Cancer Chemother Pharmacol (1997) 40: 419–424

ORIGINAL ARTICLE

L. Lundgren-Eriksson · A. Carlsson · S. Eksborg
W. Ryd · R. Vesanen · R. Hultborn

Pharmacokinetics of doxorubicin and epirubicin in mice during chlorpromazine-induced hypothermia

Received: 27 May 1996 / Accepted: 8 February 1997

Abstract Blood concentrations of doxo- and epirubicin were studied in mice after i.v. or i.p. administration under normal and hypothermic conditions. The animals either were pretreated i.p. with chlorpromazine at 15 mg/kg and allowed to cool to a rectal temperature of 28 °C or were given saline i.p. with their rectal temperature remaining at 37 °C. The anthracyclines were 14-^{14}C-labeled and were given at a dose of 0.85 mg/kg. Blood samples were taken at 5, 15, and 25 min and 2, 6, 24, and 48 hours after injection and were analyzed by liquid scintillation counting. The blood concentration related to time was similar for the two anthracyclines. The peak concentration was highest for i.v. administration and was higher for the hypothermic groups. The peak concentration and the area under the curve were highest under hypothermic conditions. The terminal half-life was longer after i.p. administration. The ratio calculated for the blood concentration under hypothermic/normothermic conditions over time was substantially increased after i.p. administration, the increase being most pronounced for epirubicin. The pharmacokinetic characteristics found might be related to the anthracycline toxicity encountered in tumor-inoculated mice treated at different body temperatures.

Key words Anthracyclines · Hypothermia · Chlorpromazine · Pharmacokinetics · Mice

L. Lundgren-Eriksson · A. Carlsson · R. Hultborn
Department of Oncology,
Göteborg University,
Göteborg, Sweden

S. Eksborg
Karolinska Pharmacy,
Stockholm, Sweden

W. Ryd
Department of Pathology,
Göteborg University,
Göteborg, Sweden

R. Vesanen
Department of Radiophysics,
Göteborg University,
Göteborg, Sweden

L. Lundgren-Eriksson (✉)
Department of Oncology,
Umeå University Hospital
S-90185 Umeå Sweden
Tel: +46 90 785000; Fax: +46 90 7852031

Introduction

The idea of using nonphysiological temperatures to achieve a change in therapeutic index in cancer chemotherapy, either favorable or detrimental, stems from two assumed opposing basic mechanisms. The thermodynamics at increased temperatures imply an increased chance of substrate and enzyme interaction, whereas on the other hand, the complex quartenary and three-dimensional enzyme structure that is vital for its affinity to the substrate is likely to be disturbed. The rates of all enzymatic reactions have their own specific temperature optima. Since there are quantitative differences between normal and malignant cells in, for example, energy production, a change in temperature may imply a different response, presumably implicating a cascade of secondary effects on cell proliferation and DNA repair, among other cellular events. These effects on a cellular level might also coincide with general physiological adaptations possibly affecting drug distribution and elimination.

The therapeutic index of cancer chemotherapy agents is low, often limiting the full expression of their antineoplastic activity. In humans, anthracycline toxicity and efficacy have been modulated by variation of administration times from bolus injection to prolonged infusion, thereby decreasing the peak blood concentration without changing the area under the plasma concentration-time curve (AUC) [14]. Continuous infusion in animals, as in humans, has been shown to reduce cardiac toxicity while maintaining antitumor activity [5, 15, 20]. Fractionated administration of doxorubicin to

tumor-bearing mice has also been shown to give less toxicity while preserving therapeutic efficacy [24]. The reduction of the toxicity emanating from these modifications of the administration has been attributed to a decrease in the maximal plasma concentration of doxorubicin. Decreased body temperature, hypothermia, has been exploited experimentally [12, 13, 16]. It was found that the acute toxicity of doxorubicin could be reduced if the drug was given to tumor-bearing mice at a body temperature of 28 °C accomplished by administration of chlorpromazine. It seemed that the antitumor efficacy was increased under these conditions [16]. The aim of the present work was to describe the influence of chlorpromazine-induced hypothermia on the pharmacokinetics of doxorubicin and epirubicin given i.v. and i.p. to mice.

