Martiala, K. (1973) Pharmacol. Rev. 53: 496–531
Martindale's Extra Pharmacopoeia (1977) 27th Ed. Pharmaceutical Press, London, p 101
Millburn, P., Smith, R. L., Willians, R. T. (1967) Biochem. J. 105: 1275–1281
Smith, R. L. (1973) The Excretory Function of Bile, Chapman and Hall, London, pp 16–34

J. Pharm. Pharmacol. 1982, 34: 597–600
Communicated February 8, 1982
0022–3573/82/090597–04 $02.50/0

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

TETSUYA TERASAKI, TATSUJI IGA, YUICHI SUGIYAMA, MANABU HANANO*, *Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan*

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.

* Correspondence.

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}

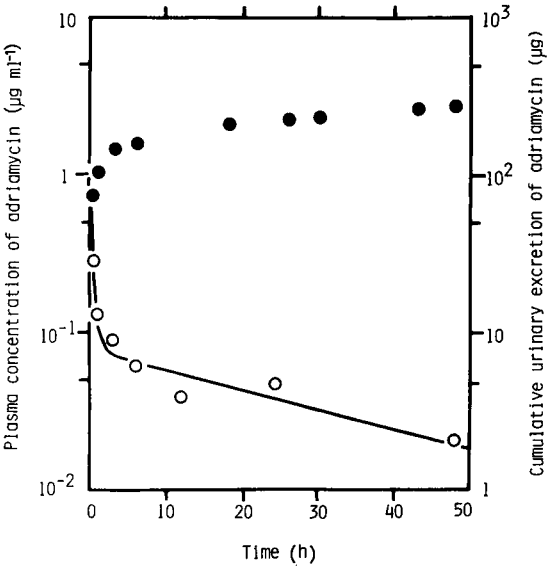

FIG. 1. Plasma disappearance and cumulative urinary excretion time course after intravenous administration of 10 mg kg^{-1} of adriamycin (ADR) in rats. Each point in urinary excretion represents the mean of 3 rats, while that in plasma concentration was obtained from the individual rat. The plasma disappearance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

Table 1. Pharmacokinetic parameters after intravenous administration of adriamycin in rats.[a,b]

Parameters	Estimated values
A ($\mu g\ ml^{-1}$)	0·741 ± 0·0001
B ($\mu g\ ml^{-1}$)	0·081 ± 0·0124
α (min^{-1})	0·04254 ± 0·00292
β (min^{-1})	0·00050 ± 0·00021
AUC [(μg min) ml^{-1}]	178·6

[a] Dose: 2·54 ± 0·05 mg/ rat.
[b] Results are given as the means ± s.e. Parameters and respective s.e. were calculated from the biexponential curve fitting by a non-linear iterative least squares method using a digital computer (see Text).

for 10 min and the concentration of ADR in plasma was determined as described. The metabolism during incubation was negligible, since no decrease was observed in the plasma concentration of ADR until 40 min.

For rabbits, the tissue DNA concentration was determined in the same manner as for the rat. In this study we did not determine the tissue concentration of ADR in rabbits. We used the values of tissue-to-plasma partition coefficients (K_p) reported by Harris & Gross (1975).

Plasma disappearance and cumulative urinary excretion curves for 48 h after intravenous administration of 10 mg kg^{-1} ADR are shown in Fig. 1. The pharmacokinetic parameters calculated from the biexponential curve fitted by an iterative least squares method using the 'SALS' program (Nakagawa et al 1978) are also listed in Table 1. The small value of β gives a long biological half-life ($t_{1/2}$), 23·1 h. The total recovery of unchanged ADR excreted in urine over 48 h was 11·1 ± 0·38% of the dose (n = 3), while Yesair et al (1972) reported a value of 6·2% of the dose over 48 h after intravenous administration of 10 mg kg^{-1} ADR in rats. Since the main pathway of ADR elimination has been reported to be the hepato-biliary route (Tavoloni & Guarino 1980), the ADR which was not excreted in urine (about 90% of the dose), was treated as the hepatic elimination.

