and 6).

Drug metabolites

Urine. The extraction procedure of urinary samples allowed recovery of about 80% of urinary radioactivity in the methanolic eluate. The distribution of extracted radioactivity in four main TLC fractions at different times after administration is presented in Fig. 2. Aglycones were always less than 3% of total radioactivity in the samples. It is clearly apparent that 13-dihydroIDR (Ig) is the main radioactive species in the urines. It represents almost 60% of

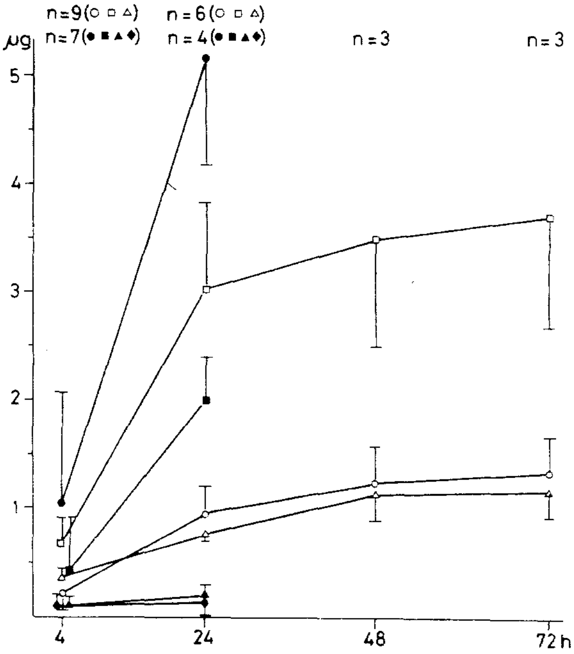

Fig. 2. Distribution of urinary radioactivity in polar metabolites (○, ●), 13-dihydroIDR (Ig) (□, ■), IDR (If) (△, ▲), and aglycones (◆), as deduced from TLC analysis with solvent system B. Data are expressed as cumulative mean microgram drug equivalents excreted per animal at indicated times (± SD, n as shown) after IV (0.25 mg/kg b.w., *open symbols*) or oral (1 mg/kg, b. w., *filled symbols*) administration of [^{14}C]IDR to male Sprague-Dawley rats

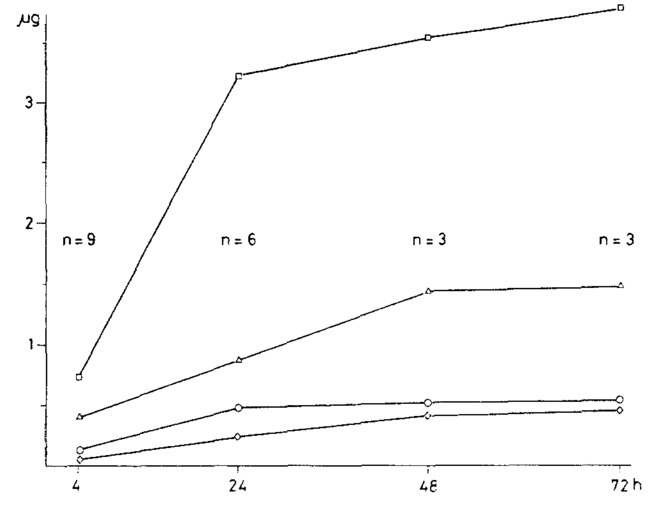

Fig. 3. Distribution of urinary radioactivity after enzymic treatment in polar metabolites (○), 13-dihydroIDR (Ig) (□), IDR (If) (△), and aglycones (◇), as deduced from TLC analysis with solvent system B. Data represent the microgram drug equivalents present in the pooled excreta of *n* animals divided by *n* at indicated times after IV administration (0.25 mg/kg b.w.) of [^{14}C]IDR to male Sprague-Dawley rats

