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SHORT COMMUNICATION

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Evaluation of anthracycline cardiotoxicity with the model of isolated, perfused rat heart: comparison of new analogues versus doxorubicin

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Abstract We have compared the cardiotoxicity of 3 anthracyclines in a model of isolated perfused rat heart using the Langendorff technique. The contractile state and ventricular compliance were studied. Doxorubicin, epirubicin and pirarubicin were perfused at concentrations of 10^{-6} and 10^{-5} M during 70 min. The cardiac accumulation of the drugs was studied by HPLC. No significant alteration of cardiac functional parameters was observed at 10^{-6} M. At 10^{-5} M, epirubicin produced a significantly greater alteration of cardiac contractility than doxorubicin, whereas pirarubicin exerted first an inotropic effect followed by a recovery to initial values at the 60th min. Anthracycline accumulation in the heart was dose-dependent; epirubicin accumulated to a 30% greater extent than doxorubicin and pirarubicin heart concentrations were 4–5 times higher than those of doxorubicin at the end of the perfusion. These results suggest that doxorubicin and epirubicin have the same intrinsic cardiac toxicity, and that their distinct clinical cardiotoxicity must be explained by pharmacokinetic differences, whereas pirarubicin is much less cardiotoxic than the other anthracyclines because of different pharmacodynamic properties.

Key words Anthracyclines · Cardiotoxicity
Isolated perfused rat heart

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Introduction

Doxorubicin is an effective anthracycline antibiotic used in the treatment of hematologic malignancies and solid tumors. However, both acute and chronic dose-related and irreversible cardiotoxicity limit the use of doxorubicin and other anthracyclines [1]. Numerous anthracycline analogues have been synthesized and tested in attempts to obtain potent anthracyclines that are less cardiotoxic [2]. A fundamental assumption of this continued development is that the cardiotoxic and cytotoxic effects of anthracyclines can be divorced from one another, that is, they are the result of different mechanisms [3]. Among the new anthracyclines developed by the pharmaceutical industry and brought to routine clinical use are epirubicin (4′-epidoxorubicin) and pirarubicin (4′-*O*-tetrahydropyranyldoxorubicin). Both of these have been shown to display similar or better antitumour activity and to cause less cardiac toxicity than doxorubicin in preclinical models as well as in early clinical trials [4–6]. No definitive explanation has been brought forward to explain this reduced cardiotoxicity. It has been hypothesized, however, that it could result from pharmacokinetic and metabolic differences between the drugs [7] or from the inability of these drugs to produce free radicals upon one-electron reduction, a reaction that is well known to occur with doxorubicin [8].

For the preclinical evaluation of anthracycline cardiotoxicity and its eventual modulation by cardioprotectors, we used the isolated, perfused rat-heart preparation of Langendorff. We compared the cardiac contractility and myocardial drug uptake obtained following the perfusion of anthracyclines in this preparation. The anthracyclines tested were the reference molecule doxorubicin and the newly clinically available anthracyclines epirubicin and pirarubicin. The objectives of the study were to evaluate the value and the limits of this ex vivo model for the prediction of anthracycline cardiotoxicity in humans. The relationships that can be established in this model between the decrease in cardiac contractility and the accumulation of anthracyclines in the heart could prove to be useful for

the preclinical screening of new anthracyclines as well as for new forms of administration of these drugs (encapsulation in liposomes, complexation with macromolecules).

Materials and methods

Drugs

Anthracyclines were obtained from Laboratoire Bellon (daunorubicin, pirarubicin) and from Farmitalia (doxorubicin, epirubicin). They were diluted to 2 mg/ml with sterile water, aliquoted, and kept frozen for subsequent use.

