Cancer Chemother Pharmacol (1996) 37: 472–478

ORIGINAL ARTICLE

Guozhu Han · Mervyn Israel · Ramakrishnan Seshadri
James T. Dalton · Trevor W. Sweatman

Pharmacology of N,N-di(*n*-butyl)adriamycin-14-valerate in the rat

Received: 19 September 1994/Accepted: 27 June 1995

Abstract. Lipophilic N-alkylanthracyclines such as AD 198 (N-benzyladriamycin-14-valerate) or AD 201 [N,N-di-(*n*-propyl)adriamycin-14-valerate], which exert their cytotoxicity through mechanisms which are not yet fully defined, possess inherent abilities to circumvent multidrug resistance *in vitro* and *in vivo*, possibly through alterations in normal intracellular drug trafficking. As part of structure-activity studies with this class of agent, we have now examined the pharmacology of AD 202 [N,N-di(*n*-butyl)adriamycin-14-valerate], another analog possessing superior antitumor activity to doxorubicin *in vivo* and an ability to circumvent multidrug resistance *in vitro*. Following the administration of AD 202 (20 mg/kg, i.v.) to anesthetized rats, rapid drug distribution ($T_{1/2}$ 5 min) was followed by more gradual elimination ($T_{1/2}$ 3.6 h). Plasma clearance of AD 202 (224 ± 63.6 ml/min per kg) and steady state volume of distribution (25.7 ± 11.1 l/kg) were indicative of extensive tissue sequestration and/or a large degree of extra-hepatic metabolism. The parent drug predominated in plasma until 20 min, thereafter N,N-di(*n*-butyl)adriamycin became the principal circulating anthracycline. The systemic exposure to this biotransformation product (area under the plasma concentration-time curve from time zero to 480 min AUC_{0-480} 28 1672 ng·min/ml) was > tenfold higher than for the other detected plasma products (N-butyladriamycin-14-valerate, N-butyladriamycin, and three unidentified fluorescent signals; P1-3). Total urinary elimination over 8 h was limited (1.9% of dose), occurring predominantly as N,N-di(*n*-butyl)adriamycin (1.2% of dose), N-butyladriamycin (0.4% of dose), and their corresponding 13-carbinol metabolites (< 0.1% of dose each). Low levels of adriamycin (ADR), aglycones and two unidentified products were also seen. Parental AD 202 was found in urine only up to 1 h. By contrast, hepatic elimination of parent drug was seen, albeit at low levels, through 8 h. Excretion by this route (22% of dose) occurred principally as N-butyladriamycin (8% of dose), N-butyladriamycinol (2.1% of dose) with lower levels of N,N-di(*n*-butyl)adriamycin (1.6% of dose), N,N-di(*n*-butyl)adriamycin (0.8% of dose), and aglycones (4.3% of dose, combined). Other products included ADR (1.1% of dose) and two unidentified signals (3.4% of dose, combined). The relatively poor mass balance in these studies is attributed to prolonged intracellular retention (elimination $T_{1/2}$ 24.2 h) of N,N-di(*n*-butyl)adriamycin. Thus, in common with other N-alkylanthracyclines, the pharmacology of AD 202 is complex but its therapeutic properties clearly are not derived from an ADR prodrug effect. Significant differences continue to be noted as to the metabolic fate of congeners of this class of anthracycline analogs.

Key words: N-Benzyladriamycin-14-valerate · Pharmacology · Anthracycline analogs

G. Han[1] · M. Israel · R. Seshadri · T.W. Sweatman (✉)
Department of Pharmacology,
University of Tennessee College of Medicine,
874 Union Avenue, Memphis, TN 38163, USA
Tel (901) 448-4586; Fax (901) 448-4587
e-mail tsweatman@utmem1.utmem.edu

J.T. Dalton
Department of Pharmaceutical Sciences, College of Pharmacy,
The University of Tennessee, Memphis, TN 38163, USA

Permanent address
[1] Department of Pharmacology, Dalian Medical College,
Dalian, China

Introduction

The present study was undertaken as part of a program to elucidate the pharmacology and metabolic fate of mono- and di-N-alkylanthracycline compounds in connection with the search for doxorubicin analogs having improved therapeutic index and also the ability to circumvent drug resistance mechanisms.

Doxorubicin (Adriamycin; ADR) remains among the most widely used antitumor agents available today [34], with broad activity against a wide range of malignancies including leukemias, lymphomas, and solid tumors including those of breast and lung [22]. Unfortunately, the full clinical potential of this agent is tempered by drug-mediated toxicities, which, in addition to acute bone marrow suppression, include irreversible cumulative dose-related cardiomyopathy [17]. In this regard, efforts to improve current anthracycline chemotherapy have centered on the use of cardioprotective agents such as dexrazoxane [24, 25], or analogs such as epirubicin (4′-epidoxorubicin) which possess somewhat lower inherent cardiotoxic potential [9] as a result of pharmacologic differences from the parental agent [9, 26, 33]. However, despite the provision for administration of an incrementally larger total anthracycline dose, neither approach has demonstrated any significant increase in therapeutic efficacy over the use of ADR alone [7, 31].