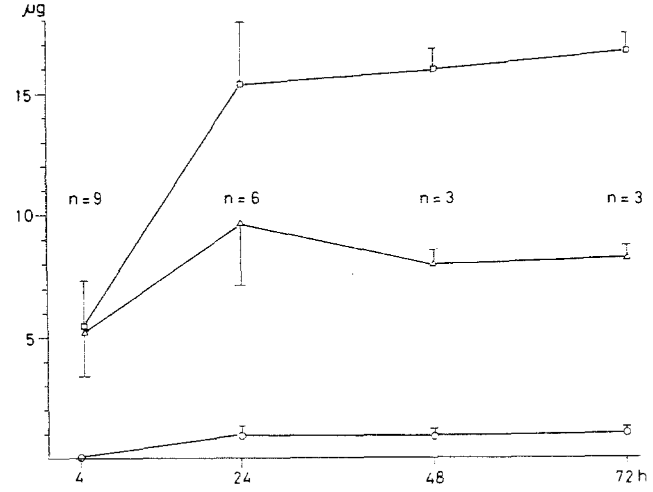

Fig. 4. Distribution of urinary radioactivity in polar metabolites (○), 13-dihydroDNR (Ii) (□), and DNR (Ih) (◇), as deduced from TLC analysis with solvent system B. Data are expressed as cumulative mean microgram drug equivalent excreted per animal at indicated times (±SD, *n* as shown) after IV administration (1 mg/kg b.w.) of [^{14}C]DNR to male Sprague-Dawley rats

total excreted radioactivity at 3 days after drug injection, whereas the unchanged drug and the polar fraction (the sum of all chromatographic peaks with R_f values lower than that of 13-dihydroIDR) each account for approximately 20%. In contrast, polar metabolites represent by far the main radioactive species excreted in the urine after oral administration. Another difference measured between the orally and IV treated animals is seen in the relative amounts of unchanged drug and dihydro derivative, the latter being in greater excess in orally treated animals. As shown in Fig. 3, the amount of polar metabolites in the urine of IV-treated animals is substantially reduced after enzymic hydrolysis, with concomitant appearance of non-polar metabolites (aglycones). The same result is obtained after enzymic hydrolysis of urines derived from orally treated animals. In both cases, conjugation of the aglycones appears to be the main phase II reaction [28] of idarubicin metabolism in the rat. However, an important fraction of the polar metabolites is not readily converted to aglycones by the beta glucuronidase-arylsulphatase treatment and therefore cannot be chemically characterized at present. On the other hand, in the urines of rats treated with [^{14}C]DNR the main radioactive species are the 13-dihydroderivative and the unchanged drug, whereas the polar fraction is less than 5% of total excreted radioactivity (Fig. 4).

Bile

The different order of magnitude in the amount of radioactive species excreted in the bile (Table 7) by the two compounds in clearly explained by the different dosage and the different rate of excretion in the bile between the two drugs. Radioactivity in the polar fractions represents almost 80% of total biliary radioactivity in [^{14}C]IDR-treated animals, whereas the said fractions account for less than 25% in the case of [^{14}C]DNR-treated ones. Because of the importance of the establishment of the chemical identity of [^{14}C]IDR-derived polar fractions, these were submitted to enzymic hydrolysis. In the conditions described in *Materials and methods*, fractions A and B failed to give less polar species, whereas fraction C gave rise to a major peak corresponding to 7-deoxy-13-dihydro-4-demethoxydaunomycinone (IId).

