Cancer Chemother Pharmacol (1981) 5:267–270

Cancer Chemotherapy and Pharmacology

© Springer-Verlag 1981

Doxorubicin Pharmacokinetics after Intravenous and Intraperitoneal Administration in the Nude Mouse

Peter B. Johansen

Royal Danish School of Pharmacy, Department of Pharmacology, 2, Universitetsparken, DK-2100 Copenhagen Ø, Denmark

Summary. *The pharmacokinetics of doxorubicin in nude mice have been investigated following intravenous and intraperitoneal administration of single doses of 12 mg/kg. The areas under the concentration curves of doxorubicin in kidney, heart, and striated muscle following intraperitoneal administration were approximately half the areas following intravenous injection, whereas plasma and liver showed nearly identical concentrations after a distribution phase of 2 h. Only minor differences in pharmacokinetics were found between nude and normal mice.*

Introduction

Doxorubicin (Adriamycin) is one of the most frequently used drugs in cancer chemotherapy. Clinically the drug is administered intravenously. In short-term experiments in mice reported in the literature both intravenous (IV) and intraperitoneal (IP) injections have been used, whereas results of long-term administration are preponderantly based upon IP administration [e.g., 2, 6, 7, 9, 12, 13]. The acute LD_{50} is significantly higher after IV administration than after IP administration (13–19 mg/kg and 7–10 mg/kg, respectively) [4]. The increased toxicity of doxorubicin administered IP as against IV may be related to peritoneal irritation [6].

The antitumor effect after IP injection has been reported to be inferior to the effect of IV injection [6, 11]. Based upon these observations a comparative investigation of the pharmacokinetics of doxorubicin in the nude mouse after IV and IP administration has been performed.

Pharmacokinetic data concerning doxorubicin in the nude mouse, which is commonly used in experimental cancer research, have not been published previously. Data obtained in the nude mouse have been compared with those recorded in normal mice.

Methods

Nude mice, nu/nu-Balb/c/ABom and normal Balb/c mice weighing 18–22 g were supplied by Friis, Bomholtgård, 8680 Ry, Denmark. Each IV and IP injection consisted of 100 µl, containing 250 µg doxorubicin hydrochloride (approximately 12 mg/kg) dissolved in normal saline. Plasma and tissue samples were frozen at −20° C and analysed within 3 weeks.

Blood samples were obtained at 5 min, 30 min, 2 h, 8 h, and 24 h after the administration of doxorubicin from the eye plexus under ether anesthesia, 10 IU heparin/ml blood being used as anticoagulant. After centrifugation (1,500 *g* for 6 min at room temperature) 200 µl plasma were removed and 50 µl phosphate buffer (pH 8.6, ionic strength $\mu = 1.0$) and 2.0 ml chloroform: methanol 9:1 were added. The aliquot was shaken on a Whirlimixer for 10 s and centrifuged (600 *g* for 15 min at 10° C), after which the water phase was discarded; 1.5 ml chloroform phase was removed and evaporated under a stream of air in siliconized test tubes. The residue was redissolved in 100 µl methanol, and 100 µl mobile phase was added. The aliquot was stored at +4° C overnight, centrifuged (1,500 *g* for 6 min at room temperature), and 100 µl clear supernatant was injected into the liquid chromatograph.

Tissue samples weighing 100–400 mg were homogenized at 0° C in 0.1 *M* phosphate buffer pH 7.4 by means of a Virtis homogenizer, 40,000 rpm for 45 s. We added 100 µl 0.1 *M* borate buffer pH 9.8 and 2.0 ml chloroform: methanol 9:1 to 100 µl homogenate. The analysis then followed the procedures described for plasma.

The determination of doxorubicin and metabolites was performed with high-pressure liquid chromatography equipment consisting of Waters pump 6000 A, Waters U6K universal injector (maximum volume 2 ml), and Schoeffel fluorescence detector FS-970 (exication wavelength 470 nm, emission wavelength 550 nm). The column dimensions were: Length 15 cm, inner diameter 4 mm, outer diameter 1/4 in. The surface-modified chromatographic support LiChrosorb RP-8 (Merck, particle diameter 5 µm) was used. The mobile phase consisted of acetonitril: 0.01 *M* phosphoric acid 35:65 adjusted to pH 2.00 and degassed before use. The flow

0344-5704/81/0005/0267/$ 01.00

rate was 1.5 ml/min, resulting in a pressure of 2500 psi. The thermostat of the chromatographic system was set at 25° C. Concentrations were measured by the peak heights on the chromatogram. Standard solutions containing doxorubicin, doxorubicinol, and doxorubicinon were analysed every sixth sample. Linearity between concentrations 0–10 µg/ml (injection volume 100 µl) and peak heights was observed. The lower detection limit was 10 ng/ml. Recovery of doxorubicin and of doxorubicinol added to plasma and liver homogenate was 80%–85%, and recovery of doxorubicinon 90%–95%.

