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EFFECT OF COLLOIDAL CARRIERS ON THE DISPOSITION AND TISSUE UPTAKE OF DOXORUBICIN: II. CONJUGATION WITH ISOBUTYL-CYANOACRYLATE NANOPARTICLES

Niteen Bapat[1] and Mehdi Boroujerdi[2]

Department of Pharmaceutical Sciences
Northeastern University
Boston, MA 02115

ABSTRACT

The effect of isobutylcyanoacrylate nanoparticles, in the size range of 100-220nm, on the disposition of doxorubicin was investigated. After intravenous injection of drug-containing nanoparticles into rats, the plasma concentration showed an initial rapid rise before a biphasic decline. The rate of the breakdown of the polymer was found to be much slower than the anticipated value. Urine flow declined significantly after injection. The concentration of drug in the liver, kidney, lung and spleen was lower than the control and did not increase over time. The low concentration may be attributed to the slow breakdown of the polymer in the body and slow release of the drug associated with the matrix of the carrier. The association of doxorubicin with the nanoparticles alters pharmacokintics of the drug in ways that could provide optimal condition for drug distribution within the systemic circulation and a therapeutic effect with a lesser probability of cardiotoxicity.

INTRODUCTION

The possibility of a role for polymeric nanoparticles in the use of anti-cancer drugs has aroused considerable interest in recent years (1-4,13,14). Two distinct areas of potential usefulness have been suggested: as carriers for the direct delivery of drugs to

1 Present Address: Proctor & Gamble Co. Miami Valley Labs., Cincinnati, Ohio.
2 Author to whom correspondence should be addressed.

tumor cells either through active/passive targeting or as reservoirs for the slow release of drugs at predetermined rates. The latter is especially applicable to drugs for which therapeutically effective plasma concentrations are required during the growth cycle of the target cells. Nanoparticles made from polyalkylcyanoacrylate were first developed by Couvreur and coworkers (5) and have been shown to be biocompatible and biodegradable (1,6,7). The absence of mutagenicity for polyalkylcyanoacrylate nanoparticles and their degradation products has been reported even at high doses (8).

In this study we have examined the effect of isobutylcyanoacrylate nanoparticles, in the size range of 100-220 nm, on the pharmacokinetics and tissue distribution of doxorubicin in rats. The rationale for using the particles is as follows. By incorporating doxorubicin in the particles, the plasma concentration of the free drug in the systemic circulation, due to the slow release of the drug from the matrix of the particles, would be reduced significantly. Therefore, the level of the uptake by the heart would be less than the conventional dosage form. Consequently the acute myelotoxicity and the development of irreversible cardiomyopathies (10) would decrease accordingly.

MATERIALS AND METHODS

CHEMICALS

Doxorubicin HCl, daunorubicin HCl, isobutylcyanoacrylate and dextran 70 were obtained from Sigma Chemicals (St. Louis, MO). All reagents and solvents were of analytical or HPLC grade.

PREPARATION OF NANOPARTICLES CONTAINING DOXORUBICIN

The nanoparticles were prepared by addition of doxorubicin to 10ml of polymerizing medium of 1% w/v citric acid and 1% w/v dextran 70 (11). The mixture,

prepared in a silanized 20ml glass beaker, had a final pH of 2.2. The monomer, isobutylcyanoacrylate, was added to the mixture while stirring at 2000 r.p.m. The stirring was continued for four hours at room temperature. The particulate suspension was filtered through a series of filters of sizes 5000, 1200, 800, 450, 220 and 100 nm (Millipore corporation, Bedford, MA). The particles retained on 100 nm filter were used for this investigation.

The nanoparticles were examined under a scanning electron microscope. The particle size was measured from the micrographs and exhibited a uniformly spherical appearance (15).