Tissue extracts

Extraction of liver, kidney, spleen, and lung tissues from IV-treated animals allowed recovery of approximately 70% of tissue radioactivity. For the 72-h tissues, recoveries were

Table 7. Distribution of radioactivity[a] recovered in rat bile excreted in the time interval 0–8 h after IV administration of [^{14}C]IDR (If) (0.23 mg/kg) or [^{14}C]DNR (Ih) (0.96 mg/kg) in different chromatographic fractions

Compound administered	Polar fractions			13-Dihydro derivative	Unchanged drug	Aglycones
	A	B	C			
[^{14}C]IDR	1.2 ± 0.7	3.3 ± 1.4	1.2 ± 0.3	0.8 ± 0.3	0.5 ± 0.2	0.4 ± 0.3
[^{14}C]DNR	0.0	15.4 ± 1.8	9.1 ± 2.8	35.2 ± 5.5	39.6 ± 17.6	5.6 ± 2.5

[a] Data are expressed as total excreted microgram drug equivalents (mean ±SD, *n* = 3)

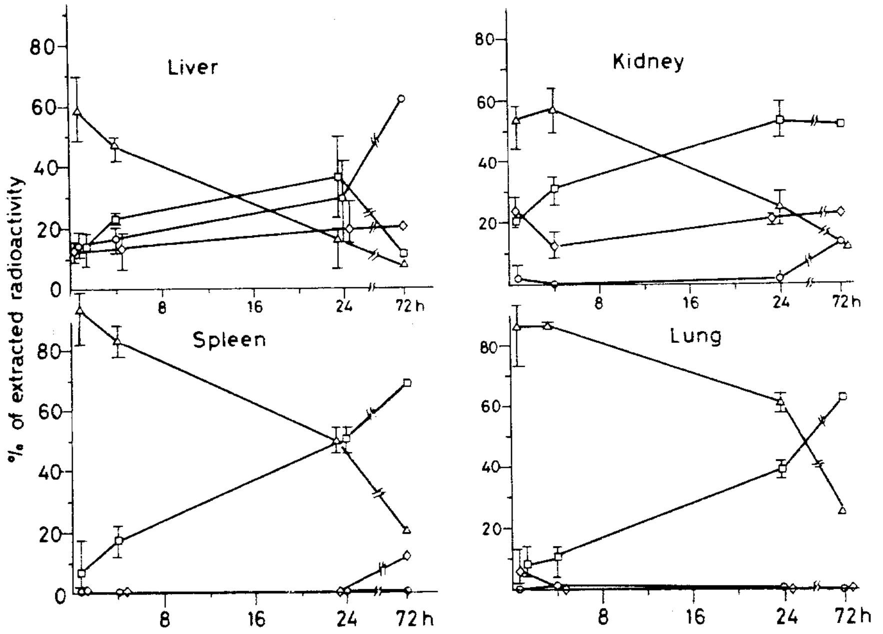

Fig. 5. Distribution of radioactivity in polar metabolites (○), 13-dihydroDNR (Ig) (□), IDR (If (△), and aglycones (◇), as deduced from TLC analysis with solvent system B, of tissues extracted [^{14}C]IDR of IV-treated rats. Data are expressed as median percent values of total extractable radioactivity ($n=3$, range). The 72-h samples were pooled for analysis

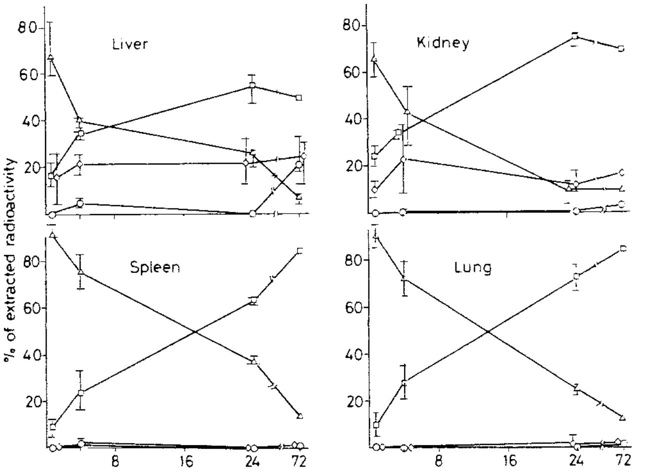

Fig. 6. Distribution of radioactivity in polar metabolites (○), 13-dihydroDNR (Ii) (□), DNR (Ih) (△), and aglycones (◇), as deduced from TLC analysis, with solvent system B, of tissues extracts of [^{14}C]DNR IV-treated rats. Data are expressed as median percent values of total extractable radioactivity ($n=3$, range). The 72-h samples of kidney, spleen, and lung were pooled for analysis