The apparent total blood clearance ($CL_{tot.B.app}$) can be expressed as

$$CL_{tot.B.app} = \frac{Dose}{R_B AUC_p} \quad (1)$$

where R_B is the blood-to-plasma concentration ratio and AUC_p is the area under plasma concentration time curve. In this study, the value of 1·91 ± 0·18 (n = 18) was obtained for R_B and no concentration dependency was observed in the plasma concentration range from 0·01 to 1·3 $\mu g\ ml^{-1}$. Using this value of R_B and the parameters listed in Table 1, $CL_{tot.B.app}$ was calculated to be 7·43 ml min^{-1} by use of equation 1. The infinite urinary excreted amount of ADR was calculated to be 14·1% of the dose by multiplying the total urinary excreted amount of ADR for 48 h by the ratio of $AUC_{p,0-\infty}/AUC_{p,0-48\ h}$. Accordingly, the apparent hepatic clearance ($CL^{LV}_{B.app}$) and apparent renal clearance ($CL^{KD}_{B.app}$) were calculated to be 6·38 and 1·05 ml^{-1}, respectively.

The organ intrinsic clearance (CL_{int}) can be expressed as

$$f_p CL_{int} = \frac{CL_{B.app} Q_B R_B}{Q_B - CL_{B.app}} \quad (2)$$

where $CL_{B.app}$ is the apparent organ clearance, Q_B is the organ blood flow and f_p is the plasma free fraction. The physiological constants for a 250 g rat used in this study are listed in Table 2. Tissue volumes except for muscle and blood were determined experimentally from the wet tissue weight by assuming a density of 1·0 for each tissue. The muscle volume was assumed to be the half of the body weight (Dedrick 1973). The blood volume was calculated according to Bischoff et al (1971) as follows:

$$V_{plasma} = 44 \times [\text{body weight (kg)}]^{0.99} \quad (3)$$

$$V_{blood} = V_{plasma}/(1 - Ht) \quad (4)$$

where Ht is the hematocrit value and was determined to be 0·40 in this study. The volume ratio of artery to venous blood was assumed to be the same as that of man, i.e. 0·5 (Benowitz et al 1974). Tissue or organ blood flow rates were obtained from the literature after correction for body weight (Sasaki & Wagner 1971; Dedrick et al 1973; Lutz et al 1977). With these constants and the values of $CL_{B.app}$ determined in this study, the $f_p CL_{int}$ was calculated to be 21·53 ml min^{-1} for liver and 2·21 ml min^{-1} for kidney, respectively, by use of equation 2. Then, the values of (f_p/R_B) CL_{int} were also calculated to be 11·27 ml min^{-1} for liver and 1·16 ml min^{-1} for kidney, respectively. In comparison with the respective organ flows, i.e. 14·7 ml min^{-1} for liver and 11·4 ml min^{-1} for kidney, it is suggested that the hepatic elimination is blood flow rate-limited, whereas renal elimination is intrinsic clearance-limited.

As an index of tissue distribution of drugs, the tissue-to-plasma partition coefficient (K_p) of the physiological pharmacokinetic model is superior to the apparent tissue-to-plasma partition coefficients ($K_{p.app}$), since K_p is corrected by elimination, blood flow and tissue weight (Chen &

Table 2. Physiological constants in rats.[a]

Tissue	Volume[b] (ml)	Blood flow[c] (ml min^{-1})
Arterial blood	6·2	39·0
Kidney	2·0	11·4
Heart	1·0	5·7
Muscle	125·0	6·8
Adipose tissue	10·0	0·4
Liver	11·0	14·7
Gut	11·1	11·0
Stomach	1·2	1·0
Spleen	1·0	0·9

[a] Based on 250 g rat.
[b] Determined experimentally from the wet tissue weight by assuming a density of 1·0 for each tissue except for muscle and arterial blood volume. The muscle volume was assumed to be the half of the body weight (Dedrick 1973) and the arterial blood volume was calculated from the body weight according to Bischoff et al (1971) (see Text).
[c] Obtained from the literature (Sasaki & Wagner 1971; Dedrick et al 1973; Lutz et al 1977) (see text).