Moreover, like many other natural product drugs, ADR is often found to be clinically ineffective against classical P-glycoprotein (transport defect)-multidrug resistance (MDR) or altered topoisomerase-MDR cell subpopulations arising naturally or as a result of previous drug exposure [5]. With regard to P-glycoprotein drug resistance, efforts have centered upon increasing intracellular anthracycline levels, primarily through the use of calcium channel blockers as modulators of drug transport. Although adjunctive clinical administration of such modulators as verapamil [20], or the less acutely toxic bepridil [14], is capable of increasing intracellular anthracycline levels, this approach is limited not only by the adverse cardiac effects of such agents at clinically effective modulatory doses, but also by their inability to change the spectrum of current anthracycline antitumor activity.

An alternative strategy is the development of anthracycline analogs with a capacity to bypass P-glycoprotein-mediated drug transport effects. N-Benzyladriamycin-14-valerate (AD 198) is particularly noteworthy in this regard, for, in addition to circumventing P-glycoprotein resistance *in vitro* and *in vivo* [8, 12, 28], this agent also bypasses altered topoisomerase [4, 5, 19, 28]. AD 198, which shows marked antitumor activity *in vitro* and *in vivo*, superior to that of ADR [8, 10, 11, 28, 32], and is noncardiotoxic in a standard laboratory animal test system [13], is thus of considerable interest as a potential Phase I clinical agent. Recent studies have, additionally, provided a clear pharmacologic rationale for this agent in the treatment of small cell and non-small cell lung carcinomas [30]. In connection with a preclinical development plan, pharmacokinetic and metabolic fate studies with AD 198 in animals have revealed a complex pattern of metabolism, with a degree of 4-O-desmethyl metabolite generation not seen with other anthracyclines [27]. Furthermore, the absence of significant systemic levels of ADR both confirmed the absence of an ADR prodrug effect and the innate biological activity of the parental drug substance [27, 30].

The findings with AD 198, however, are at considerable variance from those with a close structurally related product, N,N-dibenzyldaunorubicin, which compound is biologically inactive *in vitro* but serves as a prodrug, yielding cytotoxic products *in vivo* [1, 2, 21]. These findings have thus led us to examine the pharmacology of other mono- and di-N-alkylanthracycline derivatives in an effort to better understand the biological properties of this class of compounds. In preliminary studies with N,N-di(*n*-propyl)adriamycin-14-valerate (AD 201), another lipophilic analog with significant *in vitro* and *in vivo* antitumor activity [10], this agent, like AD 198, showed the absence of an ADR prodrug effect, but, unlike AD 198, showed no production of 4-O-desmethyl metabolites [29]. Instead, AD 201 biotransformation products underwent extensive glucuronidation, with marked alteration of the chromophore.

In continuing pharmacology studies with this class of agents, we have now extended our investigations to N,N-di(*n*-butyl)adriamycin-14-valerate (AD 202), another congener with therapeutic superiority to ADR *in vivo* and an ability to circumvent multidrug resistance *in vitro* [10]. Although AD 202 is not presently under active development, in deference to other highly active antitumor agents such as AD 198 which is in late preclinical studies, knowledge of the pharmacology of this additional N-alkylanthracycline agent is useful in further defining the effects of N-alkyl substitution upon the pattern of drug metabolism and distribution and thereby in explaining the biological properties of these agents. Thus, the present studies were undertaken to examine the preliminary pharmacology of AD 202 in plasma, urine, and bile of anesthetized rats with a view to determining the time course of disappearance of parental agent and appearance of biotransformation products; identifying the major biotransformation products; determining the extent of glucuronide formation; and examining the extent of 4-O-desmethyl metabolite production.

Materials and methods

Materials

AD 202 was prepared as previously described [10]. NCI Diluent 12 [Cremaphor EL (polyethoxylated castor oil): ethanol, 1:1 v/v] was kindly provided by the Pharmaceutical Resources Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

Animal studies

All animal experimentation was conducted in accordance with protocols approved by appropriate institutional review. Female

Sprague-Dawley rats (Harlan; 200–250 g) were anesthetized with sodium pentobarbital i.p. and placed on isothermal pads for the duration of the study. Each animal was surgically fitted with catheters in the carotid artery and femoral vein for blood sampling and drug dosing, respectively. In addition, animals underwent either a common bile duct or bilateral ureteral cannulation for the continuous collection of bile and urine, respectively. Animals received a single i.v. bolus dose of AD 202; 20 mg/kg, dissolved in 20% NCI Diluent 12:80% saline. The selection of the drug dose for the present studies was based upon established optimal murine antitumor data for this compound, adjusted for the rat body surface area. At various times following drug injection (2.5 min–8 h), blood samples (20–100 µl) were collected from the arterial catheter and the plasma derived therefrom was frozen (−70 °C) pending analysis. Where appropriate, bile or urine was collected into ice-cold preweighed containers in 30-min aliquots. At the end of each collection period, the containers were re-weighed and the contents frozen (−70 °C) for later analysis.