Perfusion of isolated rat heart

Male Sprague-Dawley rats weighing 400–450 g were heparinized i.p. (500 IU/100 g) and anaesthetized with diethyl ether. The heart was quickly excised and briefly placed in a Krebs-Henseleit solution at 4 °C. Coronary perfusion was initiated through a short cannula in the aortic root and maintained at a constant pressure of 92.8 ± 1.9 mmHg in a nonrecirculating way using the Langendorff technique as described by Lorell et al. [9]. Perfusion pressure was measured by a P_{23}Db transducer (Bentley Trantec) connected to the aortic infusion cannula. The heart was electrically paced at a rate of 300 beats/min (5 Hz) through a stimulator-activated stainless-steel electrode placed on the heart. A latex balloon attached to one end of a polyethylene catheter was placed in the left ventricle through the mitral valve. The catheter was filled with water and the other end was linked to an electronic amplifier (Thomson Medical) via a second P_{23}Db transducer. The coronary perfusion pressure and left ventricular pressure were recorded on a computer that allowed continuous monitoring of the heart rate, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), and the maximal and minimal first derivatives of the LVSP as a function of time [$LV(dP/dt)_{max}$ and $LV(dP/dt)_{min}$, respectively].

The perfusate consisted of modified Krebs-Henseleit buffer containing 118 m*M* NaCl, 4.7 m*M* KCl, 1.2 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4, 25 m*M* $NaHCO_3$, 11 m*M* glucose, 0.95 m*M* $CaCl_2$, and 10 IU insulin/l (pH 7.4). It was continuously bubbled with a mixture of 95% O_2/5% CO_2 and maintained at 37 °C. After 30 min of stabilization, the latex balloon inserted into the left ventricle was dilated with distilled water sufficiently to produce an LVEDP of 6 mmHg. The heart was perfused for an additional 70 min with the Krebs-Henseleit buffer with or without anthracycline at a concentration of 10^{-6} or 10^{-5} *M*.

Anthracycline accumulation

Intracardiac drug accumulation was estimated by high-performance liquid chromatography (HPLC) as follows. Anthracyclines were extracted from samples taken from the same area of the left ventricle and weighing 148 ± 20 mg. The samples were homogenized in physiological saline (2 ml for 100 mg tissue) with a tissue homogenizer (Ultra-Turrax) and were kept frozen at –80 °C until extraction. Anthracyclines were extracted from 0.5-ml aliquots of the homogenates according to the method of Baurain et al. [10] by the addition of 0.5 ml borate buffer (50 m*M*, pH 9.8), 9 ml chloroform/methanol (4:1, v/v), and an adequate amount of internal standard (daunorubicin). After mixing and centrifugation (10 min at 3000 *g*), the solvent layer was recovered, evaporated to dryness, and reconstituted in a small volume of methanol. A calibration curve was obtained after incubation of heart homogenates with anthracyclines in vitro for 15 min at room temperature [11]. A good linearity from 0.015 to 1.5 nmol/mg tissue was obtained.

Chromatography was performed on a μBondapak C_{18} column (Waters Associates) measuring 30 × 0.39 cm. The solvent was a mixture of ammonium formate buffer (60 m*M*, pH 4.0) and acetonitrile (66:34, v/v, for doxorubicin and epirubicin assay and 60:40, v/v, for pirarubicin assay) and was delivered at 3 ml/min. Detection was achieved with a Perkin-Elmer LS1 spectrofluorometer with the excitation and emission wavelengths set at 480 and 592 nm, respectively. Retention times and peak areas were recorded with a Perkin-Elmer LCI-100 integrator. Under these conditions, the whole duration of the chromatographic analysis of each sample did not exceed 6 min.

Statistical analysis of the data

Statistical comparisons between untreated and anthracycline-treated groups were made by Student's *t*-test; all data are expressed as mean values ± SD. Statistical significance was assessed for *P* values below 0.05.

Results

Effects of anthracyclines on cardiac functional parameters

No significant decrease was observed in the untreated group ($n = 8$) after 70 min of perfusion, whatever the cardiac functional parameter considered [LVSP, LVEDP, LVDP, $LV(dP/dt)_{max}$, or $LV(dP/dt)_{min}$]. There was no significant alteration in the different cardiac functional parameters measured during 70 min of perfusion when anthracyclines were perfused at the concentration of 10^{-6} *M*, whereas these parameters were reproducibly altered at drug concentrations of 10^{-5} *M*.

