J. Pharm. Pharmacol. 1992, 44: 58–60
Communicated June 24, 1991

Comparative pharmacokinetics of tetrahydropyranyl-doxorubicin and doxorubicin in rat isolated lung

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Abstract—Rat isolated perfused lungs (Sprague-Dawley rats, n = 20) were studied to compare the pulmonary uptake of a new anthracycline, tetrahydropyranyl-doxorubicin (THP-DXR) with that of doxorubicin (DXR). Lung perfusions were initiated with a constituted medium containing either drug at concentrations of 1, 10 or 100 μM. Lungs were perfused by recirculation for 60 min. Thirteen perfusate samples were collected over 60 min and subjected to HPLC for assay. The perfusate concentration of THP-DXR decreased to 24 ± 5% of the initial concentration and to 8 ± 2%, 20 and 60 min after the beginning of the infusion, respectively. Corresponding values for DXR were 77 ± 16 and 52 ± 15%, respectively ($P < 0.05$). During the THP-DXR perfusion, the area under the perfusate concentration vs time curve (AUC) was decreased to one-third and the clearance was increased 3-fold ($P < 0.05$). The pulmonary concentration of THP-DXR reached 0.032 ± 0.01 μmol g^{-1} 60 min after the beginning of a perfusion of 1 μM of the drug. This concentration increased to 0.379 ± 0.11 μmol g^{-1} when the initial dose concentration was 10 μM. Corresponding lung concentrations for DXR were 0.013 ± 0.001 and 0.150 ± 0.04 μmol g^{-1}, respectively ($P < 0.05$). The perfusate concentration/initial concentration ratio decreased by the same amount whether a 1 or 10 μM initial concentration of either drug was used. An initial concentration of 100 μM of THP-DXR, unlike DXR, consistently induced oedema in the perfused lung. No metabolite of either drug was revealed during the course of our study. These findings suggest: (1) a higher lung affinity for THP-DXR; (2) a correlation between lung uptake and dose consistent with a passive diffusion transport mechanism for both drugs; (3) a higher acute toxicity induced by THP-DXR; (4) the absence of metabolic activity in the lung with regards to both anthracyclines.

Doxorubicin (DXR) is a potent and well-known tumouricidal drug. Despite its broad spectrum of activity and wide range of therapeutic applications, treatment is limited by cumulative cardiotoxic effects which has encouraged investigators to pursue their search for analogues such as tetrahydropyranyl-doxorubicin (THP-DXR), reported to exhibit a lesser degree of cardiotoxicity (Majima & Ohta 1987; Raber et al 1989). As anthracyclines are administered by intravenous infusion, the perfusion of the pulmonary tissue occurs first which makes their fate in lung-tissue of interest. The purpose of the present study was to compare the pulmonary phamacokinetics of DXR and THP-DXR in the rat isolated perfused lung.

Materials and methods

Drugs. Doxorubicin, tetrahydropyranyl-doxorubicin and daunorubicin (DNR) used as internal standard for HPLC assay, were obtained courtesy of the Roger Bellon Laboratories (Neuilly, France). The reference standard used to reveal the presence of doxorubicinol (DXRol), a major metabolite of doxorubicin, was kindly supplied by Specia Laboratories (Paris, France).

Male Sprague-Dawley rats, mean weight 307 ± 35 g were purchased from Iffa Credo (l'Arbresle, France) and had free access to standard rat food and water.

Animal surgery and perfusion. Rats were anaesthetized with thiopentone sodium (Nesdonal, Specia, 60 mg kg^{-1}, i.p.), tracheotomized and ventilated (5% CO_2 in O_2). The end expiratory pressure was set at 3 cm H_2O. Heparin (1000 int. units kg^{-1}) was injected into the vena cava and the lungs were surgically removed. The pulmonary artery was canulated for lung perfusion with a Krebs-Ringer bicarbonate buffer solution (pH 7.4) supplemented with 4.5% bovine serum albumin (Sigma) (Joshi et al 1986; Camus et al 1990). The lungs were allowed to equilibrate in the perfusion apparatus (Mehendale 1982) for 10 min while a drug-free medium recirculated at a flow rate of 5 mL min^{-1}. After randomization, predetermined quantities of DXR or THP-DXR were added to the upper perfusate reservoir (100 mL) which delivered the perfusion fluid to the pulmonary artery. Initial perfusate concentrations of 1, 10 and 100 μM of DXR or of THP-DXR were obtained. Thirteen 0.4 mL samples were drawn from the perfusate reservoir: 0, 1, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50 and 60 min after the addition of the drug. Each sample collected was immediately stored at −80°C for subsequent analysis. The perfusion was stopped 60 min after the addition of the anthracycline and the lungs were weighed. We discarded from the study all specimens showing an organ-weight increase in excess of 10%, indicative of pulmonary oedema.