Sample analysis

Plasma samples were thawed immediately prior to analysis and the protein precipitated by the addition of two volumes of ice-cold ethanol containing N-trifluoroacetyladriamycin-14-octanoate (AD 28) as internal standard. Following centrifugation, samples were subjected to direct HPLC injection and analysis without further manipulation. This preparative process exhibited excellent recovery (>90%) and linearity over the anticipated concentration range (5–400 ng/injection). Bile and urine samples, diluted with water where appropriate, were also analyzed by direct HPLC injection. In addition, selected samples were either subjected to bulk extraction with ethyl acetate: *n*-propanol (9:1, by volume) or solid phase extraction using C_{18} Sep-Paks (Waters Associates, Milford, Mass.) to enrich the levels of minor metabolites.

Plasma, bile and urine samples were analyzed by reversed-phase HPLC (C_{18} Nova-Pak 4 µm, 5 mm i.d. × 10 cm radial compression column; Waters Associates) with flow fluorescence detection (482 nm excitation, 550 nm barrier emission filter; Schoeffel Model FS-970 fluorimeter, Schoeffel Instruments, Westwood, N.J.) using a gradient mobile phase (70% A/30% B to 20% A/80% B in 5 min at 1.5 ml/min; total run time 15 min) consisting of ammonium formate buffer, 0.05 M, pH 4.0 (A) and acetonitrile (B).

Quantitation for bile and urine samples was based upon standard curves of parent drug produced by direct HPLC injection. For plasma, samples were quantitated by reference to standard extraction curves produced by the processing and analysis of blank rat plasma to which appropriate quantities of AD 202 and AD 28 had been added. All results are expressed in terms of AD 202 or AD 202 equivalents; no correction has been made for differences in fluorescence quantum efficiencies between the various biotransformation products.

Identification of metabolic products

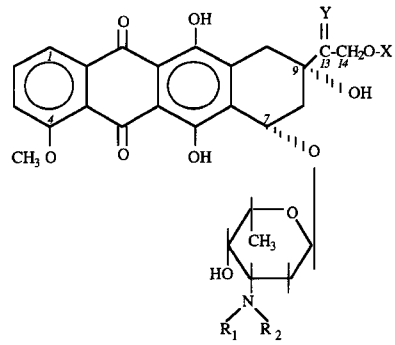
Based upon our previous experience with anthracycline metabolism, laboratory synthesis of a series of potential metabolic products of AD 202 was undertaken (AD 285, AD 284, AD 194, AD 295, AD 296) and their identity confirmed by elemental and mass spectral analysis. Identification of metabolites of AD 202 in biological extracts was made on the basis of co-chromatography of unknown and standard signals both in the reversed-phase analytical system and in a normal phase chromatography system. In this latter system, separation was accomplished on a Partisil PXS 10/25 PAC column (Whatman, Clifton, N.J.) with a gradient mobile phase (90% A/10% B to 100% B in 10 min at 2.5 ml/min) of chloroform (A) and mixed solvent (B): chloroform: methanol: glacial acetic acid: water, 85:15:5:1.5 by volume). No significant production of any of these metabolites was evident in serum samples, to which AD 202 had been added and subjected to the extraction process used for the biological samples. The chemical structures of AD 202, the principal metabolites, and N-benzyladriamycin-14-valerate (AD 198) are shown in Fig. 1.

Enzymatic and acid hydrolysis

Selected bile and urine samples were subjected to enzymatic (β-glucuronidase; Sigma Type B-1) and hydrochloric acid (0.1 N methanolic HCl) hydrolysis (both at 37 °C) to investigate the existence of conjugates as excretion products. Following incubation, samples were analyzed, as above, in comparison with untreated and control samples, to determine the extent of any differences in the respective chromatographic profiles.

Pharmacokinetic data analysis

Data from individual animals were used to calculate the mean plasma concentration for AD 202 and each of its metabolites at each time point (2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 300, 360, 420, and 480 min). For each sampling time, the coefficient of variation was calculated according to standard methods and was used as a measure of inter-animal variation. Pharmacokinetic analysis for metabolites with coefficients of variation >40% for the majority of the data points (i.e., AD 284, AD 194, and P1-3) was performed using the mean plasma concentration-time profiles. For AD 202 and AD 285, pharmacokinetics were determined using data collected from the individual animals.