IN VIVO EXPERIMENTS

Male CD rats weighing 175-200 g (Charles River Breeding Labs, Wilmington, MA) were randomly assigned to two groups of 40 animals in each group after acclimation. The animals were allowed food and water *ad libitum*. The experimental design was parallel. Doxorubicin, dissolved in normal saline, or associated with nanoparticles was injected intravenously in a volume of 1 ml as a bolus via the tail vein. The dosage forms were formulated to deliver 5 mg/kg of doxorubicin. The animals were sacrificed at 3, 10, 20, 40, 80, 150 and 300 minutes and 24 hours after the administration of the dose. The blood samples were collected in plastic test tubes containing 0.5 ml saturated sodium citrate and centrifuged at 1000 g for 20 min to pelletize the cells. The supernatants were stored frozen until assayed by HPLC. Organs such as liver, lungs, spleen, kidneys and heart were removed immediately after the collection of blood samples and rinsed with distilled water, dried with paper towel and frozen.

The animals for the 24 hour time point were placed individually in metabolism cages with food and water provided *ad libitum*. The urine samples were then collected at

1, 2, 4, 6, 10, 12 and 24 hours after the injection of the drug and frozen. At each sampling time the bottom tray of the cage was rinsed with distilled water and added to the sample. After the last urine sample, the animals were sacrificed and 24 hours blood and tissue samples were collected.

ANALYTICAL METHOD

The extraction of doxorubicin from biological samples was carried out with 4:1 chloroform:methanol mixture. The tissue samples were homogenized before the extraction, using hand held homogenizer (Biospec products, Bartesville, OK). The organic phase of the extraction was evaporated to dryness under vacuum. The residue was reconstituted in methanol and analyzed by HPLC.

Doxorubicin concentrations were determined by HPLC (Waters, Milford, MA). The stationary phase was octodecylsilane (ODS) Novapak C-18 Radial Compression Module (RCM) cartridge. The mobile phase was a 70:30 mixture of methanol and ammonium formate, pH 4.0. The chromatography was isocratic with the flow rate maintained at 2 ml/min. The detection was carried out by fluorometry (Gilson 121, Gilson Instruments, Randolph, MA) with 470 nm excitation and 540 nm emission wavelengths. The detection limit was 10 ng/ml. Daunorubicin, a closely related analog of doxorubicin, was used as the internal standard.

RESULTS AND DISCUSSION

The adsorption studies of doxorubicin on the nanoparticles suggest that the percent uptake of the drug was approximately 60% of the initial amount added (15). The drug appears to adsorb to both the matrix and the surface of the nanoparticles. Only a fraction of the drug was seen to be adsorbed to the surface. Therefore, in a conventional non-enzymatic *in vitro* release study, only the drug adsorbed to the surface would be

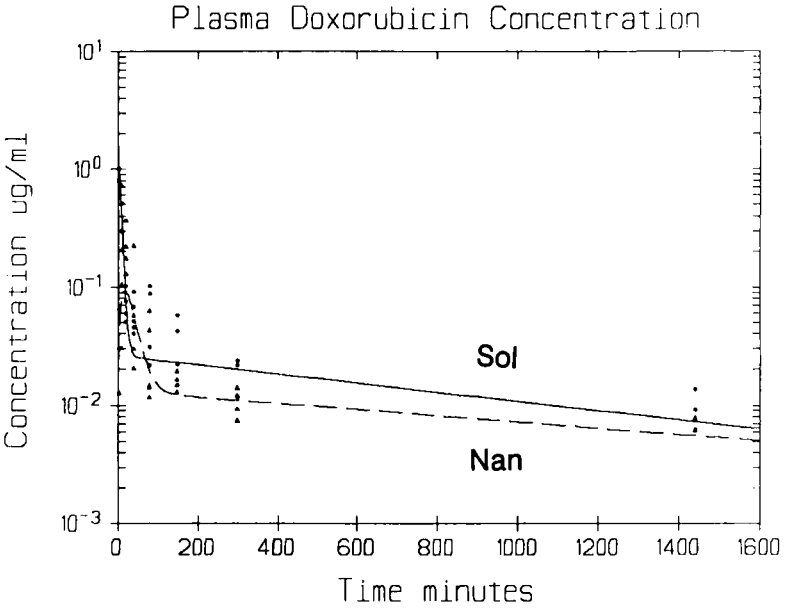

Figure 1

Plasma concentration-time curve of doxorubicin administered in normal saline (—) and associated with the nanoparticles (---).

released. However, the release of the drug *in vivo* is expected to be a function of the release from the surface of the particles as well as the rate of biodegradation of the polymer in the body. The *in vitro* release of doxorubicin from these particles in non-enzymatic buffer solutions was 0.380 day^{-1} (15).

