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Methyl-β-cyclodextrin and doxorubicin pharmacokinetics and tissue concentrations following bolus injection of these drugs alone or together in the rabbit

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Abstract

The purpose of this work was to determine the pharmacokinetics and the tissue concentrations of methyl-β-cyclodextrin (MEBCD) and doxorubicin (DOX) in rabbits following administration of MEBCD and DOX, alone or in combination. MEBCD (200 mg/kg) and DOX (1 mg/kg) were intravenously injected to white New Zealand rabbits and blood samples were obtained over a 48-h period after administration. After this period, administration was repeated and animals were killed 1, 2 or 4 h after injection. Heart, liver and kidney were then removed. MEBCD and DOX analysis in plasma and tissues was performed using two HPLC methods with fluorimetric detection. MEBCD pharmacokinetic profile was consistent with a two-compartment model ($t_{1/2}$ α: 30 min; $t_{1/2}$ β: 7 h). Co-administration with DOX did not modify the main pharmacokinetic parameters of MEBCD. However, $C_{5\ min}$, $t_{1/2}$ α, $t_{1/2}$ β and AUC_{∞} were decreased by the co-administration of DOX with MEBCD compared to DOX alone. Assays of excised tissues showed that DOX enhanced the cardiac, renal and hepatic concentrations of MEBCD. On the other hand, MEBCD did not alter the cardiac distribution of DOX, while renal and hepatic distribution profiles were modified. In this study, the pharmacokinetic parameters of MEBCD injected intravenously were determined for the first time. DOX did not enhance MEBCD pharmacokinetic profile but MEBCD reduced the distribution half-life of DOX. Tissue determination showed that MEBCD did not enhanced the cardiac accumulation of DOX, which is auspicious for further in vivo experiments using the co-administration of DOX and MEBCD.

Keywords: Methyl-β-cyclodextrin; Doxorubicin; Pharmacokinetics; Tissue distribution

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1. Introduction

Cyclodextrins (CDs) are oligosaccharide cyclic torus-shaped molecules which have the ability to form molecular inclusion compounds with a wide range of molecules, such as drugs (Hirayama and Uekama, 1987; Allegre and Deratani, 1994; Szetlji, 1994). Substituted CDs are commonly used for the optimization of biopharmaceutical properties of drugs such as solubility, delivery, membrane permeability and bioavailability (Brewster et al., 1990; Allegre and Deratani, 1994; Szetlji, 1994; Cho et al., 1995; Hovgaard and Brøndsted, 1995; Krishnamoorthy et al., 1995). Increased drug solubility suggested the application of cyclodextrins and their derivatives in parenteral dosage forms. Whereas the interest for the parenteral use of substituted CDs is increasing, only few studies described the pharmacokinetics of CDs in plasma (Frijlink et al., 1990; Giordano, 1991). However, no data are available on the pharmacokinetics of methylated-CD derivatives. Papers have reported the influence of cyclodextrin complexation on the pharmacokinetics of various molecules. For some drugs, the pharmacokinetic parameters were modified in presence of cyclodextrins (Frijlink et al., 1991; Müller and Albers, 1991) while for others, no effect was observed (Amimori and Uekama, 1987). Moreover, many authors reported that CDs (and particularly methylated-β-CDs) could alter the cell membrane cholesterol content, which plays structural roles, thus modifying the membrane fluidity and permeability between external and internal environments (Castelli et al., 1989; Irie et al., 1992; Kilsdonk et al., 1995). In previous studies, we also described the in vitro and in vivo interest of the use of methyl-β-cyclodextrin (MEBCD), alone or in combination with common antineoplastic agents such as doxorubicin (DOX), in human cancer cell lines and in human tumour xenografted mice (Grosse et al., 1997a,b,c).

DOX is a well-known intercalating agent widely used in cancer chemotherapy which presents major problems of acute and chronic toxicity like cardiotoxicity and leucopenia, and appearance of cellular multidrug-resistance (Olson and Mushlin, 1990; Muggia and Green, 1991). Owing to its low therapeutic index, a shift in its pharmacokinetics could lead to an increase of toxicity or to a lack of antitumoural activity.