Doxorubicin, doxorubicinol, and doxorubicinon were kindly supplied by FarmItalia, Milan, Italy.

Results

The plasma concentration of doxorubicin after IV and IP injections is shown in Fig. 1. Doxorubicinol was present in amounts too small for quantitation. Doxorubicinon could not be detected. The apparent half-life of doxorubicin after IV injection calculated from the 8- and 24-h values was approximately 11 h.

The concentrations of doxorubicin in various tissues are shown in Fig. 2. Doxorubicinol was present in amounts too small for quantitation. Doxorubicinon could not be detected. Two other minor peaks were observed in the chromatogram, a polar one eluted before doxorubicinol (possibly a polar conjugate), and a non-polar one eluted after doxorubicinon (possibly 7-deoxydoxorubicinon). The apparent half-lives after IV injection of doxorubicin were approximately as follows: Liver 10 h, heart 15 h,

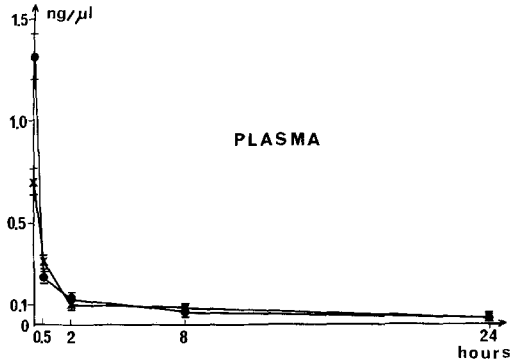

Fig. 1. Plasma concentration of doxorubicin in nude mice following 12 mg/kg IV (●) and IP (X). Each *point* represents the mean value (five animals) and each *bar* the SEM. Significant differences were found only at 5 min and 30 min after administration ($P < 0.05$, Student's *t*-test)

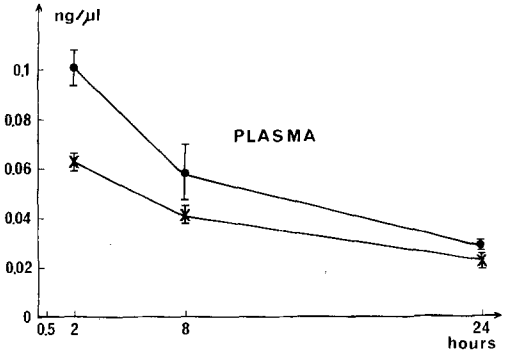

Fig. 3. Plasma concentration of doxorubicin in nude (●) and normal (X) mice following 12 mg/kg IP. Each point represents the mean value (5 animals) and each *bar* the SEM. Significant differences were found at 2 h and 24 h after administration ($P < 0.05$, Student's *t*-test)