Materials and methods

Inbred male and female mice (C57B1/6J) aged 12–16 weeks and weighing 20–27 g were used. The animals were housed at 22 °C with artifical light from 6:00 a.m. to 6:00 p.m. The experiments were approved by the Committee of Laboratory Animal Ethics at Göteborg University. Chlorpromazine hydrochloride (Hibernal; LEO Pharmaceuticals, Helsingborg, Sweden) given i.p. at 15 mg/kg was used to induce hypothermia [16]. [14-^{14}C]-Doxorubicin hydrochloride (Adriamycin; Amersham, UK), 96.8% radiochemically pure with a specific activity of 3.37 μBq/mg, was used at a dose of 0.85 mg/kg. [14-^{14}C]-4-Epidoxorubicin hydrochloride (Farmorubicin; Farmitalia Carlo Erba, Italy), 91% radiochemically pure with a specific activity of 3.18 μBq/mg, was given at a dose of 0.85 mg/kg. The mice were divided into groups of six animals (three males and three females in each group) to compare the effect of hypothermia induced by chlorpromazine versus normal temperature and i.v. administration versus i.p. administration. Comparison can thus be made in a 2 × 2 factorial fashion. All experiments were started in the morning.

Two groups of the animals were given chlorpromazine i.p. at 15 mg/kg in 0.2 ml saline and were housed at an ambient temperature of 28 °C. The rectal temperature decreased to 28–29 °C within 1 hour and remained so for at least 8 h [16]. The mice were returned to an environment of 22 °C after 24 h. The other two groups, given 0.2 ml saline i.p., were continuously housed at 22 °C. At 1 h after chlorpromazine administration, which allowed the chlorpromazine-treated mice to cool, the animals were given radiolabeled doxorubicin or epirubicin (0.85 mg/kg) in a volume of 0.5 ml i.p. or in a volume of 0.2 ml i.v. over 2–4 s via the tail vein. Blood samples taken sequentially from the same animal were collected in 20-μl heparinized capillary tubes from the tail vein by tail amputation at 5, 15, and 25 min and 2, 6, and 24 h after anthracycline administration. In the doxorubicin-treated animals the blood sample was taken at 48 h. The whole-blood sample was mixed with Soluene-100 (Packard Instruments B.V., The Netherlands) in 20-ml LSC vials and was decolorized with hydrogen peroxide prior to the addition of 16 ml Insta-Gel (Packard Instruments B.V., The Netherlands) LSC cocktail. The samples were measured with a Searle Nuclear Chicago Mark II liquid scintillation counter beginning on the day after sample preparation, which allowed a minimum of 1 day's decay of the chemiluminescence caused by the addition of hydrogen peroxide and Soluene-100 to the samples The measurement time was 2 × 100 min and the count rate was corrected for quenching with the ESR method [25] prior to calculation of the activity concentrations, which were expressed as micrograms of anthracycline per milliliter of whole blood.

Statistical evaluation

Pitman's randomization test based on Mann-Whitney's *U*-test was used for the comparison of two independent samples [7]. Median values and approximate 95% confidence intervals were calculated by the Wilcoxon signed-rank test as outlined by Tukey. All statistical tests used have been exhaustively described by Daniel [8].

Pharmacokinetic evaluation

The JANA program [10] was used for pharmacokinetic modeling of the individual blood concentration-time data and of grouped data from all animals subjected to the various treatments. The two-compartment model was applicable in all cases. The maximal plasma concentration (C_{max}) was estimated from the intersection of the fitted concentration-time curve with the Y-axis. The relevance of the parameter C_{max} at i.v. administration may be questioned and might rather be defined "the ordinate parameter of the first term of the exponential equation." The area under the blood concentration-time curve (AUC) was estimated by numerical integration of the fitted concentration-time curve. The ratio plots were constructed from the pharmacokinetic curves obtained from fitting of the grouped data.

Results

The drug concentration in whole blood was higher for both drugs in the mice with chlorpromazine-induced hypothermia, irrespective of the route of administration. Curves of blood concentration plotted versus, time are shown for doxorubicin in Fig. 1 and for epirubicin in Fig. 2. The C_{max} was consistently higher under hypothermic conditions, though differences were significant only for doxorubicin given i.v. The C_{max} found for i.p. epirubicin was one-tenth that noted for i.v. epirubicin.