The aim of the present study was to determine the pharmacokinetic profile and the tissue distribution of MEBCD, administered alone or in combination with DOX. Moreover, we investigated the influence of MEBCD on the pharmacokinetics and cardiac, renal and hepatic distribution of DOX.

2. Materials and methods

2.1. Drugs and chemicals

MEBCD (main degree of substitution: ≈ 10.4) was purchased from Sigma (St Quentin Fallavier, France). Doxorubicin was obtained from Pharmacia (St Quentin Yvelines, France). Daunorubicin was purchased from Roger Bellon laboratories (Paris, France). All other reagents were of analytical grade and were obtained from Carlo Erba (Milan, Italy) or Prolabo (Paris, France).

2.2. Protocol

New Zealand white rabbits aged 5–6 weeks (2.7–3.2 kg) obtained from a licensed supplier (INRA, Montpellier, France), were acclimatized for 1 week and observed to ensure they were healthy prior to the start of the study. Intravenous catheters (Jelco, USA) were inserted in the marginal vein of each ear for drug administration. Blood sampling was performed with an intra-arterial catheter (Jelco, USA) disposed in the central artery of the opposite ear. After dilution in normal saline, MEBCD (200 mg/kg) and DOX (1 mg/kg) were administered alone or together by simple mixing, over 1 min (injected volume:1 ml/kg). Blood sampling (2–3 ml) was performed prior to, and at 5, 15, 30, 60 min and 2, 4, 8, 24, 48 h after administration. Samples were immediately centrifuged for 10 min at $3000 \times g$ and separated plasma was stored at -20°C for later processing. Experiments were carried out in replicate ($n = 6$). Main plasmatic biochemical parameters (GOT, GPT, Creatinine, LDH and total

cholesterol) were also determined prior to and at 15 min, and 2 and 4 h after administration of either MEBCD, DOX or both ($n = 2$), using an Hitachi I-800 automatic analyzer (Tokyo, Japan). After the last blood sample (48 h), rabbits received MEBCD, DOX, or both, as described above and were killed 1, 2 or 4 h ($n = 2$) after bolus administration, by an intravenous injection of a massive dose of KCl (20%). Heart, liver and kidneys were exsanguinated and removed. Tissue samples obtained were frozen until assays. After thawing, tissue samples (1 g) were quartered and homogenized in 2 ml of purified water with a Tissue Tearor homogenizer (Biospec products, Bartlesville, OK, USA).

2.3. MEBCD and DOX analysis

The analysis of MEBCD in plasma and tissues was performed as described in a previous paper (Grosse et al., 1998). Concentrations were determined by HPLC with fluorescence detection (wavelengths, 290 nm for excitation and 360 nm for emission), after in situ complexation with 1-napthol. Briefly, an alkaline extraction of plasma or tissue homogenate was performed using chloroform. After extraction, the lower organic phase was transferred to a fresh tube and daunorubicin was added as an internal standard. Then, the organic phase was evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 250 μl of mobile phase, then 50 μl were injected onto the column. The mobile phase was a mixture of water–methanol (98:2; v/v) containing 10^{-4} M of 1-napthol as a fluorophore. The flow rate was 1 ml/min. Linear detection response was obtained for concentrations ranging from 1 to 100 μM in plasma and tissue homogenate. The intra-day and inter-day variations in precision were lower than 11%. The limit of quantitation was 0.5 μM.