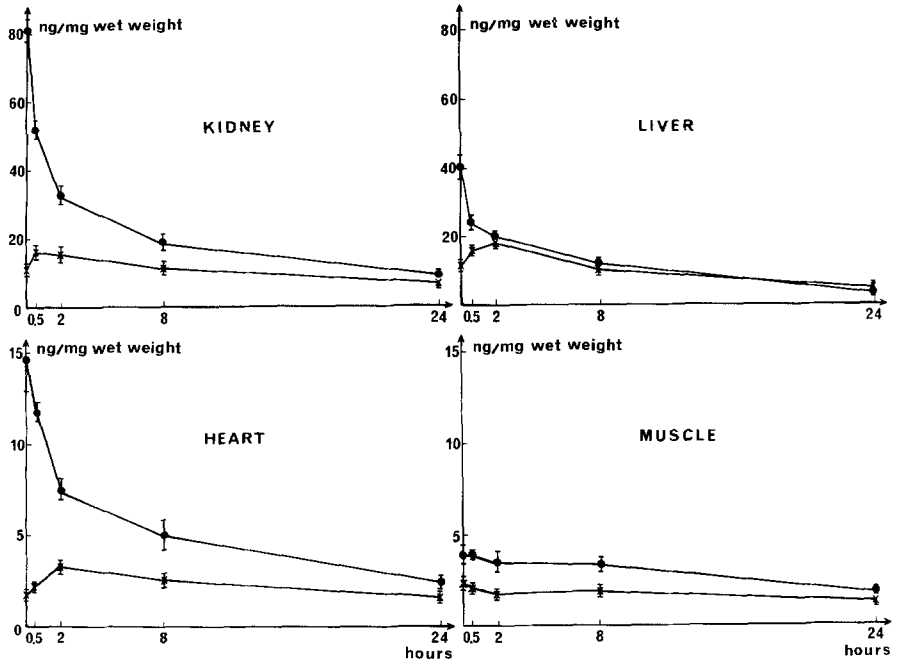

Fig. 2. Tissue concentrations of doxorubicin in nude mice following 12 mg/kg IV (●) and IP (X). Each *point* represents the mean value (five animals) and each *bar* the SEM. Significant differences ($P < 0.05$, Student's *t*-test) were found concerning kidney at all times, liver at 5 min and 30 min, heart at 5 min, 30 min, 2 h, 8 h, and muscle at 5 min, 30 min, 2 h, and 8 h. Note the differences in the range of the ordinate

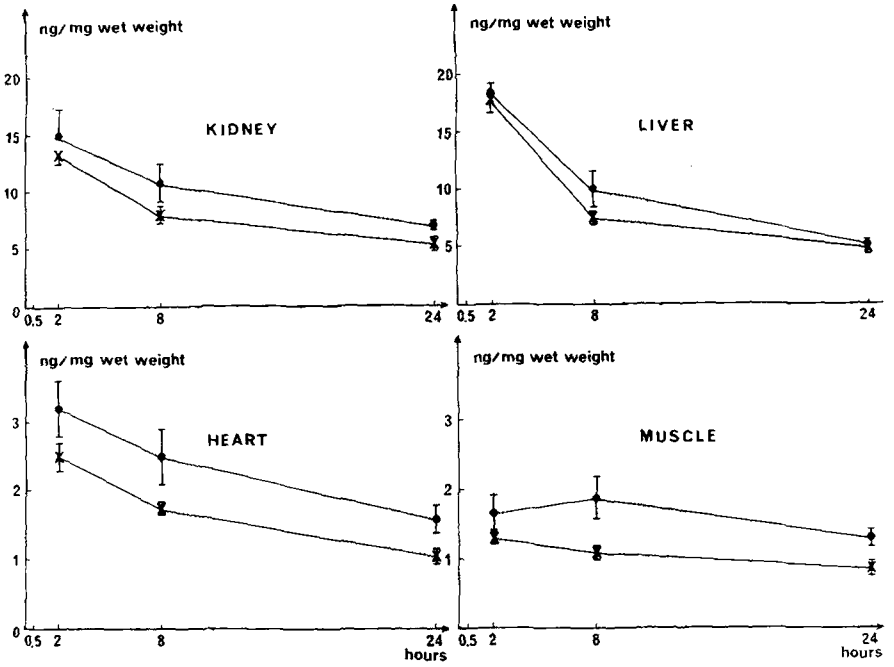

Fig. 4. Tissue concentrations of doxorubicin in nude (●) and normal (X) mice following 12 mg/kg IP. Each *point* represents the mean value (5 animals) and each *bar* the SEM. Significant differences ($P < 0.05$, Student's *t*-test) were found for kidney at 24 h, heart at 8 h and 24 h, and muscle at 8 h and 24 h. Note the differences in the range of the ordinate

kidney 15 h, and striated muscle 19 h. The ratios of areas under the concentration curve (calculated by the trapezoidal rule) after IP injection to areas after IV injection were as follows: Liver 0.9, heart 0.5, kidney 0.5, and striated muscle 0.6.

In normal mice (Balb/c) the pharmacokinetic pattern following IP administration of doxorubicin was similar to the pattern obtained in nude mice, though the plasma and tissue concentrations were generally somewhat higher in the nude mice (Figs. 3 and 4). Note the differences in the range of the ordinate in Figs. 3 and 4 compared with Figs. 1 and 2.