Code No.	R_1	R_2	X	Y
ADR	H	H	H	O
AD 198	benzyl	H	$CO(CH_2)_3CH_3$	O
N,N-Dibenzyl-ADR	benzyl	benzyl	H	O
AD 201	*n*-propyl	*n*-propyl	$CO(CH_2)_3CH_3$	O
AD 202	*n*-butyl	*n*-butyl	$CO(CH_2)_3CH_3$	O
AD 285	*n*-butyl	*n*-butyl	H	O
AD 284	*n*-butyl	H	H	O
AD 194	*n*-butyl	H	$CO(CH_2)_3CH_3$	O
AD 295	*n*-butyl	*n*-butyl	H	H, OH
AD 296	*n*-butyl	H	H	H, OH

Fig. 1 Structures of doxorubicin and N-alkylanthracycline analogs included in this study. (*ADR* adriamycin)

Pharmacokinetic analysis was performed using published model-independent methods [3, 23]. AUC_{0-last} was calculated using the linear trapezoid rule. $AUC_{last-\infty}$ was calculated as plasma concentration at 480 min divided by the absolute value of the terminal slope. The terminal slope used for these calculations was determined using linear least squares regression of the ln (mean plasma concentration) versus time plot for each analyte to minimize potential error due to individual variation in plasma concentration. $AUC_{0-\infty}$ was then calculated as the sum of these two AUC values and the half life ($T_{1/2}$) was calculated as 0.693 divided by the absolute value of the terminal slope. First moment curves [3] were constructed by plotting the product [plasma concentration] × [time] versus time. Areas under the [first] moment curve from time zero to infinity ($AUMC_{0-\infty}$) were calculated using the linear trapezoid rule. The total body clearance (CL) for AD 202 was calculated as intravenous dose divided by the $AUC_{0-\infty}$. Mean residence time (MRT) was calculated as $AUMC_{0-\infty}$ divided by $AUC_{0-\infty}$, and the volume of distribution at steady state (Vdss) was calculated as the product of MRT and CL.

Results

The mean plasma concentration-time profiles for AD 202 and principal circulating metabolites are shown in Fig. 2. For the sake of clarity, only the mean values are plotted, with the exception of the total plasma anthracycline concentrations, where error bars for standard deviation are shown. Inset on an expanded scale are AD 284, AD 194, and the unidentified plasma products (P1-3). Initially, levels of AD 202 declined rapidly, falling from ~ 8 μg/ml at 2.5 min to only 1 μg/ml by 15 min. Although AD 202 was detectable in plasma through 8 h, significant levels (> 50 ng/ml) were only evident up to 3 h. N,N-Di(*n*-butyl)adriamycin (AD 285) was the principal biotransformation product in plasma, with levels exceeding those of AD 202 by 15 min. In contrast to AD 202, AD 285 persisted at significant concentrations (> 0.5 μg/ml) throughout the study. Plasma concentrations of parental drug and AD 285 were similar between the individual animals, with median coefficients of variation of 32% and 16%, respectively (Table 1). N-Butyladriamycin (AD 284) and N-butyladriamycin-14-valerate (AD 194) were also detected as minor plasma products (see insert), although the latter was short lived, being detectable only up to 45 min. In addition, three other minor drug-derived fluorescent peaks were found in plasma (residence time 9.2, 10.8, and 11.1 min, respectively; denoted as P1, P2, and P3); the levels of these have been combined in the figure for the sake of clarity. Unfortunately, concentrations of AD 284, AD 194, and P1-P3 (combined) varied widely between animals, precluding a detailed pharmacokinetic analysis.

Pharmacokinetic parameters for AD 202 are shown in Table 2. The terminal $T_{1/2}$ for AD 202, determined from the mean concentration time profile, was 218 min. For AD 202, the AUC_{0-480} represented 92.7 ± 4.3% of the total $AUC_{0-\infty}$ (94 508 ± 20 591 ng · min/ml, mean ± SD) indicating that a significant fraction of the overall disposition of AD 202 was accounted for within the time scale of the present studies. This fact is further corroborated by the relatively short MRT for AD 202 (115 ± 33.4 min). The clearance of AD 202 (224 ± 63.6 ml/min per kg) was significantly higher than

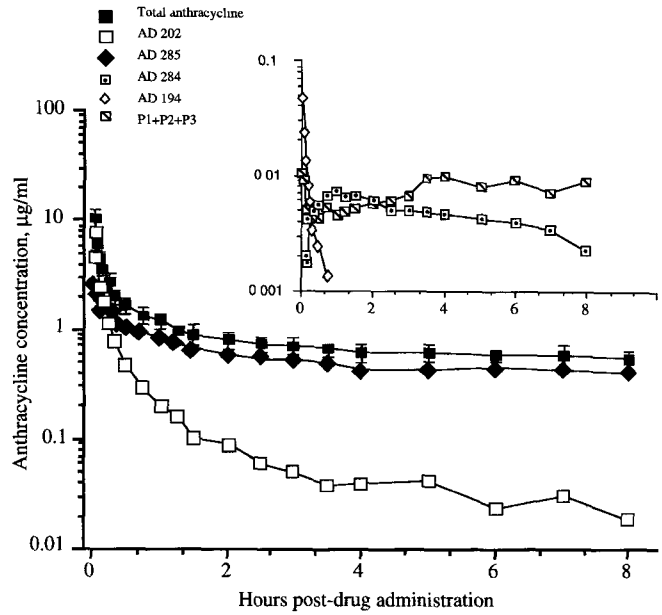


Fig. 2 Mean circulating concentrations of total anthracycline (mean ± SD), parent drug and metabolites, as AD 202 equivalents, determined by HPLC analysis of rat plasma following the administration of AD 202; 20.0 mg/kg, i.v.