Under doxorubicin perfusion, the LVDP decreased to 75% ± 4.5% of the initial value at the 70th min ($P < 0.001$ versus control; Fig. 1 A). After the same perfusion period, epirubicin induced a greater decrease in this parameter: 51% ± 5.4% of the initial value ($P < 0.001$). In contrast, pirarubicin induced a transient 15%–40% increase in the LVDP, which persisted until the 30th min and was followed by a recovery to the baseline value at the 60th min of perfusion.

$LV(dP/dt)_{max}$ and $LV(dP/dt)_{min}$ reflect the contractility and the relaxation state, respectively, of the left ventricular isovolumic myocardium. Doxorubicin induced a statistically significant decrease in both parameters, $LV(dP/dt)_{max}$ being lowered to 61% ± 4.2% ($P < 0.001$; Fig. 1 B) and $LV(dP/dt)_{min}$, to 72.4% ± 5.8% of the initial values ($P < 0.001$; Fig. 1 C). Epirubicin induced a significantly greater decrease in these parameters than did doxorubicin; the $LV(dP/dt)_{max}$ decreased to 43.5% ± 4.4% ($P < 0.001$; Fig. 1 B) and the $LV(dP/dt)_{min}$, to 46.9% ± 5.8% ($P < 0.001$; Fig. 1 C) of the initial values. Pirarubicin, in contrast, induced first a transient 20%–40% ($P < 0.01$) increase in both $LV(dP/dt)_{max}$ (Fig. 1 B) and $LV(dP/dt)_{min}$ (Fig. 1 C) at between 10 and 30 min of perfusion, followed by a recovery to the initial values after the 60th min.

Cardiac accumulation of anthracyclines

Anthracycline accumulation in the heart was highly dependent on the dose present in the perfusion liquid; when the anthracycline concentration was increased from 10^{-6} to 10^{-5} *M*, the accumulation was augmented by a factor of