DOX concentrations in plasma and tissues were determined by HPLC with fluorescence detection as described elsewhere (Muller et al., 1993). After addition of daunorubicin as an internal standard and 0.05 M borate buffer (pH 9.8), plasma or tissue homogenate samples were extracted by a chloroform–methanol mixture (4:1; v/v). After centrifugation at $3000 \times g$, the organic phase was removed and evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 150 μl of mobile phase and 50 μl were injected onto the column. The excitation and emission wavelengths were 478 and 590 nm, respectively. The elution was performed using a mobile phase consisting of 0.05 M phosphate buffer, acetonitrile, tetrahydrofuran and triethylamine (59.8:35:5:0.2; v/v) at pH 2.5. The flow rate was 1 ml/min. Linear detection response was obtained for concentrations ranging from 5 to 2000 ng/ml in plasma and tissue homogenate. The intra-day and inter-day variations in precision was lower than 10%. The limit of quantitation was 1 ng/ml.

2.4. Pharmacokinetic parameters and statistical analysis

Pharmacokinetic parameters were calculated using the SIPHAR software (Gomeni and Gomeni, 1987). The area under the plasma concentration–time curve (AUC_{∞}) was calculated from 0 to the last time point using the trapezoidal rule with extrapolation to infinity. Total plasma clearance (CL) was calculated by dividing the dose by the AUC. The apparent volume of distribution (V_d) was the ratio of the total clearance to the apparent rate constant of elimination. The values for each parameter are reported as mean values $\pm$ S.D. Between-group parameters were compared using Student's t-test. The null hypothesis was rejected at $P < 0.05$.

3. Results

3.1. Pharmacokinetics of MEBCD

Experimental results of MEBCD concentrations in plasma over time showed that the best fit was obtained with a two-compartment model and a linear elimination kinetic. This model did not produce systemic deviations in the residuals and provided the lowest Akaike criterion. Mean plasma concentration versus time curve is shown in Fig. 1. The co-administration of MEBCD with

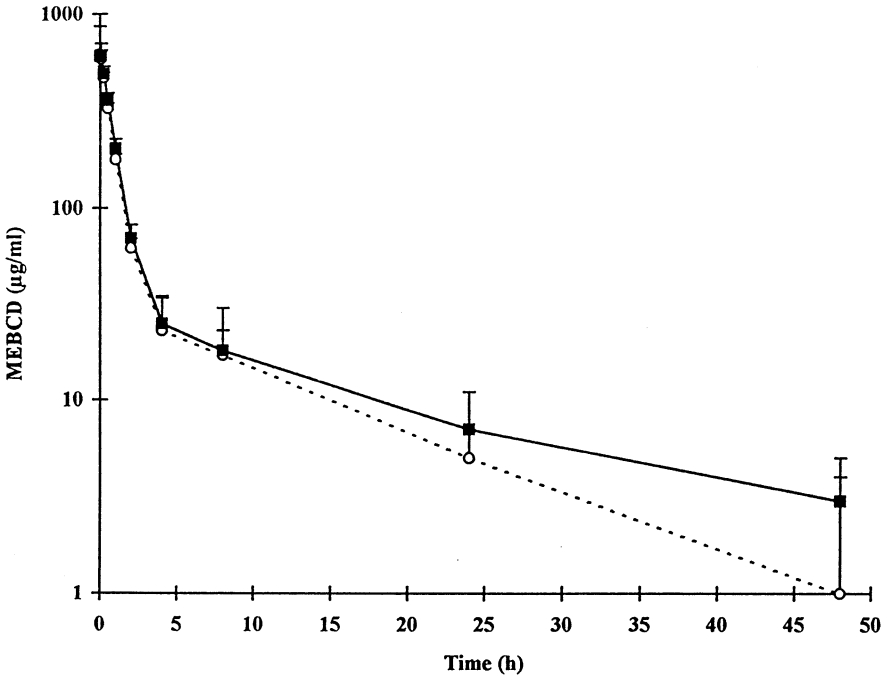

Fig. 1. Mean plasma concentration/time curve for MEBCD following administration by intravenous bolus of MEBCD alone (○) or simultaneously with DOX (■) in rabbits. Error bars represent S.D.

DOX did not modify the pharmacokinetic profile of MEBCD. Main pharmacokinetic parameters are summarized in Table 1. MEBCD pharmacokinetics is characterized by distribution and elimination half-lives of about 30 min and 7 h, respectively. The apparent volume of distribution was relatively low, about 3 l/kg. None of the calculated parameters were significantly modified by the co-administration of MEBCD with DOX. Plasma biochemical parameters in rabbits treated with MEBCD associated or not with DOX, were not changed compared to control.