Table 1 Inter-animal variability in plasma anthracycline concentrations, (*cv* coefficient of variation)

Identity	Range of CVs	Median CV
AD 202	15.8–82.0	31.8
AD 285	13.1–42.9	16.0
AD 284	64.4–230	79.0
AD 194	21.7–232	109
P1-3	69.9–319	108

Table 2 Pharmacokinetic parameters for AD 202. (*CL* total body clearance, *MRT* mean residence time, *Vdss* volume of distribution at steady state)

Animal number	CL (ml/min per kg)	MRT (min)	Vdss (l/kg)
1	184	104	19.2
2	175	116	20.2
3	173	137	23.7
4	221	104	23.1
5	386	136	52.8
6	172	162	28.0
7	241	82.1	19.8
8	203	108	22.0
9	199	61	12.2
10	186	164	30.4
11	299	134	40.0
12	247	69.6	17.2
Mean ± SD	224 ± 63.6	115 ± 33.4	25.7 ± 11.1
CV	28.4%	29.1%	43.2%

the hepatic blood flow in the rat (56 ml/min per kg; [6]), suggesting that AD 202 undergoes rapid extra-hepatic metabolism. In addition, extensive tissue sequestration is indicated by the .Vdss for AD 202 (25.7 ± 11.1 l/kg).

In comparison, the terminal $T_{1/2}$ for AD 285, calculated from the mean data, was 1449 min and, in consequence, the AUC_{0-480} represented only 24.6 ± 2.9% of the $AUC_{0-\infty}$. The terminal $T_{1/2}$ for AD 284, AD 194, and P1-3 could not be calculated accurately owing to the large variation in plasma concentrations (Table 1). In order to avoid potential errors due to extrapolation and variable plasma concentrations, the pharmacokinetic parameters for all metabolites were calculated using data obtained only up to 480 min. Thus, metabolite data presented below should be viewed with the consideration that they may represent a small percentage of the overall disposition of each component following AD 202 administration. Additional studies of a more extended duration would be necessary to more fully characterize these individual components. Nevertheless, the AUC_{0-480} values for these metabolites can be used as a measurement of their relative exposure following AD 202 administration. For AD 285, AD 284, AD 194, and P1-3 these values were 281672, 19418, 10655 and 14324 ng·min/ml, respectively. Thus, systemic exposure to AD 285 was > tenfold higher than for the other metabolites, suggesting a more rapid conversion of AD 202 to AD 285 than to the other metabolites or a slower AD 285 clearance. Given that the terminal $T_{1/2}$ of AD 285 was significantly larger than that of AD 202, and the fact that AD 285 appears in the plasma almost instantaneously following AD 202 administration, slower clearance of AD 285 is the most likely contributor to the increased exposure. A slow clearance of AD 285 would also account for the poor anthracycline mass balance (23%) in the present study, i.e., a large amount of AD 285 was resident in the tissue at 480 min. The MRT for AD 285, AD 284, AD 194, and P1-3 (calculated as mean $AUMC_{0-480}$/mean AUC_{0-480}) were 2001, 221, 202, and 268 min, respectively.

Figure 3 shows total (mean ± SD) urinary anthracycline elimination together with the individual urinary components (mean only, for the sake of clarity). In keeping with the lipophilic nature of the parent drug and the rapid conversion of the parent drug principally to AD 285, a metabolite with a high MRT, elimination by this route was extremely limited, with only 1.9% of the dose recovered over 8 h. Despite the presence of intact AD 202 in plasma throughout the study, the parent drug was only evident in the earliest urine fractions (up to 1 h) and then at insignificant concentrations (0.006% of dose). In common with plasma, AD 285 was the principal metabolic product, accounting for 1.2% of the dose or 64% of urinary material. Other urinary excretion products included AD 284, aglycones,