Anthracycline determination. Drug concentrations in the lung and perfusion fluid were determined using HPLC separation procedures as previously described for DXR by Chauffert et al (1988). Briefly, to 200 μL of perfusate or a 200 μL sample of a mixture containing a weighed fraction of wet lung-tissue homogenized with 2 mL of distilled water, we added the internal HPLC standard (DNR), 300 μL of borate buffer (pH 9.8) and 9.5 mL of chloroform–methanol (4:1, v/v). Following mixing and centrifugation, the organic phase was evaporated under nitrogen. The residue was dissolved in 200 μL of the HPLC mobile phase and centrifuged. A 50 μL sample of the supernatant was submitted to chromatographic analysis. The stationary phase was Microbondapack C18 (150 × 3.9 mm) 5 μm (Millipore Waters, Milford, USA). The mobile phase consisted of an isocratic mixture of acetonitrile and 0.1% ammonium formate, pH 4 (35:65, v/v). The HPLC system included a 6000 A pump with a U6K injector (Waters) and a Jasko FP 210 fluorescence detector which was operated at an excitation wavelength of 480 nm and an emission wavelength of 560 nm. The retention times for DXRol, DXR, DNR and THP-DXR were: 1.3, 1.8, 3.2 and 3.7 min, respectively. The determination limit inherent to this method was 0.03 nmol for DXR and 0.008 nmol for THP-DXR. The accuracy obtained was 4% (DXR) and 7% (THP-DXR) while the precision was 7% (DXR) and 7% (THP-DXR).

Kinetic and statistical analysis. The concentrations of DXR or THP-DXR present in the lung perfusate 0, 1, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50 and 60 min after the addition of the drug were determined as well as the quantity of the drug accumulated in the lung 60 min after the beginning of the infusion. From these data we: (1) estimated (trapezoidal rule) the area under the perfusate concentration vs time curve (AUC); (2) calculated the clearance (dose added/AUC); (3) obtained the anthracycline lung uptake vs time profile after dividing the difference between the initial

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quantity instilled and that remaining in the perfusate, by the weight of the lung; (4) derived the L/P ratio (lung concentration/perfusate concentration, at 60 min); (5) followed the perfusate concentration/initial concentration ratio over time. Our statistical evaluation included one-way analysis of variance for pharmacokinetic parameters and two-way analysis of variance for curves.

Results

An experiment without lungs allowed the perfusate to circulate in the perfusion apparatus to ensure that there was no binding of the drugs to the apparatus. The concentration vs time curve of DXR or THP-DXR in the perfusate of rat lungs perfused with a 10 μM admixture of either drug is represented in Fig. 1A. The decrease of drug concentration in the perfusate was significantly higher for THP-DXR. Our results show that the concentration of THP-DXR in the perfusate dropped to 24 ± 5% of its initial value 20 min after the beginning of the infusion and further decreased to 8 ± 2% at 60 min. The perfusate concentration of DXR was still up to 77 ± 16% at 20 min and 52 ± 15% at 60 min. We obtained similar results when a 1 μM admixture of DXR or THP-DXR was perfused through the lung. The decrease of the drug concentration was also significantly higher for THP-DXR. The lung uptake over time for THP-DXR was significantly higher (Fig. 1B). When perfused with 10 μM drug the quantity of THP-DXR in the lung reached 77% of the initial dose at 20 min. The corresponding value for DXR was 14%.

Whether the initial concentration of anthracycline was 1 or 10 μM, comparison of: (1) area under the perfusate concentration vs time curve; (2) clearance; (3) lung concentration at 60 min; (4) L/P ratio, always led to significantly different results between the two drugs. Table 1 summarizes these data. Lower AUC, higher clearance, lung concentration and L/P ratio support our conclusion that THP-DXR uptake is higher. Table 1 includes values obtained during 100 μM infusions of DXR. We were unable to pursue our experiments when perfusing with 100 μM initial concentration of THP-DXR as the lung invariably showed signs of severe oedema a few minutes after the addition of the drug. Toxic effects could already be observed with 30 μM concentration of THP-DXR.