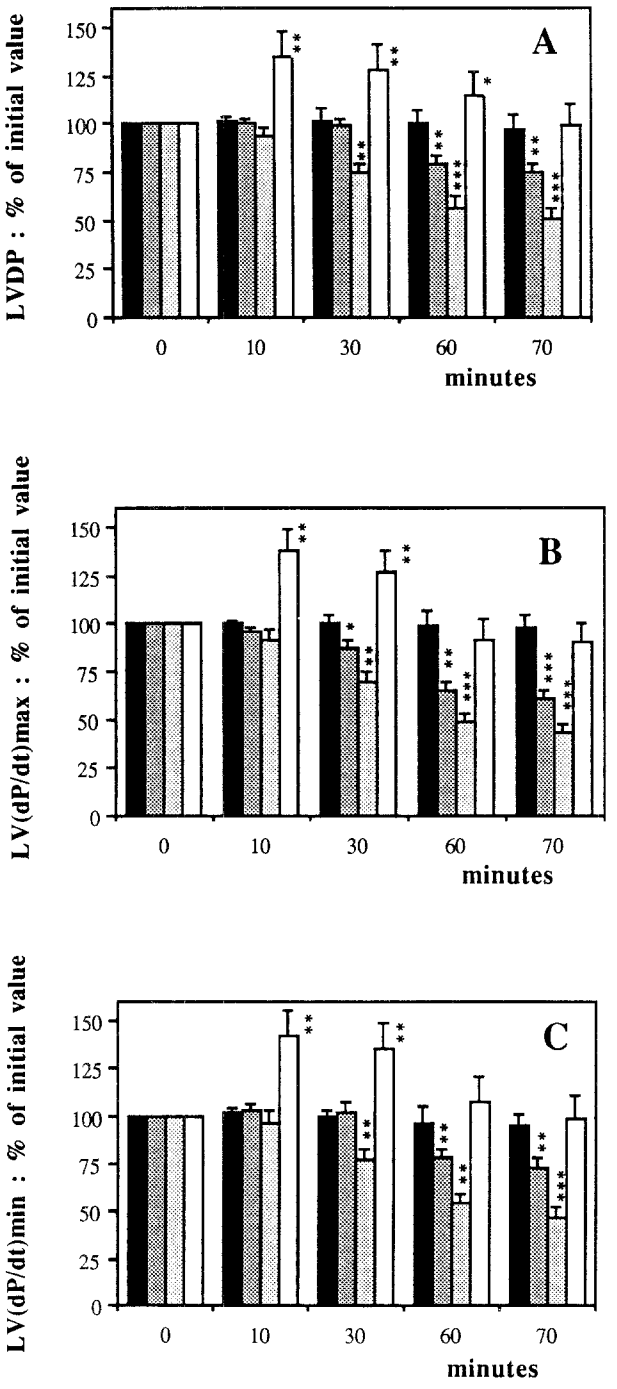

Fig. 1A–C Effects of once-through perfusion of anthracyclines on the cardiac functional parameters **A** LVDP, **B** $LV(dP/dt)_{max}$, and **C** $LV(dP/dt)_{min}$. After 30 min of stabilization, the heart was perfused with the Krebs-Henseleit solution without drug (■, $n = 8$) or with doxorubicin (▨, $n = 7$), epirubicin (▨, $n = 6$), or pirarubicin (□, $n = 7$) at 10^{-5} M as described in Materials and methods. Data are expressed as mean values ± SD. Significant differences as compared with controls: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

7.5–8 (Table 1). It seems that cardiac uptake must be very rapid, since no difference in cardiac accumulation of doxorubicin at 10^{-6} M occurred between 30 and 60 min of perfusion (data not shown). Epirubicin accumulation in the heart was 30% higher than that of doxorubicin, and pirarubicin accumulation was even larger; its concentrations in the left ventricle were 4–5 times higher than those of doxorubicin after 70 min of perfusion of equimolar doses (Table 1).

Discussion

We showed in this study that it was possible to follow the acute cardiac toxicity of anthracyclines with the isolated, perfused rat heart. The anthracycline concentrations capable of generating reproducible alterations in cardiac function are in the range of 10^{-6}–10^{-5} M, which is pharmacologically relevant in view of the circulating amounts of these drugs observed after an i.v. bolus [12].

The adverse effects of doxorubicin on cardiac function have been extensively described [13–16] but the mechanisms of this cardiac toxicity remain unclear. It is likely, however, that these mechanisms are different from those involved in the cytotoxicity of this drug and its analogues [3, 17], and sustained research efforts aimed at dissociating these two activities are therefore worthwhile.

Paradoxically, epirubicin, which has shown a lower degree of cardiac toxicity than doxorubicin in animal models, including the rat [18, 19], and in clinical use [20], exerts in our model a greater effect on cardiac contractility than does doxorubicin. This might be due to the observation that the disposition of these drugs in the whole body are different, epirubicin having a higher volume of distribution and a shorter half-life than doxorubicin [21, 22]; under these conditions, the plasma concentrations of epirubicin are systematically lower than those of doxorubicin after equimolar administration. In our model of isolated, perfused rat heart, the more pronounced cardiac effects of epirubicin could be due to its increased tissue uptake relative to doxorubicin. If the two molecules have similar toxicity for equivalent intracardiac concentrations, then the reduced cardiac toxicity of epirubicin in vivo would be essentially due to pharmacokinetic reasons [23]. In contrast, pirarubicin accumulates in the myocardium to an even greater extent than epirubicin, probably due to its very fast tissue uptake [24, 25]. Despite the extent of this uptake, pirarubicin produced lesser effects, which would indicate that it is much less pharmacologically active than doxorubicin and epirubicin against the myocardium; its reduced cardiotoxicity can therefore be attributed to pharmacodynamic reasons.

