Cancer Chemother Pharmacol (1987) 20: 267–269

Cancer Chemotherapy and Pharmacology

Short communication

Doxorubicin concentration time course in the myocardium after single administration to the dog

Possible role in the cardiac effects

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Summary. Three hours after i. v. administration of doxorubicin, concentrations of the drug in the myocardium are much higher (about 50 times) and decrease much more slowly (drug still detected 21 days later) than those in the plasma, so that storage results from too early readministration, with possible toxic signs.

Introduction

Doxorubicin has been shown in experimental animals [3, 8] and in humans [2, 17] to disappear rapidly from the plasma because of rapid uptake by tissues: its distribution volume is very high (about 20 l/kg body weight) and its concentration is 20- to 100-fold the plasma concentration in several organs, such as the lungs, kidneys, liver, and heart, 15–20 min after i. v. administration.

In its pharmacokinetics and chemical structure, doxorubicin is not much different from daunorubicin, another anthracycline antibiotic, the high concentrations of which in leukaemic cells are responsible for the cytostatic effects [14, 22]. Although both drugs will be taken up by non-neoplastic cells, such as fibroblasts, to a lesser degree, as suggested by in vitro studies [15, 16], it seemed possible that histopathological alterations with function deficiency might result from an excess of these drugs in the cellular medium. This study was undertaken to find whether cardiotoxicity could be related to such an excess.

Materials and methods

Acute experiments. The uptake of doxorubicin by the heart was first investigated within the 3 h after i. v. infusion of 3 mg/kg dissolved in water (3 ml/kg) over 15 min. The study was performed in six healthy mongrel dogs weighing between 14 and 22 kg, of either sex, anaesthetised with sodium thiopental (5 mg/kg) and chloralose (100 mg/kg). The animals were intubated and artificially ventilated with an air-oxygen mixture, and their temperature was maintained at 39° C by an external infrared heater. A thoracotomy designed to facilitate heart sampling when necessary was made before the drug infusion and electrocardiogram and mean blood pressure were monitored. Actually, no substantial change is observed when the infusion rate does not exceed 0.2 mg kg^{-1} min^{-1}, as in the present experiments: the electrocardiogram did not show any conduction disorder or ectopic rhythm, and the mean blood pressure did not fall by more than 20 mmHg within the minutes following the end of the infusion, which was not enough to affect pharmacokinetics to a large extent.

Several blood samples were taken to define the plasma pharmacokinetics and, in addition, a fragment of left ventricular free wall was obtained according to the 'drill biopsy' technique [19]. Myocardial biopsies were carried out last because they were transmural biopsies and the lungs, kidneys and liver, and a lymph node had been sampled before, for comparison with the heart.

Chronic experiments. Doxorubicin was assayed both in the heart and in the organs cited above at 4, 7, 14 and 21 days after a single i. v. infusion of 1.5 mg/kg over 15 min (the 3 mg/kg dose had proved to be lethal before the first determination, generally 3 days after administration, with serious gastrointestinal disorders: vomiting, diarrhoea, sometimes intestinal haemorrhage), each time in two conscious dogs. Sampling was carried out under the same conditions as in the acute experiments in anaesthetised animals.

Doxorubicin assay. Doxorubicin was assayed in the plasma and tissue fragments by high-performance liquid chromatography according to the method described by Strauss et al. [21]. These fragments, weighing 200–400 mg, were kept at −30° C and homogenised at 0° C. The assay was performed shortly after defrosting, and the results were expressed as nanograms per gram of wet weight. The limit of detection was 1 ng/ml for the plasma and 20 ng/g for the tissues, the difference being explained by the dilution necessary for preparation of the homogenates. Recovery was checked to reach 95%, as defined by Strauss et al. [21].

Results

The doxorubicin concentrations, which declined rapidly in the plasma [2], appeared at 3 h (Fig. 1) to be much lower (82.9 ± 11.4 (SEM) ng/ml, than in the tissues, such as the heart (4066 ± 306 ng/g; $P < 0.001$), but also the lungs (3596 ± 272 ng/g), the kidneys (7304 ± 918 ng/g), the liver (5976 ± 368 ng/g) and the lymph nodes (2266 ± 316 ng/g). The concentration ratio of tissue to plasma is therefore close to 50 for the heart, higher than for the lungs (45) and lymph nodes (30) but not as high as for the kidneys (90) and liver (75).