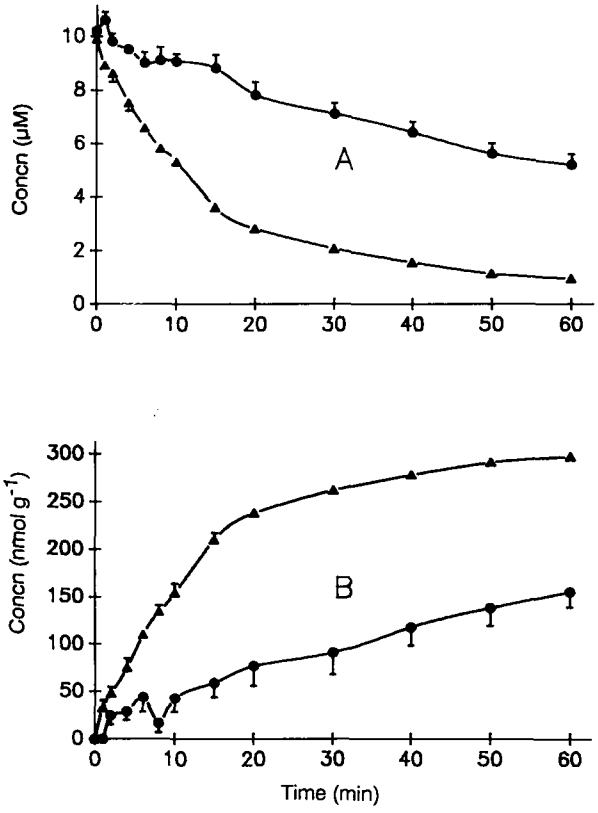

FIG. 1. Mean perfusate concentrations (A) and mean lung concentrations (B) with an infusion of isolated lung up to 60 min with an initial concentration of 10 μM DXR (●) or THP-DXR (▲). Mean values ± s.e. were obtained from 3 to 5 experiments. THP-DXR and DXR curves are significantly different.

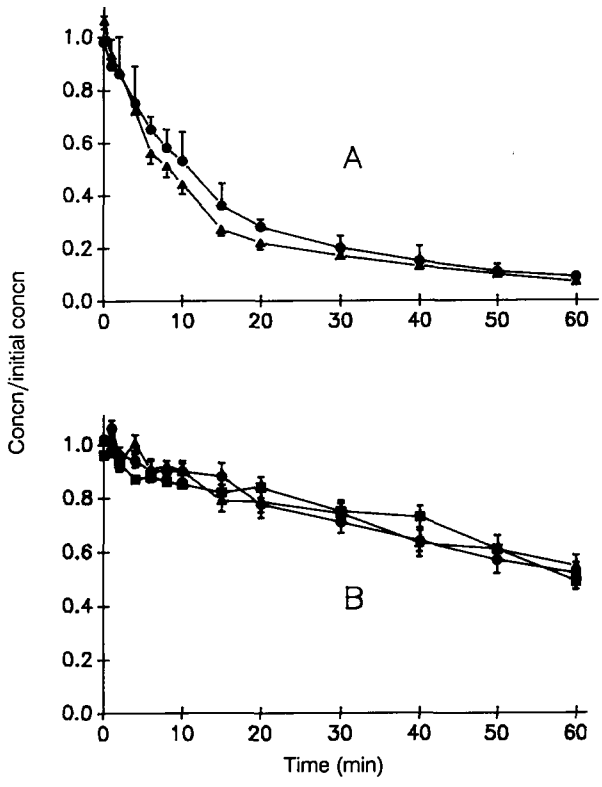

FIG. 2. Mean ratio of concentration observed at each time/initial concentration: 1 (▲) and 10 μM (●) THP-DXR (A) or 1 (▲), 10 (●) and 100 μM (■) DXR (B). Mean values ± s.e. were obtained from 3 to 5 experiments.

The perfusate concentration/initial concentration ratios for each collection time obtained during 1 and 10 μM infusions of THP-DXR were not significantly different (Fig. 2A). Neither were they significantly different when we compared corresponding values obtained during 1, 10 and 100 μM infusions of DXR (Fig. 2B).

HPLC analysis did not reveal the presence of any other substance besides the parent drugs. Comparison of the quantity of anthracycline infused with that incorporated by the lung and that remaining in the circulating medium ruled out the presence of any non-detectable metabolite. We also verified that no metabolite had the same retention time as that of the HPLC internal standard. Care was taken to freeze all biological samples immediately after collection as a non-negligible amount of THP-DXR was observed to be transformed into DXR in-vitro.