The decrease we observed in heart contractility can be entirely attributed to the drugs perfused. It should be emphasized that there was no evident production of any of the 13-dihydro metabolites in the myocardium, which excludes the possibility of their role in the alteration in cardiac function observed in our study. It has been suggested by Olson et al. [17] and Mushlin et al. [26] that doxorubicinol contributes to the cardiac toxicity of doxorubicin in the rabbit. In view of the lower metabolic conversion of epirubicin to epirubicinol, Sweatman and Israel [27] have suggested that this metabolic difference

Table 1 Relationship between cardiac dysfunction and anthracycline accumulation in the heart after 70 min of perfusion. At the end of heart perfusion with anthracycline, a sample of myocardium was taken from the left ventricle to determine drug accumulation as described in Materials and methods. In the control group, the $LV(dP/dt)_{max}$ decreased to 97.8% ± 6.8% of the initial value. Data are expressed as described in Materials and methods and represent mean values ± SD for at least six independent experiments

Drug	10^{-6} *M*		10^{-5} *M*	
	Drug accumulation (nmol/mg tissue)	$LV(dP/dt)_{max}$, % of initial value (70 min)	Drug accumulation (nmol/mg tissue)	$LV(dP/dt)_{max}$, % of initial value
Doxorubicin	0.040 ± 0.003	104 ± 8	0.32 ± 0.03	61.0 ± 4.2*
Epirubicin	0.055 ± 0.003**	103 ± 4	0.41 ± 0.04**	43.5 ± 4.4*
Pirarubicin	0.190 ± 0.005**	96 ± 6	1.43 ± 0.13**	90.0 ± 10.6

*$P < 0.001$ as compared with the control value
**$P < 0.001$ as compared with the value obtained with doxorubicin

could be the cause of the decreased cardiac toxicity of this drug as compared with doxorubicin. However, in our model, the absence of the 13-dihydro metabolites means that the difference in cardiac toxicity observed between doxorubicin and epirubicin cannot be explained by metabolic considerations.

Pirarubicin did not reduce cardiac contractility in our model. It is noteworthy that it was not transformed into doxorubicin in the myocardium. Doxorubicin is known to be a metabolite of pirarubicin in humans, although its concentration after pirarubicin administration rules out the consideration of pirarubicin as a prodrug of doxorubicin [28]. In view of the absence of cardiac toxicity of pirarubicin in the isolated heart, the congestive heart failures observed in humans treated with cumulative doses of pirarubicin exceeding 1000 mg/m^2 [6] could well be due to the presence of significant amounts of doxorubicin in the circulation after pirarubicin administration.

All contractile parameters were altered by the perfusion of anthracyclines at a concentration of 10^{-5} *M*. We did not explore in this study the electrophysiological properties and the spontaneous heart rate; when alterations in these parameters are produced by anthracyclines, they are generally transient and reversible and are therefore not indicative of a functional alteration in the myocardium. Using pirarubicin in the isolated, perfused rat heart, Del Tacca et al. [29] observed a transient positive inotropic effect followed by a considerable decrease in developed tension to values inferior to those obtained in control experiments. Although we also observed a transient positive inotropic effect with this drug, we observed no subsequent decrease in developed pressure. This discrepancy can be explained by the observation that we applied a constant stimulation to the heart throughout our experimental protocol; several studies have shown that electrical pacing is desirable, allowing a constant heart rate and a precise and reproducible measurement of cardiac function [30]. Moreover, the measurement of developed tension of cardiac fibers in the isolated whole heart, which has been used by Del Tacca et al. [29], might not be reliable because the ventricular myocardium does not feature the homogeneous alignment of muscle fibers seen in other parts of the heart such as the papillary muscle or atria. Hirano et al. [31] and Temma et al. [32] also observed a positive inotropic effect with pirarubicin, without any decrease in cardiac contractile force; our results are in agreement with these findings. At the doxorubicin concentration used in this study (10^{-5} *M*), we did not observe the positive inotropic effect described by some authors [13, 31]. This discrepancy is probably due to differences in the models or techniques used.