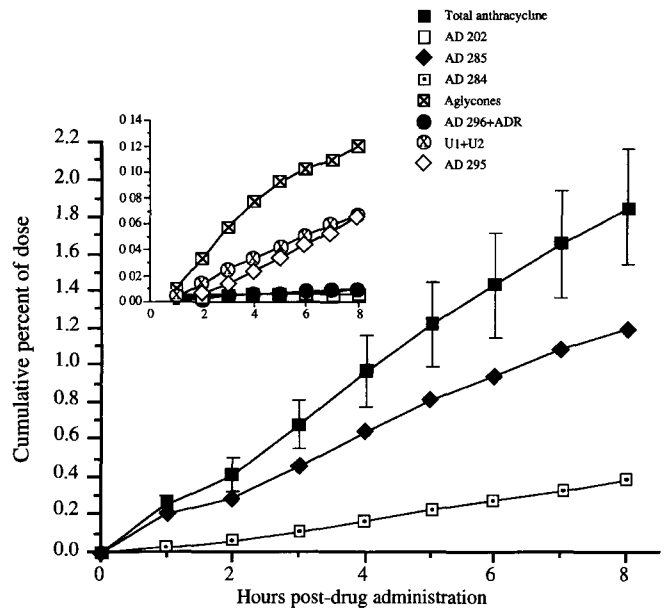


Fig. 3 Mean concentrations of total anthracycline (mean ± SD), parent drug, and metabolites, as AD 202 equivalents, determined by HPLC analysis of rat urine following the administration of AD 202; 20.0 mg/kg, i.v.

and traces of N,N-di(*n*-butyl)adriamycinol (AD 295), N-(*n*-butyl)adriamycinol (AD 296), and ADR. A further two unidentified urinary products were evident. Based upon the chromatographic retention time (9.2 min), one of these may be identical to one of the three unknowns seen in plasma. Enzymatic and acid hydrolysis failed to reveal the presence of any anthracycline conjugates in urine; no differences were seen between treatment and control samples.

Mean elimination of anthracycline biotransformation products in bile is shown in Fig. 4, together with total (mean ± SD) dose recovery by this route. In contrast to urinary anthracycline elimination, excretion of drug-related fluorescence in bile amounted to ~22% of the dose over 8 h. Parental AD 202 was found only in the earliest samples (< 1 h) and then only at extremely low concentrations. Unlike plasma and urine where AD 285 predominated, AD 284 was the principal biotransformation product in bile throughout the study, accounting for 8% of the dose or 36.6% of total recovered anthracycline. The extensive nature of hepatic processing was evidenced by the detection of a further seven identified products. In order of importance, these included: the aglycones AD 8 (adriamycinone) and AD 31 (adriamycinone-14-valerate), 4.3% of dose, combined; AD 296, 2.1% of dose; AD 285, 1.6% of dose; ADR, 1% of dose; AD 295, 0.8% of dose; and AD 194, 0.03% of dose. A further four minor products, one of which showed an identical retention time (9.2 min) to unidentified products in plasma and urine, were also found. For the sake of clarity, only the two most prevalent are shown in Fig. 4. In common with urine, no

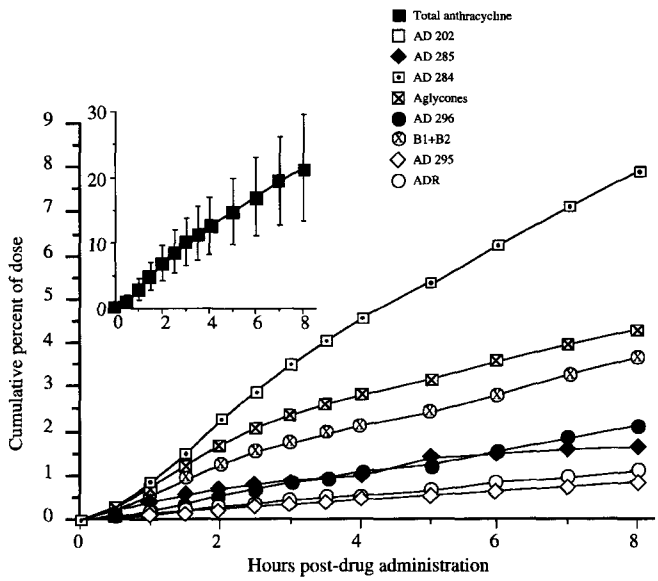


Fig. 4 Mean concentrations of total anthracycline (mean ± SD), parent drug, and metabolites, as AD 202 equivalents, determined by HPLC analysis of rat bile following the administration of AD 202; 20.0 mg/kg, i.v.

evidence of conjugated elimination products was found in any bile samples.

Discussion

The present study demonstrates that AD 202 undergoes extensive biotransformation to a number of predictable metabolites based upon the chemical structure of the parent compound. Principally, these involve sequential N-dealkylation, 14-deacylation, and 13-carbonyl reduction. In this respect, the pharmacology of this analog is comparable to that observed with the other related congeners. Thus, the low distribution $T_{1/2}$ of AD 202 and AD 198 (5 min versus 3.5 min) results from the ease of membrane passage of these highly lipophilic agents. Unlike ADR which is only slowly intracellularized, with localization primarily in the nucleus, lipophilic N-alkylanthracycline analogs such as AD 198 and AD 202 undergo almost instantaneous equilibration [15, 16, 28], with extensive sequestration in the perinuclear region of the cytoplasm. This process appears to be related to the extra degree of hydrophobicity imparted by the intact 14-valerate side chain [18] resulting in altered intracellular drug trafficking and sequestration relative to ADR. With regard to the question of an ADR prodrug effect, AD 202, like AD 201 and AD 198, does not give rise to meaningful concentrations of ADR, thereby confirming the intrinsic activity of the parental agents and their principal circulating biotransformation products.