3.2. Pharmacokinetics of DOX

Mean plasma concentration of DOX versus time curve is shown in Fig. 2. The data were fitted using a two-compartment model and a linear elimination kinetic. The main calculated parameters of DOX are presented in Table 2. The pharmacokinetic of DOX is characterized by a very short distribution phase ($t_{1/2}$ α: 3 min) and a long phase of elimination ($t_{1/2}$ β: 24 h). Co-administration of DOX with MEBCD did not modify significantly the pharmacokinetic profile of DOX in the rabbit. However, some differences between pharmacokinetic parameters were observed between the administration of DOX alone and together with MEBCD. Indeed, the DOX concentration 5 min after injection, the distribution half-life and the area under curve were decreased by 31, 45 and 8%, respectively, when DOX was associated with MEBCD, but only the difference observed in the $t_{1/2}$ α was statistically significant. On the other hand, the apparent volume of distribution was slightly increased (16%) by the co-administration of DOX with MEBCD. Moreover, plasma biochemical parameters in rabbits injected with DOX associated or not with MEBCD, were not changed compared to those found prior the drug administration.

3.3. Tissue distribution of MEBCD and DOX

Tissue assays are shown in Fig. 3. Results indicated that DOX enhanced the cardiac and renal

Table 1
MEBCD pharmacokinetic parameters calculated in groups treated with MEBCD alone or in combination with DOX

Parameter	MEBCD group (Mean ± S.D. ($n = 6$))	MEBCD–DOX group (Mean ± S.D. ($n = 6$))	t-test
$C_{5\ min}$ (μg/ml)	688 ± 45	711 ± 82	N.S.
$t_{1/2}$ α (min)	31.2 ± 2.3	27.6 ± 14.2	N.S.
$t_{1/2}$ β (h)	6.3 ± 0.6	8.4 ± 2.7	N.S.
CL (ml/min per kg)	3.8 ± 0.9	3.7 ± 0.7	N.S.
V_d (l/kg)	2.5 ± 0.7	3.9 ± 1.5	N.S.
AUC_∞ (μg/ml·min)	56 800 ± 16 730	56 880 ± 9075	N.S.

concentrations of MEBCD, 4 h after the co-administration of DOX and MEBCD. Moreover, the hepatic accumulation of MEBCD, 1 h after bolus injection was enhanced by the co-administration with DOX (Fig. 3A).

DOX intra-cardiac concentrations were slightly decreased 2 h after co-administration with MEBCD. Renal and hepatic concentrations of DOX were modified by MEBCD; bolus injection of DOX and MEBCD led to a later decrease of renal DOX concentration and to an earlier decrease of hepatic DOX concentration, compared to tissue distribution obtained after administration of DOX alone (Fig. 3B).