The mechanisms of cardiac toxicity of anthracyclines remain elusive and cannot be inferred from our work. However, there are marked differences between epirubicin and pirarubicin in their effect on cardiac contractility. These could be due to differences in the generation of toxic free-radical species or lipid peroxides or could result from perturbations of cytosolic calcium homeostasis; the model of isolated, perfused rat heart provides a tool for the understanding of these processes. These approaches are presently being investigated in our laboratory.

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References

1. Unverferth DV, Magorien RD, Leier CV, Balcerzak SP (1982) Doxorubicin cardiotoxicity. Cancer Treat Rev 9: 149
2. Casazza AM (1986) Preclinical selection of new anthracyclines. Cancer Treat Rep 70: 43
3. Cummings J, Anderson L, Willmott N, Smyth JF (1991) The molecular pharmacology of doxorubicin in vivo. Eur J Cancer 27: 532
4. Ganzina F (1983) 4′-Epi-doxorubicin, a new analogue of doxorubicin: a preliminary overview of preclinical and clinical data. Cancer Treat Rev 10: 1
5. Maehara Y, Sakaguchi Y, Kusumoto T, Kusumoto H, Sugimachi K (1989) 4′-*O*-Tetrahydropyranyladriamycin has greater antineoplastic activity than doxorubicin in various human tumours in vitro. Anticancer Res 9: 387
6. Hérait P, Poutignat N, Marty M, Bugat R (1992) Early assessment of a new anticancer drug analogue. Are the historical comparisons obsolete? The French experience with pirarubicin. Eur J Cancer 28 A: 1670
7. Ganzina F, Di Pietro N, Magni O (1985) Clinical toxicity of 4′-epi-doxorubicin (epirubicin). Tumori 71: 233
8. Keizer HG, Pinedo HM, Schuurhuis GJ, Joenje H (1990) Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. Pharmacol Ther 47: 219