The relatively long total anthracycline terminal $T_{1/2}$ following AD 202 and AD 198 administration (14.3 versus 15.2 h, respectively) appears to be related primarily to the prolonged intracellular retention of the respective principal circulating 14-deacyl biotransformation products, AD 285 and AD 288. This, in turn, results in low hepatic anthracycline recovery of drug fluorescence. In the present study, the MRT value calculated for AD 285 (33.35 h) was supported by separate but related studies of AD 202 tissue distribution, where AD 285 was found to be both the principal and the most persistent intracellular anthracycline species in all tissues examined (heart, liver, lung, spleen, muscle, intestines, fat, brain, kidney) through 24 h following AD 202 administration.

Notwithstanding these common findings, a wide diversity in metabolic processing appears to exist amongst these various N-alkyl ADR congeners. Contrary to AD 201, but in common with AD 198, AD 202 does not elicit any detectable glucuronide conjugation. For the respective parental agents, this may relate to steric hindrance effects at the amino-nitrogen substituent. However, since all three of these agents undergo sequential N-dealkylation, in this manner removing any hindrance, this metabolic difference may better relate to differences in intracellular drug localization relative to metabolizing enzymes. Likewise, AD 201 and AD 202 do not generate detectable concentrations of 4-O-desmethyl metabolites, as were evident with AD 198. Thus, the investigation of three N-alkyl-substituted ADR analogs has yielded a metabolic diversity not seen previously in structure-activity studies with ADR-derived compounds. In view of the superior antitumor activity of these analogs, compared with parental ADR, and their ability to overcome drug resistance, the underlying rationale for these major differences, and their effects upon the biological activity of this class of anthracyclines, continues to warrant further investigation.

References

1. Acton EM (1980) N-Alkylation of anthracyclines. In: Crooke ST, Reich SD (eds) Anthracyclines, current status and new developments. Academic Press, New York, p 15
2. Acton EM, Jensen RA, Peters JH (1982) Factors in the selection of new anthracyclines. In: Muggia FM, Young CW, Carter SK (eds) Anthracycline antibiotics in cancer therapy. Nijhoff, Boston, p. 205
3. Benet LZ, Galeazzi RL (1979) Noncompartmental determination of the steady state volume of distribution. J. Pharm Sci 68: 1071
4. Bodley A, Liu LF, Israel M, Seshadri R, Koseki Y, Giuliani FC, Silber R, Kirschenbaum S, Potmesil M (1989) DNA topoisomerase II interaction of doxorubicin and daunorubicin congeners with DNA. Cancer Res 49: 5969
5. Danks MK, Yalowich JC, Beck WT (1987) Atypical multiple drug resistance in a human leukemic cell line selected for resistance to teniposide. Cancer Res 47: 1297
6. Davies B, Morris T (1993) Physiological parameters in laboratory animals and humans. Pharm Res 10: 1093