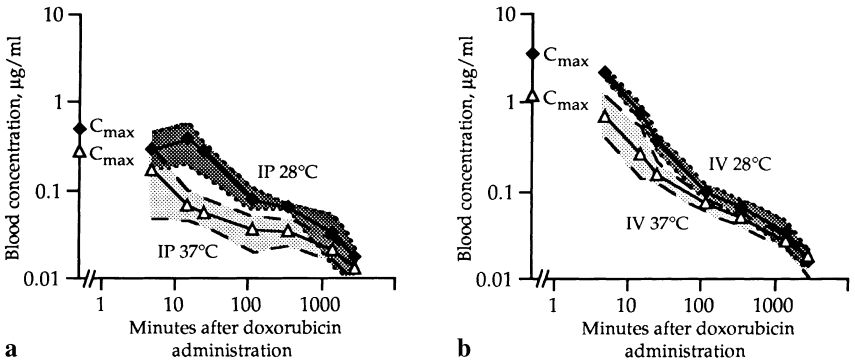

Fig. 1 a Blood concentration curve generated for doxorubicin after i.p. (*IP*) administration of 0.85 mg/kg to mice. Data represents median values ±87% confidence intervals (◆ 28 °C – $n = 4$, Δ 37 °C – $n = 5$). **b** Blood concentration curve generated for doxorubicin after i.v. (*IV*) administration of 0.85 mg/kg to mice. Data represent median values ±94% confidence intervals (◆ 28 °C – $n = 6$, Δ 37 °C – $n = 5$)

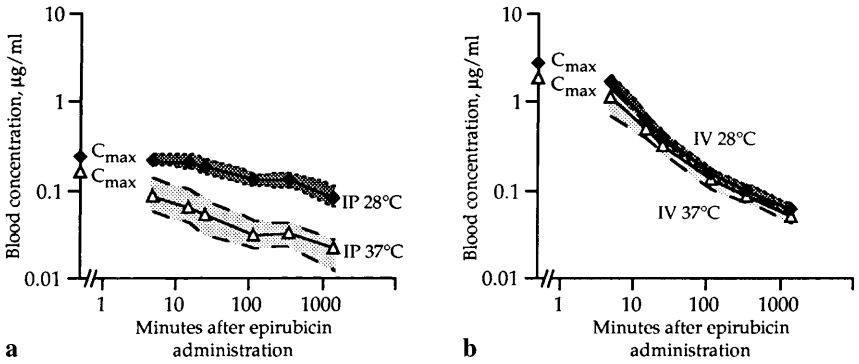

Fig. 2 a Blood concentration curve generated for epirubicin after i.p. (*IP*) administration of 0.85 mg/kg to mice. Data represent median values ±94% confidence intervals (◆ 28 °C – $n = 6$, Δ 37 °C – $n = 5$). **b** Blood concentration curve generated for epirubicin after i.v. (*IV*) administration of 0.85 mg/kg to mice. Data represent median values ±94% confidence intervals (◆ 28 °C – $n = 6$, Δ 37 °C – $n = 6$)

Table 1 Pharmacokinetic parameters calculated for [14-^{14}C]-doxorubicin after i.p./i.v. administration of 0.85 mg/kg to mice, expressed as median values and 95% confidence intervals[a]

	AUC (µg min ml^{-1})		
	i.p.	i.v.	
28 °C	2.37 (1.32–3.34) 87%	2.99 (2.21–3.55)	$P = 0.28$
37 °C	1.78 (1.3–1.97)	2.12 (1.82–2.41)	$P = 0.06$
	$P = 0.14$	$P = 0.03$	
	$t_{1/2}$ (min)		
	i.p.	i.v.	
28 °C	19.0 (17.0–23.0) 87%	16.1 (13.5–22.7)	$P = 0.64$
37 °C	35.5 (24.6–46.4)	19.4 (17.8–23.9)	$P = 0.008$
	$P = 0.02$	$P = 0.30$	
	C_{max} (µg/ml)		
	i.p.	i.v.	
28 °C	0.5 (0.24–0.8) 87%	3.44 (3.09–4.07)	$P = 0.05$
37 °C	0.27 (0.07–0.65)	1.2 (0.56–1.83)	$P = 0.02$
	$P = 0.23$	$P = 0.02$	