The plasma data were normalized with respect to the weight of the animal and to the dose injected. The plasma concentrations of doxorubicin associated with the nanoparticles showed an initial rapid rise in concentration-time curve before a biphasic decline (Fig. 1). The rise in the concentration can be attributed to the release of the drug adsorbed on the surface of the nanoparticles. The drug adsorbed to the matrix would be released after the enzymatic breakdown of the polymers. Judging from the plasma

TABLE I

Comparison of Pharmacokinetic Parameters of Doxorubicin After Intravenous Injection of Solution (Control) and Nanoparticle Colloid in Rats

	Control	Nanoparticles
AUC (mg.min/l)	38.23	25.69
T½ (hr)	13.13	19.25
k_{10} (hr^{-1})	2.42	0.53
Vd (l)	29.74	64.87
Clearance (ml/min)	26.18	36.07
MRT (hr)	5.81	6.16

AUC : Area under plasma concentration-time curve from 0 - ¥
T½ : Biological half-life ($(T_{1/2})_\beta$)
k_{10} : Overall elimination rate constant
Vd : Apparent volume of distribution
MRT : Mean Residence Time

concentration of the drug, the rate of the breakdown of the polymer was slower than the anticipated value.

The area under the plasma concentration-time curve (AUC) was calculated by the linear trapezoidal rule. The apparent volume of distribution, Vd, and clearance, Cl, were calculated as follows:

$$Vd = Dose/ (AUC * \beta)$$

$$Cl = Dose/ AUC$$

where β is the terminal rate constant. The pharmacokinetic data are reported in Table I. All parameters and constants were calculated by nonlinear iterative least squares regression with PCNONLIN. Due to the initial rapid rise before a biphasic decline, the

plasma data were fitted to a two-compartment model with first-order release in the central compartment. The calculated Akaike value for the model was less than the two-compartment model with instantaneous input.

The volume of distribution of doxorubicin associated with the nanoparticles is nearly twice of the control. This difference is a manifestation of the binding of the drug to the carrier. A lower concentration of the free drug in the central compartment causes smaller AUC and larger volume of distribution. The longer half-life may indicate the slow release of the drug from the particles in the plasma. The maximum plasma concentration of the free drug after the administration of nanoparticles was one tenth of the maximum concentration reached with the drug in normal saline.

The injection of doxorubicin solution (normal saline) resulted in a higher concentrations in the heart (Fig 2). The area under the free doxorubicin concentration-time curve in the heart tissue was 20.43 mg.min/g for the solution and 3.25 mg.min/g for the nanoparticles (Table II). The reduction in the uptake may be due to a lower concentration of free doxorubicin in the systemic circulation.

The liver is the largest organ of the reticuloendothelial system and is expected to remove colloids from the systemic circulation. Also, the nanoparticles, with hydrophobic exterior, are expected to be taken up by the liver. However, the concentration of doxorubicin in the liver after the administration of nanoparticles was lower than the control and did not increase over time (Fig 3). The decline of the concentration was slow and the drug could still be detected at the 24 hour time point. The low concentration of the drug could be the result of the slow breakdown of the polymer in the liver and, hence, the slow release of the drug associated with the matrix of the nanoparticles.