higher than 74% except for liver and kidney of [^{14}C]IDR-treated animals, for which lower extraction yields were recorded. Examination of analytic radiochromatographic data of tissue extracts from IV-treated animals (Figs. 5 and 6) showed that in spleen and lung the 13-dihydro derivatives of both [^{14}C]IDR and [^{14}C]DNR became the predominant species at 72 h. The same behavior was observed in kidney extracts, in which, however, an aglycone fraction was present at all times studied. An important difference between the two drugs can be seen in the liver extracts. In addition to the dihydro derivative and to an aglycone fraction present in both cases, in the [^{14}C]IDR-treated animals significant percentage of liver radioactivity was represented by unidentified polar metabolites, which became the prevalent radioactive fraction at 72 h after treatment. Recoveries in the range of 60.5%–98.1% were obtained in the extraction of tissues from orally treated rats. Radiochromatographic analysis showed the presence of the parent drug and its 13-dihydroderivative as the main radioactive species in spleen and lung tissues as already observed in the IV-treated animals. In contrast the 4-h liver and kidney extracts showed a marked increase of the polar fraction compared with the corresponding extracts from the IV-treated animals. At 24 h, the composition of kidney extracts is different from that from IV-treated animals, because of the higher amount of aglycones and polar metabolites. Generally, the level of the unchanged drug in the tissues analyzed is clearly lower in the orally treated animals than in the IV-treated ones, by a factor in the range of 0.3–0.6. This appears not to be related to an enhanced level of the 13-dihydro metabolite, which in the orally treated animals is generally lower than or equal to that in the IV-treated animals.

Discussion

The almost complete recovery of radiolabel in the excretion studies points out the usefulness of ^{14}C labelling for the quantitative evaluation of the fate of antitumor anthracyclines. The much lower level of $^{14}CO_2$ in the expired air than after administration of [^{14}C]doxorubicin and [^{14}C]4'-epidoxorubicin [5] is in agreement with the higher chemical stability of the methylketone side-chain present in IDR and DNR. The lower rate of biliary elimination of [^{14}C]IDR-derived than [^{14}C]DNR-derived radioactivity might be related to the distinctly higher lipophilicity of the 4-demethoxyglycosides than of the parent compounds.

On the basis of urinary excretion, the oral absorption of [14C]IDR must be approximately 50% of the administered dose. The difference in the extent of biotransformation following PO versus IV administration suggested by the tissue recovery data is in agreement with the urine analysis, indicating a greater amount of polar radioactivity in the case of oral administration.

It is clearly apparent that [^{14}C]IDR and [^{14}C]DNR differ considerably from labelled doxorubicin and 4'-epidoxorubicin [21] in the rate of elimination of radiolabel from rat tissues after IV administration. The difference is particularly striking in such tissues as kidney, lung, and spleen. Also, the rate of elimination of radioactivity in heart tissues is higher with IDR- and DNR-derived species than the above-mentioned compounds. The presence of the hydroxyl group at 14-C is therefore an important molecular determinant of the retention of antitumor anthracyclines and/or their metabolites in animal tissues.