Discussion

Malignant tumours, particularly metastases, frequently develop in the lung. Anthracyclines used in the treatment of lung metastatic diseases are administered by intravenous infusion

Table 1. Comparison of area under the perfusate anthracycline concentration curves (AUC), perfusate clearances, lung concentrations at 60 min and lung/perfusate concentration ratio after 60 min of lung infusion of doxorubicin (DXR) or tetrahydropyranyl-doxorubicin (THP-DXR). Results are expressed as means ±s.d. from 3 to 5 experiments. Statistical difference between DXR and THP-DXR infused with the same initial concentration (1 or 10 μM) is indicated by $*P<0.05$, $**P<0.01$, $***P<0.001$.

	AUC (μmol L^{-1})	Clearance ($L\ h^{-1}$)	Lung concn (μmol g^{-1})	L/P
DXR (1 μM)	0·737±0·149	0·068±0·014	0·013±0·001	26·75±9·78
THP-DXR (1 μM)	0·258±0·052***	0·201±0·043***	0·031±0·009**	439±210·35**
DXR (10 μM)	7·305±1·247	0·070±0·025	0·150±0·039	31·33±15·04
THP-DXR (10 μM)	2·947±0·472***	0·173±0·025***	0·379±0·116*	336·8±59·63***
DXR (100 μM)	74·679±10·127	0·068±0·009	1·254±0·030	26±4·58

and reach these metastases through the pulmonary arterial tree. The same route of administration is utilized when extrapulmonary sites are targeted, so that the perfusion of the lung tissue precedes that of other tissues. These considerations led us to investigate pulmonary extraction of DXR and THP-DXR in the rat isolated perfused lung.

DXR uptake was relatively low in our study, in agreement with a previous report by Minchin & Boyd (1983) where 4% lung extraction was observed 20 min post-infusion with a 1 μM admixture of DXR, with a pulmonary concentration being 12·5 nmol g^{-1}. To our knowledge, the pulmonary uptake of THP-DXR has not been investigated.

Kunimoto et al (1984) and Umezawa et al (1987) compared cellular incorporation of DXR and THP-DXR in lymphoblastoma and reported that THP-DXR uptake was four times higher than that of DXR at 20 min. Our findings in the rat isolated lung are similar. The affinity of normal lung tissue for THP-DXR is of the same order as that of non-resistant tumour cells. These results suggest that the removal of THP-DXR from the circulation by the lung following an intravenous infusion in man, would be appreciable and probably extensive; treatment of non-resistant pulmonary metastases would be enhanced.

Yoshida et al (1989) studied the lung uptake of various basic drugs and suggested the existence of a specific binding mechanism without active transport for these drugs. We observed a linear increase in pulmonary drug concentrations with increased doses of DXR or THP-DXR, which is consistent with this finding. The pK_a, similar for both compounds, cannot explain the significantly different quantities of substances extracted by the lung. Another consideration concerns lipophilicity of anthracyclines. In octanol and water (pH 7·0), the partition ratio of DXR is 0·1 (Kessel 1979) as opposed to 65 for THP-DXR (manufacturer's data). Higher lipophilicity for THP-DXR might be responsible for its higher lung uptake (Yoshida et al 1989). This observation is consistent with the higher degree of toxicity witnessed during THP-DXR perfusions. Although clinically relevant concentrations of the drug would be considerably less than those investigated in our study, appropriate considerations should accompany THP-DXR administration in man.

We were unable to identify any metabolite of DXR or THP-DXR during the course of our study. This finding is in agreement with our results on DXR distribution in rat tissue reported earlier (Chauffert et al 1988). As suggested by Majima & Ohta (1987), the liver would be the only site of THP-DXR metabolism. In contrast to the above studies, other investigators (Minchin & Boyd 1983), identified two metabolites of DXR in the effluent of rat isolated perfused lung following a 10 min perfusion with DXR.

The present study has shown that lung affinity for THP-DXR is higher than it is for DXR. The relationship between drug uptake by the lung and dose infused suggests that both anthracyclines gain access to the cell through a diffusion mechanism. The lung exhibits higher acute toxicity when perfused with THP-DXR than when infused with DXR. It is doubtful that any metabolic activity involving DXR or THP-DXR takes place in the lung.

This work has been supported by the University of Burgandy, Faculty de Médecine (tranche B_2).

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