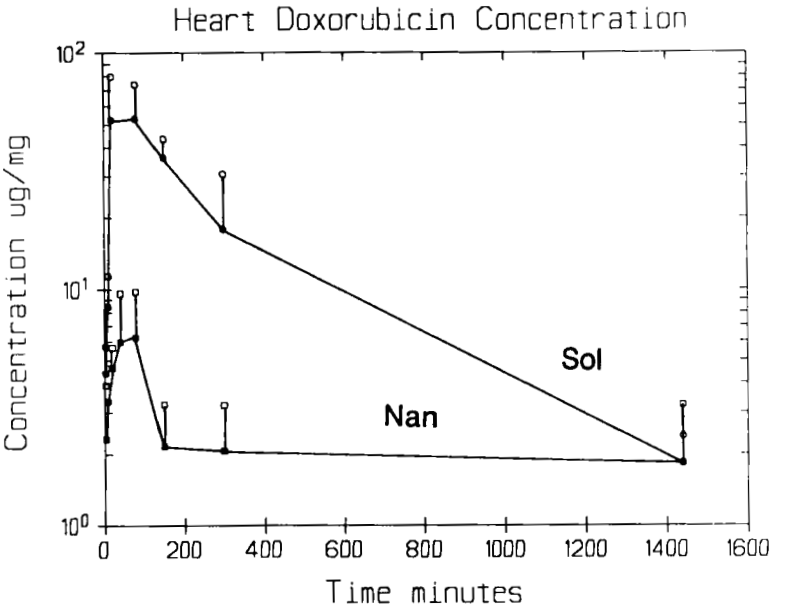

Figure 2

Concentration-time profile of doxorubicin in the heart after the intravenous administration of doxorubicin in normal saline (o) and the nanoparticles (□).

TABLE II

Comparison of the Area under the Tissue Concentration-Time Curves for Major Organs after the Inrtavenous Injection of Doxorubicin Solution and Doxorubicin Associated with Nanoparticles in Rats

	Control (ug min/g)	Nanoparticles (ug min/g)
Heart	20.43 ± 11.75	3.25 ± 2.00 *
Liver	28.63 ± 19.41	13.74 ± 5.81
Kidney	24.58 ± 12.78	11.36 ± 5.38
Spleen	15.70 ± 8.34	3.81 ± 2.06 *
Lung	8.14 + 6.84	1.58 + 0.80

* $p < 0.05$

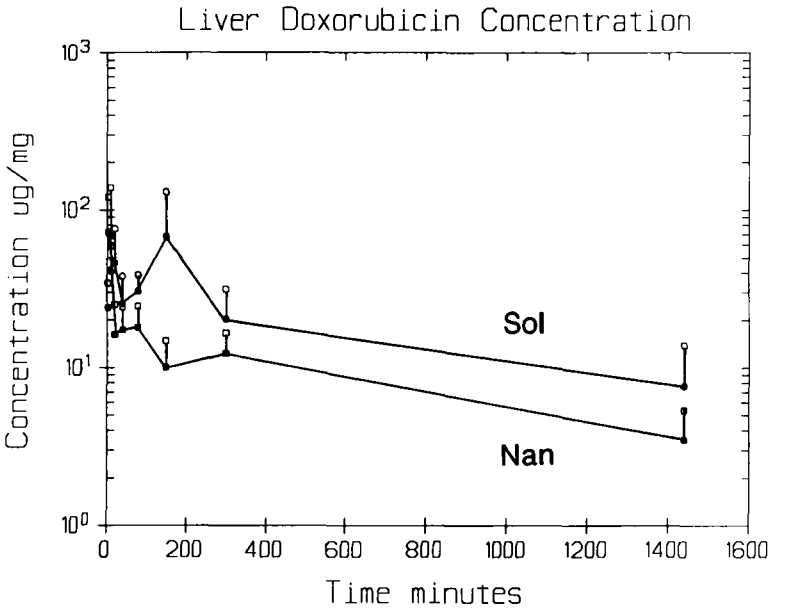

Figure 3

Concentration-time profile of doxorubicin in the liver after the intravenous administration of doxorubicin in normal saline (o) and the nanoparticles (□).

Doxorubicin associated with the nanoparticles did not produce high concentrations in the kidneys (Fig 4) or the spleen (Fig 5). Lungs are reported to be selective in entrapping particles of particular size range. In humans this range has been reported to be 7 to 12 u. The nanoparticles did not show any effect on the concentration of the drug in the lungs of rats (Fig. 6).