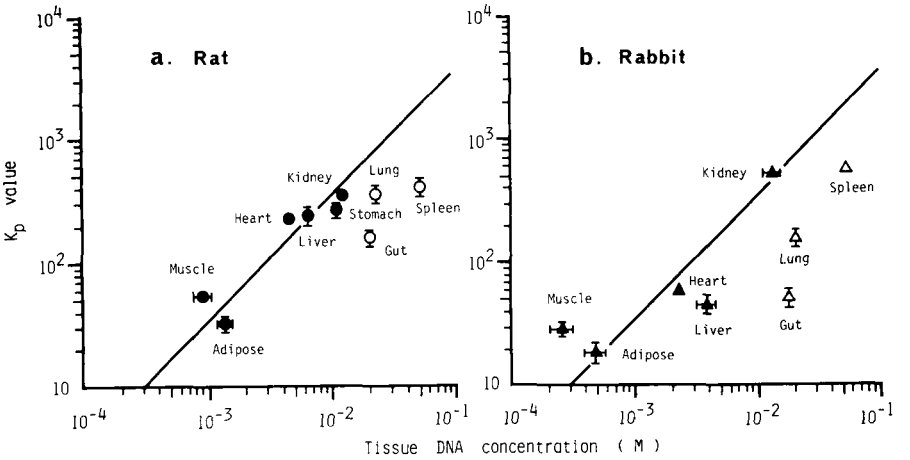

FIG. 2. Correlation between K_p value and tissue DNA concentration in rats (a) and rabbits (b). Each point and vertical bar represents the means ± s.e. of 3–7 independent experiments. The point without vertical bar includes it within the circle or triangle. Lines were obtained from the data points except for those of lung, spleen and gut by an iterative least squares regression analysis (see Text).

Gross 1979). In this study, considering the distribution to blood cells, we have further developed the equations of K_p for tissues, proposed by Chen & Gross (1979). The equations of K_p for lung, liver, kidney and nondisposing tissue or organ except for lung are respectively,

$$K_p^{LU} = \frac{K_{p,app}^{LU}}{1 - (\beta V_{AB}/Q_B^{LU})} \quad (5)$$

and

$$K_p^{LV} = \frac{(R_B Q_B^{LV} + f_p CL_{int}^{LV})\, K_{p,app}^{LV}}{\beta V_{LV} K_{p,app}^{LV} + R_B[(Q_B^{LV} - Q_B^{SP} - Q_B^{GT} - Q_B^{SM}) + (K_{p,app}^{SP}/K_p^{SP})\, Q_B^{SP} + (K_{p,app}^{GI}/K_p^{GT})\, Q_B^{GT} + (K_{p,app}^{SM}/K_p^{SM})\, Q_B^{SM}]} \quad (6)$$

and

$$K_p^{KD} = \frac{(1 + f_p CL_{int}^{KD}/R_B Q_B^{KD})\, K_{p,app}^{KD}}{1 + (\beta V_{KD}/R_B Q_B^{KD})\, K_{p,app}^{KD}} \quad (7)$$

and

$$K_p^{T} = \frac{K_{p,app}^{T}}{1 + (\beta V_T K_{p,app}^{T}/R_B Q_B^{T})} \quad (8)$$

where super- and subscriptions LU, AB, LV, KD, SP, GT, SM and T represent lung, arterial blood, liver, kidney, spleen, gut, stomach and tissue, respectively.

The tissue-to-plasma unbound concentration ratio ($K_{p,f}$) is expressed as

$$K_{p,f} = \frac{C_t}{C_{p,f}} = \frac{K_p}{f_p} \quad (9)$$

where C_t is the total tissue concentration of ADR and $C_{p,f}$ is the plasma unbound concentration of ADR. To compare the species difference in ADR distribution, the $K_{p,f}$ should be used. However, in this study we intended to compare the tissue difference of ADR distribution, therefore we used K_p instead of $K_{p,f}$.