7. French Epirubicin Study Group (1988) A prospective randomized phase III trial comparing combination chemotherapy with cyclophosphamide, fluorouracil and either doxorubicin or epirubicin. J Clin Oncol 6: 679
8. Ganapathi R, Grabowski D, Sweatman TW, Seshadri R, Israel M (1989) N-Benzyladriamycin-14-valerate versus progressively doxorubicin-resistant murine tumours: cellular pharmacology and characterisation of cross-resistance *in vitro* and *in vivo*. Br J Cancer 60: 819
9. Goldin A, Venditti JM, Geran R (1985) The effectiveness of the anthracycline analogue 4′ epi-adriamycin in the treatment of experimental tumors: a review. Invest New Drugs 3: 3
10. Israel M, Seshadri R (1986) N-Alkyl and N-benzyladriamycin derivatives. US Patent 4 610 977, US Patent Office, Washington, DC
11. Israel M, Seshadri R, Koseki Y, Sweatman TW, Idriss JM (1987) Amelioration of adriamycin toxicity through modification of drug-DNA binding properties. Cancer Treat Rev 14: 163
12. Israel M, Sweatman TW, Seshadri R, Koseki Y (1989) Comparative uptake and retention of adriamycin and N-benzyladriamycin-14-valerate in human leukemic lymphocyte cell cultures. Cancer Chemother Pharmacol 25: 177
13. Israel M, Koseki Y, Jenkins JJ (1991) Murine cardiotoxicity assay of the mechanistically novel adriamycin analogue, N-benzyladriamycin-14-valerate (AD 198). Proc Am Assoc Cancer Res 32: 423
14. Kalken CK van, Hoeven JJ van der, Jong J de, Giaccone G, Schuurhuis GJ, Maessen PA, Blokhuis WM, Vijgh WJ van der, Pinedo HM (1991) Bepridil in combination with anthracyclines to reverse anthracycline resistance in cancer patients. Eur J Cancer 27: 739
15. Krishan A, Sauerteig A, Gordon K, Swinkin C (1986) Flow cytometric monitoring of cellular anthracycline accumulation in murine leukemia cells. Cancer Res 46: 1768
16. Lameh J, Chuang LF, Israel M, Chuang RY (1988) Mechanistic studies on N-benzyladriamycin-14-valerate (AD 198), a highly lipophilic alkyl adriamycin analogue. Anticancer Res 8: 689
17. Lenaz L, Page JA (1976) Cardiotoxicity of adriamycin and related anthracyclines. Cancer Treat Rev 3: 111
18. Lothstein L, Sweatman TW, Dockter ME, Israel M (1992) Resistance to N-benzyladriamycin-14-valerate in mouse J774.2 cells: P-glycoprotein expression without reduced N-benzyladriamycin-14-valerate accumulation. Cancer Res 52: 3409
19. Maniar N, Krishan A, Israel M, Samy TSA (1988) Anthracycline-induced DNA breaks and resealing in doxorubicin-resistant murine leukemia P388 cells. Biochem Pharmacol 37: 1763
20. Ozols RF, Cunnion RE, Klecker RW Jr, Hamilton TC, Ostchega Y, Parrillo JE, Young RC (1987) Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients. J Clin Oncol 5: 641
21. Peters JH, Ross G, Kashiwase D, Acton EM, Hunt A (1983) Metabolic disposition of N,N-dibenzyldaunorubicin in the rat. Cancer Res 43: 1477
22. Riggs E (1992) Antitumor antibiotics and related compounds. In: Perry MC (ed) The chemotherapy source book. Williams and Wilkins, Baltimore, p 318
23. Rowland M, Tozer TN (1980) Clinical pharmacokinetics, concepts and applications, Lea and Febiger, Philadelphia
24. Speyer JL, Green MD, Kramer E, Rey M, Sanger J, Ward C, Dubin N, Ferrans V, Stecy P, Zeleniuch-Jacquotte A, et al (1988) Protective effects of the bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. N Engl J Med 319: 745
25. Speyer JL, Green MD, Zeleniuch-Jacquotte A, Wernz JC, Rey M, Sanger J, Kramer E, Ferrans V, Hochster H, Meyers M, et al (1992) ICRF-187 permits longer treatment with doxorubicin in women with breast cancer. J Clin Oncol 10: 117
26. Sweatman TW, Ahmed NK, Israel M (1986) Relative activity of daunorubicin (DNR), adriamycin (ADR), and 4′epi-adriamycin (epiADR) with human liver reductases. Proc Am Assoc Cancer Res 27: 247
27. Sweatman TW, Seshadri R, Israel M (1989) Metabolism and elimination of N-benzyladriamycin-14-valerate (AD 198) in the rat. Proc Am Assoc Cancer Res 30: 619
28. Sweatman TW, Israel M, Seshadri R, Koseki Y, Beck WT (1990) Cytotoxicity and cellular pharmacology of N-benzyladriamycin-14-valerate in mechanistically different multidrug-resistant human leukemic cells. J Cell Pharmacol 1: 96
29. Sweatman TW, Pawlik C, Seshadri R, Israel M (1991) Metabolism and elimination of N,N-di(*n*-propyl)adriamycin-14-valerate (AD 201) in the rat. Proc Am Assoc Cancer Res 32: 345
30. Sweatman TW, Seshadri R, Israel M (1992) Selective retention of N-benzyladriamycin-14-valerate (AD 198) in the lung following intravenous drug administration. Proc Am Assoc Cancer Res 33: 520
31. The Italian Multicentre Breast Study with Epirubicin (1988) Phase III randomized study of fluorouracil, epirubicin and cyclophosphamide vs. fluorouracil, doxorubicin and cyclophosphamide in advanced breast cancer: an Italian multicentre trial. J Clin Oncol 6: 976
32. Traganos F, Israel M, Seshadri R, Kirschenbaum S, Potmesil M (1985) Effect of new N-alkyl analogues of adriamycin on *in vitro* survival and cell-cycle progression. Cancer Res 45: 6273
33. Weenen H, Maanen JM van, Planque MM de, McVie JG, Pinedo HM (1984) Metabolism of 4′-modified analogues of doxorubicin. Unique glucuronidation pathway for 4′-epi-doxorubicin. Eur J Cancer Clin Oncol 20: 919
34. Weiss RB (1992) The anthracyclines: will we ever find a better doxorubicin? Semin Oncol 19: 670