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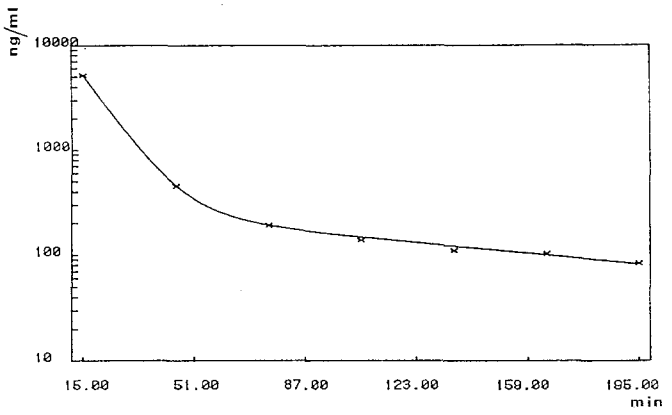

Fig. 1. Doxorubicin concentration time course in plasma after infusion of 3 mg/kg over 15 min. Computer tracing. Time 15, end of the infusion. Mean values in six dogs

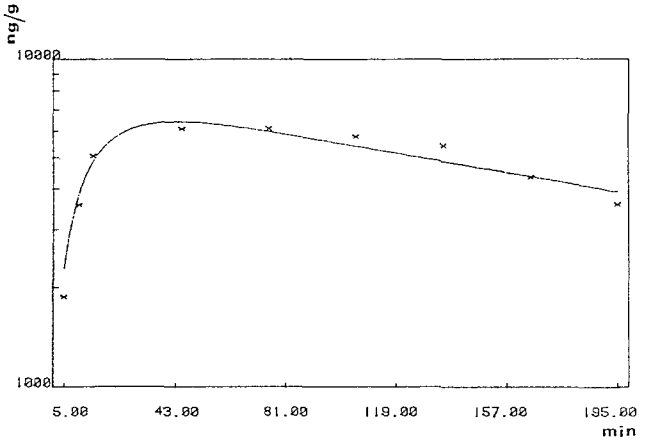

Fig. 2. Doxorubicin concentration time course in pulmonary tissue during and after the infusion of 3 mg/kg over 15 min. Computer tracing. Time 0, start of the infusion. Mean values in six dogs

As myocardial concentrations were determined in full-thickness left ventricular wall samples, the tissue concentration time course could not be investigated in the heart from the onset of infusion to the end of the experiment. Such a determination was possible in the lungs, however, in which the time course was probably similar, since the final concentration at 3 h did not differ notably in the two organs. A few minutes after the end of the infusion, the level observed in the lungs was approximately equal to the plasma level, which was very high at this time (Fig. 2), probably because of the passive diffusion which governs the penetration into the cells [14, 16] and ensures an equilibrium between the extracellular and the intracellular medium with a short delay. However, the subsequent fall in doxorubicin concentration in the lungs was much slower than in the plasma, as is apparent on comparison of Figs. 1 and 2.

Similarly, after chronic administration, doxorubicin would not disappear from the cardiac tissue in the same way as it disappears from plasma, since a single administration gives rise to myocardium drug contents which remain at 161 ng/g on day 7 and 45 ng/g on day 14, the drug still being detected at the 21st day (Table 1). Even

Table 1. Doxorubicin concentration time course in several organs (ng/g) (result obtained in two dogs each time) and in plasma (ng/ml) after a single i. v. injection of 1.5 mg/kg

	Day 4	Day 7	Day 14	Day 21
Plasma	2.3	0	0	0
Heart	240	161	45	28
Lungs	580	351	50	37
Kidneys	456	215	46	22
Liver	279	160	80	20
Lymph node	1474	472	248	109

though a strict comparison of the concentration time course in the heart and plasma is questionable in view of the much lower plasma level which complicates detection, there was an obvious difference between the rate of elimination from the plasma and cardiac tissue from 3 h to 21 days after the administration. Assuming similar pharmacokinetics in the different animals, the elimination from tissues might be characterised by an elimination rate constant and a half-life particular for each: concerning the heart, the former would be below 0.0086 h^{-1} and the latter above 81 h, namely about 4-fold lower and higher, respectively, than the corresponding parameters for the plasma [2, 8]. However, the elimination from the lungs and especially from the lymph nodes was even slower (Table 1), so that the concentrations found at the 21st day in the lymph nodes, where the half-life reached 148 h, were the highest, at above 100 ng/g.

Discussion

This study shows that doxorubicin reaches much higher levels in the myocardial fibres than in the plasma. These concentrations in tissues must obviously be taken into account in the effects, toxic as well as cytostatic, exerted by the drug: these effects are undoubtedly developed inside the cells and even their nuclei [6, 10, 20], whether they are founded on free radical induction [1, 3], lipid peroxidation [12, 13], inhibition of guanylate cyclase activity [2, 11] or modification of calcium handling [7, 18].

There is not only uptake, but also lasting retention, of doxorubicin in tissues such as the myocardial tissue. The uptake is less marked than that of daunorubicin [15, 16], but the retention is much more prolonged [16], since the differences in the tissues and plasma half-lives are considerable in one case and barely significant in the other [14, 22]. Every administration performed prior to complete elimination from the tissues, i. e. sooner than 14 or 21 days after the previous dose, will lead to the drug storage and, if the amounts administered are substantial and numerous, the rise in the myocardial drug content can be expected to result in signs of cardiotoxicity, taking the form of congestive heart failure or conduction disorders related to damage to myofibrils and mitochondrial changes [4, 9].

With such a drug, attention should be paid to the cell concentration rather than to the plasma concentration, which after the first minutes following the i. v. administration corresponds to only a minor part of the dose administered and falls relatively rapidly to zero, while the cardiac tissue is likely to contain substantial amounts, increasing with each premature readministration.

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Received July 30, 1986/Accepted April 30, 1987