Substitution of the physiological constants listed in Table 2 and the values of $f_p CL_{int}$, β and R_B determined in this study into equations 5–8, allowed the values of K_p for nine tissues to be calculated (Table 3). The values for rabbits from the literature (Harris & Gross 1975) are given in Table 3 for comparison with those of rats obtained in this study. In both species, large values of K_p (20–500) were obtained and the affinities of ADR for the tissues are high for kidney and spleen, but low for adipose tissue and muscle. However, in rats the ratio of K_p for kidney to those for liver and heart is 1·5, whereas in rabbits it is 10—a remarkable species difference. The tissue DNA concentrations determined are also listed in Table 3. Fig. 2 shows the relationship between the K_p and the tissue DNA concentration (mole per wet tissue weight) for each tissue from rats (panel a) and rabbits (panel b). In both species, good correlations were observed for muscle, adipose tissue, heart, liver, stomach and kidney, whereas spleen, lung and gut showed a large discrepancy from the positive correlation.

From these findings, it is suggested that for the tissues which show a good correlation, the tissue DNA concentration might be a primary determinant of the tissue distribution of ADR. It is also suggested that the remarkable species difference of K_p for liver and heart might be explained by the species difference of the DNA concentration in these tissues (Table 3). On the other hand, a permeability-limited transport mechanism was suggested for spleen in rabbits (Harris & Gross 1975), and a carrier-mediated influx and an active efflux mechanism were suggested for Ehrlich Ascites tumor cells (Skovsgaard 1978). Accordingly, possible mechanisms, such as a lower capacity to bind to DNA than that of other tissues and/or existence of a barrier, is suggested for tissues which show a

Table 3. K_p values of adriamycin and tissue DNA concentration in rats and rabbits.

Tissue	K_p values[a]		Tissue DNA concentration[a]	
	Rat[b]	Rabbit[c]	Rat[d,f] (M)	Rabbit[e,f] (M)
Lung	354 ± 54	155 ± 24	$2{\cdot}35 \times 10^{-2} \pm 0{\cdot}04 \times 10^{-2}$	$2{\cdot}00 \times 10^{-2} \pm 0{\cdot}08 \times 10^{-2}$
Kidney	349 ± 27	512 ± 45	$1{\cdot}25 \times 10^{-2} \pm 0{\cdot}09 \times 10^{-2}$	$1{\cdot}28 \times 10^{-2} \pm 0{\cdot}12 \times 10^{-2}$
Heart	230 ± 17	57 ± 5·0	$4{\cdot}59 \times 10^{-3} \pm 0{\cdot}38 \times 10^{-3}$	$2{\cdot}24 \times 10^{-3} \pm 0{\cdot}08 \times 10^{-3}$
Muscle	54 ± 4·2	29 ± 3·6	$9{\cdot}15 \times 10^{-4} \pm 1{\cdot}59 \times 10^{-4}$	$2{\cdot}60 \times 10^{-4} \pm 0{\cdot}53 \times 10^{-4}$
Adipose	33 ± 4·4	19 ± 3·5	$1{\cdot}35 \times 10^{-3} \pm 0{\cdot}15 \times 10^{-3}$	$4{\cdot}89 \times 10^{-4} \pm 0{\cdot}88 \times 10^{-4}$
Liver	242 ± 39	45 ± 8·3	$6{\cdot}43 \times 10^{-3} \pm 0{\cdot}38 \times 10^{-3}$	$3{\cdot}81 \times 10^{-3} \pm 0{\cdot}51 \times 10^{-3}$
Gut	161 ± 23	51 ± 7·7	$2{\cdot}05 \times 10^{-2} \pm 0{\cdot}08 \times 10^{-2}$	$1{\cdot}77 \times 10^{-2} \pm 0{\cdot}10 \times 10^{-2}$
Stomach	258 ± 29	N.D.[g]	$1{\cdot}11 \times 10^{-2} \pm 0{\cdot}04 \times 10^{-2}$	$7{\cdot}77 \times 10^{-3} \pm 0{\cdot}47 \times 10^{-3}$
Spleen	404 ± 72	556 ± 46	$5{\cdot}41 \times 10^{-2} \pm 0{\cdot}38 \times 10^{-2}$	$5{\cdot}13 \times 10^{-2} \pm 0{\cdot}27 \times 10^{-2}$