Discussion

The present investigation has demonstrated significant differences between tissue concentrations of doxorubicin after IV and after IP administration of single doses of doxorubicin. The concentrations of doxorubicin in plasma and liver indicate a nearly complete absorption following IP administration. The lower concentrations in other tissues following IP administration than after IV administration may be the result of a first-pass metabolism and a partial biliary excretion of the metabolites.

The results are consistent with those of previous investigations [1, 3, 5] in normal mice. Ozols et al. [8], however, reported much lower concentrations of doxorubicin in kidney and liver of normal mice (C3H/FeJ) following IV and IP administration, whereas the concentrations in the heart were similar to those recorded in the present investigation. These discrepancies cannot be explained. It should be noted that the present investigation has demonstrated only minor differences between the pharmacokinetics in nude and normal mice with the Balb/c background in common. Reich and Bachur [16] reported a plasma half-life in NGP(S) mice of 26 h and Di Fronzo et al. [3] reported a half-life in CDl mice of 32 h. The shorter half-life (11 h) found in the present investigation may result from the fact that the plasma concentrations have been followed for only 24 h, which does not exclude a slower terminal elimination phase. Similary Siemann and Sutherland [12] reported longer half-lives in tissues when the concentrations were followed for longer periods than in the present investigation.

In conclusion, the present investigation has demonstrated considerable differences of pharmacokinetics following single doses of doxorubicin in nude mice between IV and IP administration. Whether these differences may be of significance in long-term administration cannot be predicted.

Furthermore, it cannot be predicted whether the observed differences in pharmacokinetics may be of importance to the antitumor effect of the drug in tumors of different size and degree of vascularization.

Acknowledgement. This work was supported by the Danish Medical Research Council (grant no. 512–16172).

References

1. Arena E, d'Alessandro N, Dusonchet L, Gebbia N, Gerbani F, Palazzoadriano M, Raineri R, Rausa L (1971) Analysis of the pharmacokinetic characteristics, pharmacological, and chemotherapeutic activity of 14-hydroxy daunomycin (Adriamycin) a new drug with an antitumor activity. Arzneim Forsch 21: 1258
2. Deprez-de Campeneere D, Jaenke R, Trouet A, Baudon H, Maldague P (1980) DNA-Anthracycline complexes. II. Comparative study of the acute lesions induced in mice after intravenous administration of free and DNA bound adriamycin. Eur J Cancer 16: 987
3. Di Fronzo G, Gambetta RA, Lenaz L (1971) Distribution and metabolism of adriamycin in mice. Comparison with daunomycin. Rev Eur Et Clin Biol 16: 572
4. FarmItalia Research Institute, Milan (1970) Unpublished results. Report no. 272
5. Harrison K, Wagner H (1978) Biodistribution of intravenously injected ^{14}C-doxorubicin and ^{14}C-daunomycin in mice: Concise communication. J Nucl Med 19: 84
6. Lenaz L, Di Marco A (1972) On the reported lack of effectiveness of daunomycin (NSC-82151) and adriamycin (NSC-123127) on solid tumors. Cancer Chemother Rep 56: 431
7. Martini A, Donelli MG, Mantovani A, Pacciarini MA, Fogar-Ottaviano E, Morasca L, Garattini S, Spreafico F (1977) Antineoplastic activity and pharmacokinetics of adriamycin and daunomycin in tumor-bearing mice. Oncology 34: 173
8. Ozols FR, Locker GY, Doroshow JH, Grotzinger KR, Meyers CE, Young RC (1979) Pharmacokinetics of adriamycin and tissue penetration in murine ovarian cancer. Cancer Res 39: 3209
9. Pacciarini MA, Barbieri B, Colombo T, Broggini M (1978) Distribution and antitumor activity of adriamycin given in a high-dose and a repeated low-dose schedule to mice. Cancer Treat Rep 62: 791
10. Reich SD, Bachur NR (1976) Alterations in adriamycin efficacy by phenobarbital. Cancer Res 36: 3803
11. Shaeffer J, El-Mahdi AM (1977) Comparative effect of intravenous and intraperitoneal adriamycin on metastatic Dunn osteosarcoma. Proc Am Assoc Cancer Res 68: 23
12. Siemann DW, Sutherland RM (1979) A comparison of the pharmacokinetics of multiple and single dose administrations of adriamycin. Int J Radiat Oncol Biol Phys 5: 1271
13. Siemann DW, Sutherland RM (1980) The interaction between adriamycin and radiation in a solid murine tumor. Radiat Res 83: 345

Received December 22, 1980/Accepted February 12, 1981