[a]Pitman's randomization test

Table 2 Pharmacokinetic parameters calculated for [14-^{14}C]-epirubicin after i.p./i.v. administration of 0.85 mg/kg to mice, expressed as median values and 95% confidence intervals[a]

	AUC (µg min ml^{-1})		
	i.p.	i.v.	
28 °C	7.53 (5.09–31.8)	4.05 (3.47–4.64)	$P = 0.001$
37 °C	3.8 (1.40–6.49)	3.30 (2.87–3.74)	$P = 0.41$
	$P = 0.02$	$P = 0.02$	
	$t_{1/2}$ (min)		
	i.p.	i.v.	
28 °C	37.6 (23.8–153.1)	16.3 (15.4–17.2)	$P = 0.001$
37 °C	32.7 (24.2–101.9)	15.9 (8.8–20.89)	$P = 0.001$
	$P = 0.32$	$P = 0.41$	
	C_{max} (µg/ml)		
	i.p.	i.v.	
28 °C	0.24 (0.16–0.28)	2.66 (2.52–3.18)	$P = 0.001$
37 °C	0.16 (0.08–0.30)	1.79 (1.17–8.03)	$P = 0.001$
	$P = 0.12$	$P = 0.5$	

[a]Pitman's randomization test

The difference was qualitatively the same for doxorubicin but was not as great (Tables 1, 2). The half-life ($t_{1/2}$) was consistently longer when the drugs were given i.p. as compared with i.v. for both anthracyclines and at both temperatures, though differences were not significant for doxorubicin during hypothermia. For doxorubicin the $t_{1/2}$ was shorter under hypothermic conditions, which was not the case for epirubicin (Tables 1, 2).

The AUCs recorded for both drugs were greater during hypothermia, significantly so for i.v. administration and for epirubicin given i.p., where the difference was substantial (Tables 1, 2). The ratio calculated for blood concentration under hypothermic/normothermic conditions over time was higher after i.p. administration,

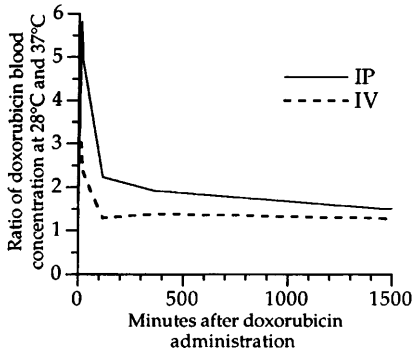

Fig. 3 Ratio of doxorubicin blood concentrations measured under hypothermic/normothermic conditions

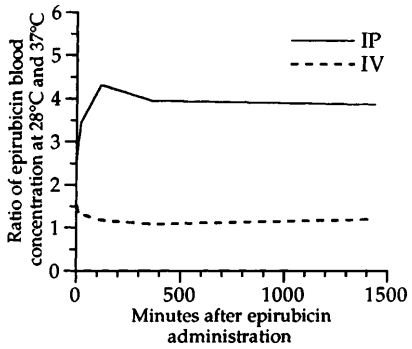

Fig. 4 Ratio of epirubicin blood concentrations measured under hypothermic/normothermic conditions

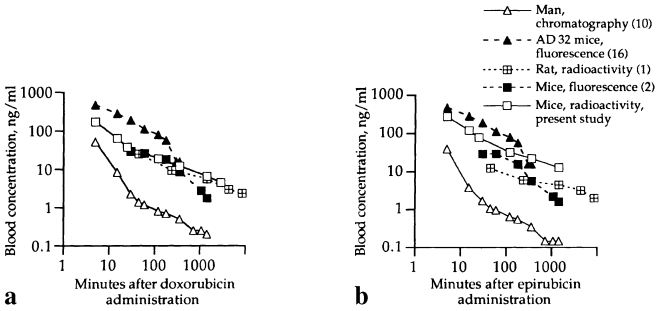

Fig. 5 a Comparative blood concentration curves generated for a doxorubicin dose recalculated to 1 mg/m^2 in various species after i.v. administration at normal body temperature. Data on fluorescence analysis of AD 32 are included for comparison. **b** Comparative blood concentration curves generated for an epirubicin dose recalculated to 1 mg/m^2 in various species after i.v. administration at normal body temperature. Data on fluorescence analysis of AD 32 are included for comparison

especially for epirubicin, but the ratio determined after i.v. administration was in the range of 1 (Figs. 3, 4).