[a] The mean ± s.e. of each experiments.
[b] n = 4.
[c] From the literature (Harris & Gross 1975).
[d] n = 4–7.
[e] n = 3.
[f] Calculated using the calf thymus DNA (mol wet 330·9; sodium salt of nucleotide) as the standard. M: mol litre^{-1}.
[g] Not determined.

discrepancy from the positive correlation.

In conclusion, the results suggest that the characteristic tissue distribution of ADR might be associated with tissue DNA concentration.

The authors thank Dr Yasufumi Sawada for his helpful discussion. The authors also thank Kyowa Hakko Kogyo Co, Ltd for the gift of adriamycin hydrochloride.

REFERENCES

Alberts, D. S., Bachur, N. R., Holtzman, J. L. (1971) Clin. Pharmacol. Ther. 12: 96–104

Bachur, N. R., Hildebrand, R. C., Jaenke, R. S. (1974) J. Pharmacol. Exp. Ther. 191: 331–340

Benowitz, N., Forsyth, R. P., Melmon, K. L., Rowland, M. (1974) Clin. Pharmacol. Ther. 16: 87–98

Bischoff, K. B., Dedrick, R. L., Zaharko, D. S., Longstreth, J. A. (1971) J. Pharm. Sci. 60: 1128–1133

Burton, K. (1956) Biochem. J. 62: 315–323

Chen, H. S. G., Gross, J. F. (1979) J. Pharmacokinet. Biopharm. 7: 117–125

Cradock, J. C., Egorin, M. J., Bachur, N. R. (1973) Arch. Int. Pharmacodyn. 202: 48–61

Dedrick, R. L., Zaharko, D. S., Lutz, R. J. (1973) J. Pharm. Sci. 62: 882–890

Di Marco, A., Arcamone, F., Zunino, F. (1975) in: Corcoran, J. W., Hahn, F. E. (eds) Antibiotics Vol. III Mechanism of Action of Antimicrobial and Antitumor Agents. Springer-Verlag New York, Heidelberg, Berlin, pp 101–128

Egorin, M. J., Hildebrand, R. C., Cimico, E. F., Bachur, N. R. (1974) Cancer Res. 34: 2243–2245

Harris, P. A., Gross, J. F. (1975) Cancer Chemother. Rep. Part I 59: 819–825

Lutz, R. J., Dedrick, R. L., Matthews, H. B., Eling, T. E., Anderson, M. W. (1977) Drug Metab. Dispos. 5: 386–396

Nakagawa, T., Koyanagi, Y., Togawa, H. (1978) 'SALS, a computer program for statistical analysis with least squares fitting'. Library Program of the University of Tokyo Computer Center, Tokyo, Japan

Sabeur, G., Genest, D., Aubel-Sadron, G. (1979) Biochem. Biophys. Res. Commun. 88: 722–729

Sasaki, Y., Wagner, H. N., Jr (1971) J. Appl. Physiol. 30: 879–884

Schneider, W. C. (1946) J. Biol. Chem. 164: 747–751

Skovsgaard, T. (1978) Biochem. Pharmacol. 27: 1221–1227

Tavoloni, N., Guarino, A. M. (1980) Pharmacology 21: 244–255

Watson, E., Chen, K. K. (1976) Cancer Treat. Rep. 60: 1611–1618

Yesair, D. W., Schwartzbach, E., Shuck, D., Denine, E. P., Asbell, M. A. (1972) Cancer Res. 32: 1177–1183

Zunino, F., Gambetta, R., Di Marco, A., Zaccara, A. (1972) Biochim. Biophys. Acta 277: 489–498