4. Discussion

Methylated cyclodextrins are cyclic oligosaccharides that have been shown to interact with lipid components of biological membranes, modifying their fluidity and their permeability (Hirayama and Uekama, 1987; Brewster et al., 1990; Allegre and Deratani, 1994; Szetlji, 1994; Cho et al., 1995; Hovgaard and Brøndsted, 1995; Krishnamoorthy et al., 1995). This interaction is correlated with the degree of methylation of the CD. However, methylated-β-CDs with high degrees of subtitution (i.e. Di-MEBCD and Tri-MEBCD), were found to be toxic when given parenterally, due to their hemolytic activity (Brewster et al. 1990; Giordano, 1991). In previous studies (Grosse et al., 1997a,b,c, 1998), only MEBCD (degree of substitution: ≈ 10.4) presented a particular interest in experimental in vitro and in vivo cancer therapy without involving toxicity on cells or animals. Indeed, doses of MEBCD up to 800 mg/kg, weekly administered i.p. in xenografted mice did not revealed any toxicity or behavioural, physical and physiological changes compared to control, throughout the study. The determination of plasmatic and tissue pharmacokinetics parenterally administered MEBCD has never been performed, probably because of the difficulty in obtaining a specific, sensitive and reproducible method to determine non-reactive products like MEBCD in biological matrices. Pharmacokinetics of β-CD and its hydroxy-propyl derivative have already been described (Frijlink et al., 1990), but the relatively high limit of quantitation did not allow the determination of plasma concentrations beyond 2 h after an intravenous injection of MEBCD at 200 mg/kg in the rat. The authors found an elimination half-life of about 30 min. In this study, we report data obtained using a sensitive and reproducible HPLC method previously validated (Grosse et al., 1998). The detection was based on the enhancement of fluorescence resulting of the formation of inclusion complexes between MECBD and the 1-napthol fluorophore, and allowed a low limit of quantitation (0.5 μg/ml) and a good specificity. This method was found to be suitable to determine plasma and tissue concentrations collected during pharmacokinetic investigations. The computed-calculated parameters showed that the pharmacokinetics of MEBCD administered intravenously at 200 mg/kg in the rabbit, followed a two-compartment model with a linear elimination. The elimination half-life was found at about 7 h. Soluble substituted cyclodextrins are known to be not metabolized and to be rapidly excreted by the kidneys (Giordano, 1991; Szetlji, 1994). These data are in agreement with the high MEBCD renal concen-

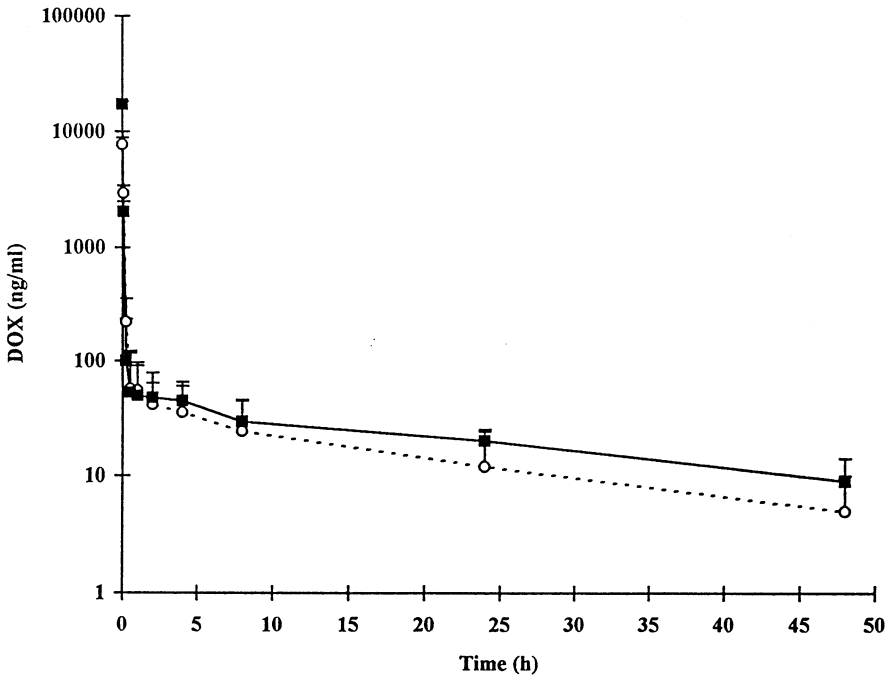

Fig. 2. Mean plasma concentration/time curve for DOX following administration by intravenous bolus of DOX alone (○) or simultaneously with MEBCD (■) in rabbits. Error bars represent S.D.