Discussion

The study was performed as part of a series of studies to determine whether hypothermic conditions might increase the therapeutic index of cancer chemotherapy. Gailis [12] and Hultborn et al. [16] found that moderate hypothermia of up to 28 °C decreased the toxicity of doxorubicin and also might have increased its antitumor efficacy. The mode of action producing this result is unknown and could be mediated several ways. Since hypothermia was accomplished by administration of chlorpromazine, a pharmacological interaction is possible [13]. However, the toxicity-protective effect of chlorpromazine-induced hypothermia was reversed by increasing the body temperature to normal in the presence of chlorpromazine [12]. Furthermore, if any interaction is reported, it seems to be additive to various cytotoxic drugs [29].

Apart from the effects of temperature or chlorpromazine per se at the cellular level, we found it necessary to explore whether any pharmacokinetic differences for the anthracyclines would occur in the two situations. The pharmacokinetics of anthracyclines have been investigated in various species (e.g., [1, 2, 4, 18, 30–32]). Does measurement of the ^{14}C activity accurately reflect the biologically active compound? Israel et al. [18] studied the pharmacokinetics of AD 32, an anthracycline with a trifluoroacetamide to which ^{14}C was bound. They measured radioactivity as well as fluorescence in blood and found a protracted level of radioactivity in contrast to the decline in fluorescence, the latter mimicking our results. Broggini et al. [2] also measured fluorescence in the serum of mice given doxo- and epirubicin and obtained results similar to ours. Arcamone et al. [1] studied the anthracycline, labeled as in our study, in rats and obtained similar results. Recalculated data from the animal experiments mentioned above and the human data of Eksborg et al. [11] are illustrated in Fig. 5. If one assumes that the fluorescence derives from the active molecule, it seems relevant to use radioactivity data when ^{14}C is bound to the 14-position as suggested by Zini et al. [32]. Pharmacokinetics studies using high-performance liquid chromatography (HPLC) techniques to identify the native drug and metabolites have also shown plasma concentrations similar to our findings (e.g., [4, 30, 31]).

From the blood concentration versus time curves, three pharmacokinetic parameter were computed: C_{max}, $t_{1/2}$, and AUC, the latter being dependent on the two former parameters. We found blood concentration curves to be greater and C_{max} values to be higher under hypothermic conditions for i.v. as well as i.p. administration. In hypothermia the metabolism decreases and the blood flow through the organs is decreased. Mortensen and Dale [23] have shown that the enzymatic processes involved in activation and detoxification of drugs are temperature-dependent and different for various enzymes. If our results were due to a decreased excretion/metabolism, this would rather be seen as a progressive divergence of the two curves, which was not at all the case. Furthermore, the $t_{1/2}$ would be increased at least when the drugs were given i.v., which contrasts with our findings. Moreover, the largest differences occur after i.p. administration, which is unlikely to reflect the above mentioned mechanism. Before reaching the bloodstream, the drugs must pass the peritoneum into the portal vascular system and liver after i.p. administration [9]. The resulting blood concentration will be balanced by resorption and excretion and, thus, the C_{max} will be lower and the $t_{1/2}$, longer for this route of administration. The considerably higher blood concentrations seen in hypothermia are not easily explained, but chlorpromazine might facilitate resorption across the peritoneal barrier [3, 17, 19] and, possibly, affect peritoneal blood perfusion. Chlorpromazine also causes peripheral vasodilation, which might retain the drug in the blood circulation.