9. Lorell BH, Wexler LF, Momomura S, Weinberg E, Apstein CS (1986) The influence of pressure overload left ventricular hypertrophy on diastolic properties during hypoxia in isovolumically contracting rat hearts. Circ Res 58: 653
10. Baurain R, Deprez-De Campaneere D, Trouet A (1979) Rapid determination of doxorubicin and its fluorescent metabolites by high pressure liquid chromatography. Anal Biochem 94: 112
11. De Jong J, Guérand WS, Schoofs PR, Bast A, Vijgh WJF van der (1991) Simple and sensitive quantification of anthracyclines in mouse atrial tissue using high-performance liquid chromatography and fluorescence detection. J Chromatogr 570: 209
12. Piazza E, Natale N, Trabattoni A, Mariscotti C, Mosca L, Libretti A, Ottolenghi L, Morasca L (1981) Plasma and tissue distribution of adriamycin in patients with pelvic cancer. Tumori 67: 533
13. Pelikan PCD, Weisfieldt ML, Jacobus WE, Miceli MV, Bulkley BH, Gerstenblith G (1986) Acute doxorubicin cardiotoxicity: functional, metabolic, and morphologic alterations in isolated, perfused rat heart. J Cardiovasc Pharmacol 8: 1058
14. Rabkin SW (1983) Interaction of external calcium concentration and verapamil on the effects of doxorubicin (adriamycin) in the isolated heart preparation. J Cardiovasc Pharmacol 5: 848
15. Lee V, Randhawa AK, Singal PK (1991) Adriamycin-induced myocardial dysfunction in vitro is mediated by free radical. Am J Physiol 261 H: 989
16. Kusuoka H, Futaki S, Koretsune Y, Kitabatake A, Suga H, Kamada T, Inoue M (1991) Alterations of intracellular calcium homeostasis and myocardial energetics in acute adriamycin-induced heart failure. J Cardiovasc Pharmacol 18: 437
17. Olson RD, Mushlin PS, Brenner DE, Fleischer S, Cusack BJ, Chang BK, Boucek RJ (1988) Doxorubicin cardiotoxicity may be caused by its metabolite, doxorubicinol. Proc Natl Acad Sci USA 85: 3585
18. Llesuy SF, Milei J, Molina H, Boveris A, Milei S (1985) Comparison of lipid peroxidation and myocardial damage induced by adriamycin and 4′-epiadriamycin in mice. Tumori 71: 241
19. Cini-Neri G, Neri B, Bandinelli M, Del Tacca M, Danesi R (1991) Anthracycline cardiotoxicity: in vivo and in vitro effects on biochemical parameters and heart ultrastructure of the rat. Oncology 48: 327
20. Bonfante V, Ferrari L, Brambilla C, Rossi A, Villani F, Crippa F, Valagussa P, Bonadonna G (1986) New anthracycline analogs in advanced breast cancer. Eur J Cancer Clin Oncol 22: 1379
21. Weenen H, Lankelma J, Penders PGM, McVie JG, Bokkel Huinink WW ten, De Planque MM, Pinedo HM (1983) Pharmacokinetics of 4′-epi-doxorubicin in man. Invest New Drugs 1: 59
22. Vijgh WJF van der, Maessen PA, Pinedo HM (1990) Comparative metabolism and pharmacokinetics of doxorubicin and 4′-epidoxorubicin in plasma heart and tumor of tumor-bearing mice. Cancer Chemother Pharmacol 26: 9
23. Andersson M, Domellöf L, Eksborg S, Häggmark S, Johansson G, Reiz S, Herslöf A (1989) Pharmacokinetics and central haemodynamic effects of doxorubicin and 4′-epi-doxorubicin in the pig. Acta Oncol 28: 709
24. Iguchi H, Tone H, Ishikura T, Takeuchi T, Umezawa H (1985) Pharmacokinetics and disposition of 4′-*O*-tetrahydropyranyladriamycin in mice by HPLC analysis. Cancer Chemother Pharmacol 15: 132
25. Kunimoto S, Miura K, Takahashi Y, Takeuchi T, Umezawa H (1983) Rapid uptake by cultured tumor cells and intracellular behavior of 4′-*O*-tetrahydropyranyladriamycin. J Antibiot 36: 312
26. Mushlin SP, Cusack BJ, Boucek RJ, Andrejuk T, Li X, Olson RD (1993) Time-related increases in cardiac concentrations of doxorubicinol could interact with doxorubicin to depress myocardial contractile function. Br J Pharmacol 110: 975
27. Sweatman TW, Israel M (1987) Comparative metabolism and elimination of adriamycin and 4′-epiadriamycin in the rat. Cancer Chemother Pharmacol 19: 201
28. Robert J, David M, Huet S, Chauvergne J (1988) Pharmacokinetics and metabolism of pirarubicin in advanced cancer patients. Eur J Cancer Clin Oncol 24: 1289
29. Del Tacca M, Danesi R, Solaini G, Bernardini MC, Bertelli A (1987) Effects of 4′-*O*-tetrahydropyranyl-doxorubicin on isolated perfused rat heart and cardiac mitochondrial cytochrome C oxidase activity. Anticancer Res 7: 803
30. Leiris J de, Harding DP, Pestre S (1984) The isolated perfused rat heart: a model for studying myocardial hypoxia or ischaemia. Basic Res Cardiol 79: 313
31. Hirano S, Agata N, Hara Y, Iguchi H, Shirai, Tone H, Urakawa (1991) Effects of pirarubicin, an antitumor antibiotic, on the cardiovascular system. Cancer Chemother Pharmacol 28: 266
32. Temma K, Akera T, Chugun A, Kondo H, Hagane K, Hirano S (1993) Comparison of cardiac actions of doxorubicin, pirarubicin and aclarubicin in isolated guinea-pig heart. Eur J Pharmacol 234: 173