tration observed in this study, compared to the low hepatic concentration. In previous studies, we described the potentiation of the cytotoxic activity of some antineoplastic agents such as DOX, induced by the co-administration with non-cytotoxic concentrations of MEBCD, in human cancer cell lines and in nude tumour xenografted mice (Grosse et al., 1997a,b,c). Therefore, it could be useful to determine the possible influence of the simultaneous administration of MEBCD with DOX on the pharmacokinetics and the tissue distribution of MEBCD and DOX. This co-administration does not involve any complexation between DOX and MEBCD. Indeed, several studies showed that this complexation is not possible, as the cavity size of MEBCD is not wide enough to include any part of the DOX molecule (Bekers et al., 1990; Grosse et al., 1997a). Otherwise, we showed that the in vitro effects of MEBCD and DOX on cell membrane were independent and that MEBCD could act as an enhancer of the cell membrane permeability which allows to further facilitate DOX penetration in cell (Grosse et al., 1997a). In the present study, the co-administration of DOX with MEBCD did not alter the pharmacokinetic profile of MEBCD. However, tissue concentration determination showed mainly an increase of cardiac and renal MEBCD accumulation.

Plasma and tissue pharmacokinetics of DOX have been widely studied and we found pharmacokinetic parameters in agreement with the literature (Brenner et al., 1984; Muller et al., 1993; Cusack et al., 1993), with an elimination half-life of about 24 h. After an intravenous injection of DOX, the hepatic elimination is preponderant (80%) while the renal elimination is relatively low (20%) (Brenner et al., 1984; Muggia and Green, 1991). In this study, some pharmacokinetic parameters of DOX, such as $C_{5\,min}$,, $t_{1/2}\ \alpha$ and AUC_{∞} were decreased when rabbits received the co-administration of MEBCD with DOX. Due to a large inter-individual variability, only the difference observed on $t_{1/2}\ \alpha$ was statistically significant. On the other hand, mean volume of distribution of DOX was increased by MEBCD. These data