Changes in biliary excretion rates might be amplified following i.p. administration as compared with i.v. administration. Skibba et al. [28] showed that hyperthermia decreased the biliary excretion rate, resulting in a longer $t_{1/2}$. Doxorubicin and epirubicin behave generally similarly, with some quantitative differences, possibly due to the greater lipophilicity of epirubicin and the additional degradation pathways for this compound [27]. Epirubicin might be more susceptible to delayed liver metabolism as the drug is more rapidly converted to its metabolites. Our results show that the blood concentration over time and the AUC were higher during chlorpromazine-induced hypothermia and were most pronounced for epirubicin given i.p. Animal studies have shown unchanged plasma concentrations of doxorubicin during both whole-body hyperthermia (WBH) and local hyperthermia [21, 22], but in humans, Riggs et al. [26] found higher plasma concentrations of doxorubicin during WBH. The combination of hyperthermia and doxorubicin acts synergistically both in vivo and in vitro [6]. The AUC, presumably reflecting the biological effect, is the result of the blood concentration and elimination rate. The pharmacokinetics of epirubicin, expressed as AUC values, differ from those of doxorubicin when the drugs are given i.p. under hypothermic conditions. Whether the pharmacokinetic results presented herein explain the decreased doxorubicin toxicity found by Hultborn et al. [16] during chlorpromazine-induced hypothermia or the unpublished findings in our laboratory of differences in the in vivo toxicity of the two anthracyclines remains to be elucidated.

Acknowledgements We are grateful to Ragnhild Bernefors for typing the manuscript. This work was supported by grants from the King Gustaf V Jubilee Clinic Cancer Research Foundation in Gothenburg, and the radiolabeled doxorubicin and epirubicin were kindly donated by Farmitalia Carlo Erba, Italy.