If the variations in radioactivity with time in different tissues are compared, different types of behavior are found to have been recorded on the basis of statistical analysis. The data indicate that compared with such tissues as left auricle and lymph nodes, radioactivity derived from [^{14}C]IDR concentrates more markedly than that derived from [^{14}C]IDR concentrates more markedly than that derived from [^{14}C]DNR, possibly because of its superior antileukemic properties [14, 16]. The different pharmacokinetic behavior is particularly evident when the quantitative and qualitative aspects of biliary excretion of the radioactive species derived from either [^{14}C]IDR or [^{14}C]DNR are considered (see below). Particular importance attaches to the levels of radioactivity measured in the heart tissues, because of the cardiotoxic properties of the anthracyclines. The lower cardiotoxic properties of idarubicin at pharmacologically effective doses [14, 16] might be explained with reference to the data reported here, insofar as concentration levels may be related to the expression of myocardial lesions induced by the drug. As for the different rates of elimination in different heart tissues, the picture is similar to that already recorded for doxorubicin [5], which is itself in agreement with histological findings concerning the severity, distribution, and evolution of myocardial lesions [26]. The tissue distribution at 4 h after oral administration suggests that at this time an active absorption process is still present. If the 4-h data are compared with those found at the same time interval after IV treatment, variations are noted that support the above-mentioned conclusions. The great difference in the ratios of plasma to tissue (e.g., heart) radioactivity observed at 4 h after the two treatments is explained by the different extent of biotransformation of idarubicin observed after oral administration. In fact, the composition of urinary radioactivity already indicates the presence of a larger amount of polar fractions in the orally treated animals, whereas the amount of drug plus dihydroderivative excreted at this time is lower when compared with the IV treated ones (0.5 versus 1.0 mg). The different ratios of heart to plasma concentrations of radiolabel between [^{14}C]IDR and [^{14}C]DNR might be related to different molecular properties induced by the absence of a methoxy group in position 4 in IDR, possibly resulting in a lower binding of the drug to some tissue constituents.

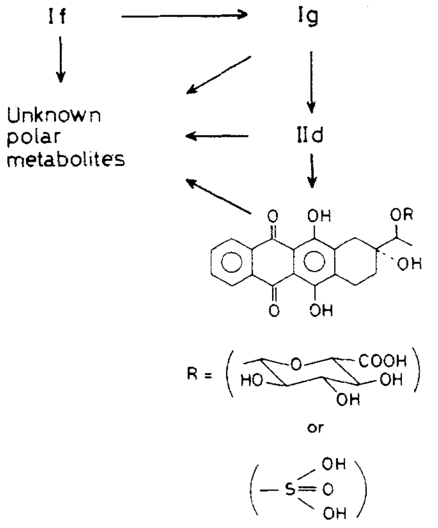

Fig. 7. Proposed metabolic pathway of IDR in the rat

The high percentage of polar metabolites found in urines of [^{14}C]IDR-treated rats indicates a divergence from the behavior of other clinically useful anthracyclines, such as DNR, doxorubicin, and 4'-epidoxorubicin. In the case of daunorubicin, conjugation involves substitutions of the C-4 methyl group with a glucuronyl or sulfonyl residue [2]. This reaction is not possible in IDR-derived metabolites, and therefore a different position must be involved in the glucuronidation (and/or sulfate conjugation) step. From the analytic results obtained with the bile samples it might be deduced that the conjugation takes place at the C-13 hydroxyl group in the 7-deoxy-13-dihydroIDRA. The latter compound should originate from the reductive deglycosidation of 13-dihydroIDR according to a pathway already established for related anthracyclines [15, 21]. A reasonable scheme is presented in Fig. 7. The quantitative importance of phase II reaction products in [^{14}C]IDR metabolism is related at least in part to the higher lipophilicity of the 4-demethoxy analogue, this property being apparently also causative of the lower biliary excretion rate of 13-dihydroIDR than of 13-dihydroDNR. Results of tissue extract experiments are in full agreement with what has been outlined above. The relative importance of the different radioactive fractions in the tissues is consistent with an efficient transformation of the parent drugs to the corresponding 13-dihydrometabolites, and with the extensive further metabolism of IDR-derived species generating polar fractions especially in the liver and hence in the bile.

Recent work with human patients [4] has allowed the isolation of 13-dihydroIDR. Because this metabolite was found to be the main IDR-derived, biologically active species in man, our findings confirm the adequacy of the rat as a model for the toxicological evaluation of IDR.

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