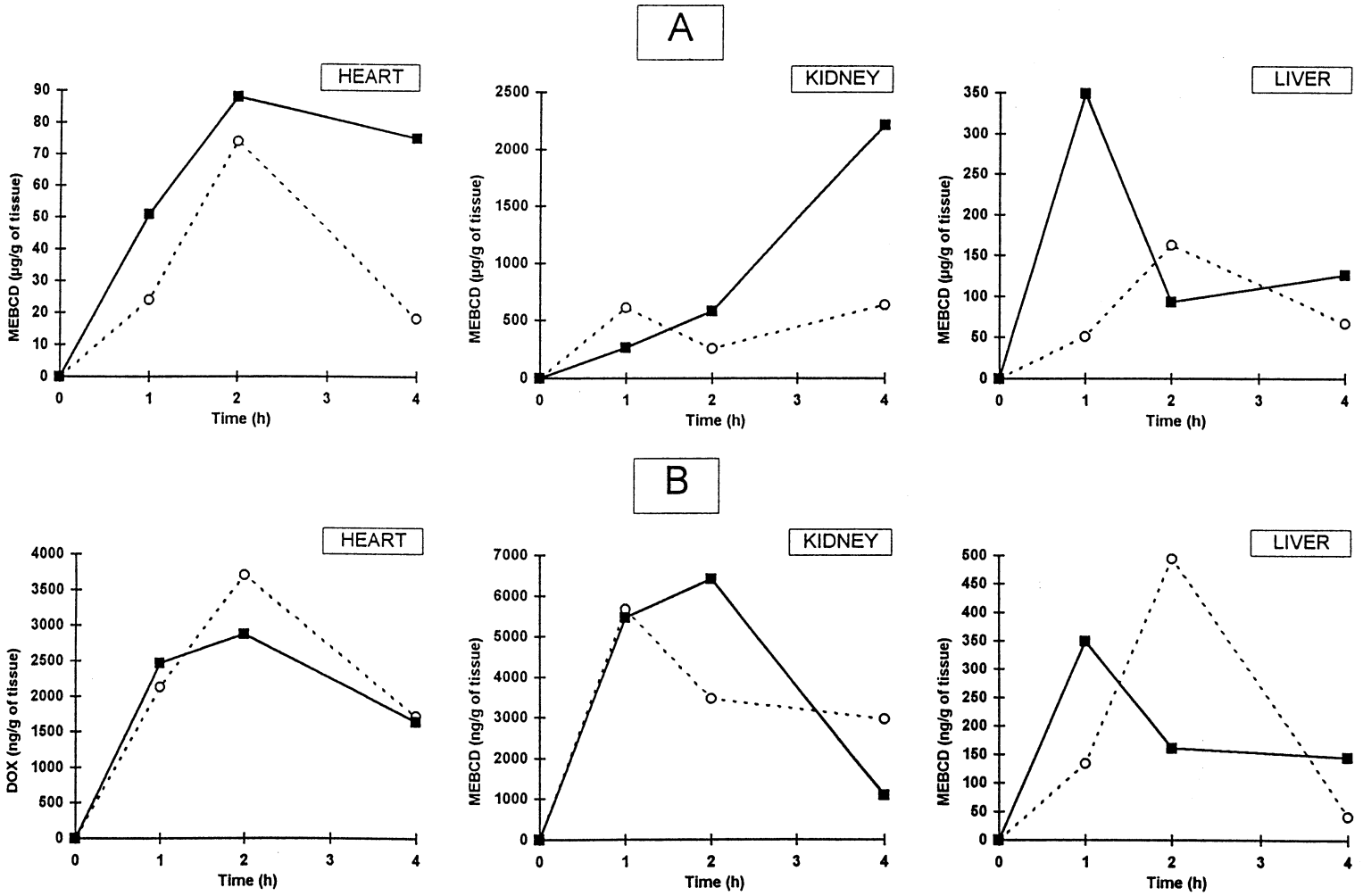

Fig. 3. Mean intratissular kinetics of MEBCD (A) or DOX (B), in rabbits after treatment with bolus injection of the drugs alone (○) or together (■).

Table 2
DOX pharmacokinetics parameters calculated in groups treated with DOX alone or in combination with MEBCD

Parameter	DOX group (Mean ± S.D. ($n=6$))	DOX–MEBCD group (Mean ± S.D. ($n=6$))	t-test
$C_{5\,min}$ (ng/ml)	2991 ± 787	2054 ± 614	N.S.
$t_{1/2}$ α (min)	3.1 ± 0.9	1.7 ± 0.7	$P<0.05$
$t_{1/2}$ β (h)	24.3 ± 4.3	20.9 ± 4.4	N.S.
CL (ml/min per kg)	10.2 ± 3.5	12.8 ± 5.3	N.S.
V_d (l/kg)	24.1 ± 9.5	28.2 ± 7.4	N.S.
AUC_∞ (ng/ml min)	103 350 ± 28 747	95 260 ± 31 877	N.S.

allowed us to think that MEBCD could enhance tissue diffusion of DOX. This hypothesis is in accordance with the results obtained in our in vitro previous studies (Grosse et al., 1997a,b). DOX determination in tissue showed that the renal and the hepatic concentrations of DOX were modified by MEBCD. Bolus injection of DOX associated with MEBCD led to a later decrease of renal DOX concentrations and to an earlier decrease of hepatic DOX concentrations, compared to those obtained with DOX alone. On the other hand, DOX intra-cardiac concentrations were slightly decreased after co-administration with MEBCD. As the peak concentration obtained after an intravenous bolus administration of DOX is generally admitted to be partially responsible for the acute cardiotoxicity (Garnick et al., 1983; Cusack et al., 1993; Muller et al., 1993), the 31% reduction of the DOX cardiac concentration, 1 h after injection, and the decrease of the $C_{5\,min}$ could offer interesting perspectives. This association could allow major antitumour effects to be obtained without an increase of the problematic cardiotoxicity.

In conclusion, we described for the first time the pharmacokinetic parameters of MEBCD injected intravenously. DOX did not modify MEBCD pharmacokinetic profile but MEBCD reduced significantly the distribution half-life of DOX. Tissue determination showed that MEBCD did not enhanced the cardiac accumulation of DOX, which is auspicious for further in vivo experiments of the association of MEBCD and DOX.

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