References

1. Arcamone F, Lazzati M, Vicario GP, Zini G (1984) Disposition of ^{14}C-labelled 4′-epidoxorubicin and doxorubicin in the rat. Cancer Chemother Pharmacol 12: 157
2. Broggini M, Colombo T, Martini A, Donelli MG (1980) Studies on the comparative distribution and biliary excretion of doxorubicin and 4′-epidoxorubicin in mice and rats. Cancer Treat Rep 64: 897c
3. Cohen K, Thomas CG Jr (1963) Enhancement of the effectiveness of chemotherapeutic agents by modification of cell membrane permeability. Proc Am Assoc Cancer Res 4: 11
4. Cummings J, Merry S, Willmott N (1986) Disposition kinetics of adriamycin, adriamycinol and their 7-deoxyaglycones in AKR mice bearing a subcutaneously growing Ridgeway osteogenic sarcoma (ROS). Eur J Cancer Clin Oncol 22: 451
5. Cusack BJ, Young SP, Driskell J, Olson RD (1993) Doxorubicin and doxorubicinol pharmacokinetics and tissue concentrations following bolus injection and continuous infusion of doxorubicin in the rabbit. Cancer Chemother Pharmacol 32: 53
6. Dahl O, Mella O (1990) Hyperthermia and chemotherapeutic agents. In: Field SB, Hand JW (eds) An introduction to the practical aspects of clinical hyperthermia. Taylor & Francis, London New York Philadelphia, p 108
7. Dallal GE (1988) PITMAN: a FORTRAN program for exact randomization tests. Comput Biomed Res 221: 9
8. Daniel WW (1978) Applied nonparametric statistics. Houghton Mifflin, Boston
9. Dedrick RL, Myers CE, Bungay PM, DeVita VT Jr (1978) Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep 62: 1
10. Dunne A (1985) JANA: a new iterative polyexponential curve stripping program. Comput Methods Programs Biomed 20: 269
11. Eksborg S, Stendahl U, Lönroth U (1986) Comparative pharmacokinetic study of adriamycin and 4′epi-adriamycin after their simultaneous intravenous administration. Eur J Clin Pharmacol 30: 629
12. Gailis L (1986) Protection against doxorubicin toxicity in mice by chlorpromazine hypothermia. Res Commun Chem Pathol Pharmacol 53: 319
13. Gailis L, Dumas L, Page M (1988) Ambiguous effect of chlorpromazine on doxorubicin activity against P388D$_1$ tumours in mice. Eur J Cancer Clin Oncol 24: 169
14. Greidanus J, Willemse PHB, Uges DRA, Oremus ETHGJ, De Langen ZJ, De Vries EGE (1988) Continuous infusion of low-dose doxorubicin, epirubicin and mitoxantrone in cancer chemotherapy: a review. Pharm Weekbl [Sci] 10: 237
15. Hortobagyi GN, Frye D, Buzdar AU, Ewer MS, Fraschini G, Hug V, Ames F, Montague E, Carrasco CH, Mackay B, et al (1989) Decreased cardiac toxicity of doxorubicin administered by continuous intravenous infusion in combination chemotherapy for metastatic breast carcinoma. Cancer 63: 37
16. Hultborn R, Lundgren-Eriksson L, Ottosson-Lönn S, Ryd W, Weiss L (1990) Chlorpromazine-induced hypothermia in tumour-bearing mice, acute cytotoxic drug lethality and long-term survival. Acta Oncol 29: 941
17. Indraprasit S, Sooksriwongse C (1985) Effect of chlorpromazine on peritoneal clearances. Nephron 40: 341
18. Israel M, Karkowsky AM, Pegg WJ (1980) Pharmacologic studies with radiolabeled *N*-trifluoroacetyladriamycin-14-valerate (AD 32). Cancer Chemother Pharmacol 4: 79
19. Kalra OP, Aggarwal HK, Mahajan SK, Seth RK (1992) Effect of vasodilator and surface active drugs on the efficacy of peritoneal dialysis. J Assoc Physicians India 40: 233
20. Legha SS, Benjamin RS, Mackay B, Ewer M, Wallace S, Valdivieso M, Rasmussen SL, Blumenschein GR, Freireich EJ (1982) Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. Ann Intern Med 96: 133
21. Magin RL, Cysyk RL, Litterst CL (1980) Distribution of adriamycin in mice under conditions of local hyperthermia which improve systemic drug therapy. Cancer Treat Rep 64: 203
22. Mimnaugh EG, Waring RW, Silic BI, Magin L, Drew R, Litterst CL, Gran TE, Guarino AM (1978) Effect of whole-body hyperthermia on the disposition and metabolism of adriamycin in rabbits. Cancer Res 38: 1420
23. Mortensen B, Dale O (1995) Effects of hypothermia on the elimination of ethanol, diazepam and oxazepam in rat liver slice incubations. Acta Anaesthesiol Scand 39: 199
24. Pacciarini MA, Barbieri B, Colombo T, Broggini M, Garattini S, Donelli MG (1978) Distribution and antitumour activity of adriamycin given in a high-dose and a repeated low-dose schedule to mice. Cancer Treat Rep 62: 791
25. Rapkin E (1970) Development of the modern scintillation counter In: Bransome ED Jr (ed) The current status of liquid scintillation counting. Grune and Stratton, New York, p 63
26. Riggs CE Jr, Egorin MJ, Akman SR, Angelou JE, Van Echo DA, Whitacre M, Bachur NR (1982) Doxorubicin metabolism is altered by combination therapy with cyclofosphamide and whole-body hyperthermia. Proc Am Soc Clin Oncol 1: 26
27. Robert J, Gianni L (1993) Pharmacokinetics and metabolism of anthracyclines. Cancer Surv 17: 219

28. Skibba JL, Jones FE, Condon RE (1982) Altered hepatic disposition of doxorubicin in the perfused rat liver at hyperthermic temperatures. Cancer Treat Rep 66: 1357
29. Stewart DJ, Evans WK (1989) Non-chemotherapeutic agents that potentiate chemotherapy efficacy. Cancer Treat Rev 16: 1
30. Vijgh WJF van der, Maessen PA, Pinedo M (1990) Comparative metabolism and pharmacokinetics of doxorubicin and 4′-epidoxorubicin in plasma, heart and tumor of tumor-bearing mice. Cancer Chemother Pharmacol 26: 9
31. Webster LK, Cosson EJ, Stokes KH, Millward MJ (1996) Effect of the paclitaxel vehicle, Cremophor EL, on the pharmacokinetics of doxorubicin and doxorubicinol in mice. Br J Cancer 73: 522
32. Zini G, Vicario GP, Lazatti M, Arcamone F (1984) Radioactive species in rat urine and tissues after (^{14}C) AD 32 administration. Cancer Chemother Pharmacol 12: 154