The results reported in this paper demonstrate that doxorubicin associated with the nanoparticles of isobutylcyanoacrylate releases slowly in systemic circulation and the tissues. The biological (disposition) half-life, when associated with nanoparticles, is similar to the control and in agreement with reported values (12). The half-life of the free drug in the plasma does not reflect the half-life of doxorubicin-nanoparticles complex. The

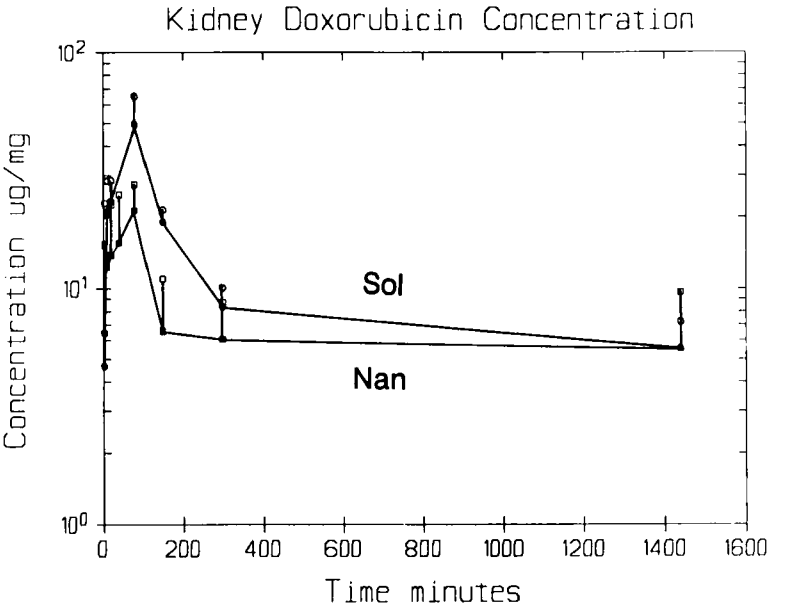

Figure 4

Concentration-time profile of doxorubicin in the kidney after the intravenous administration of doxorubicin in normal saline (o) and the nanoparticles (□).

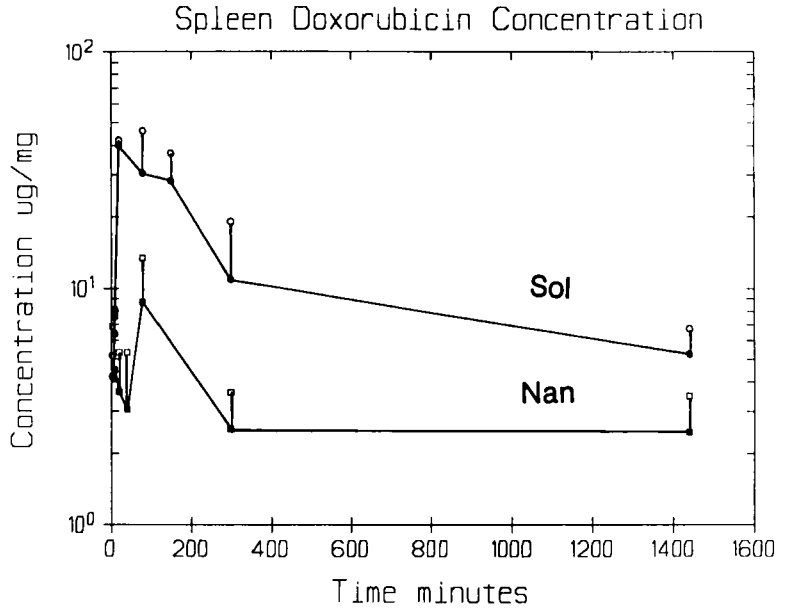

Figure 5

Concentration-time profile of doxorubicin in the spleen after the intravenous administration of doxorubicin in normal saline (o) and the nanoparticles (□).

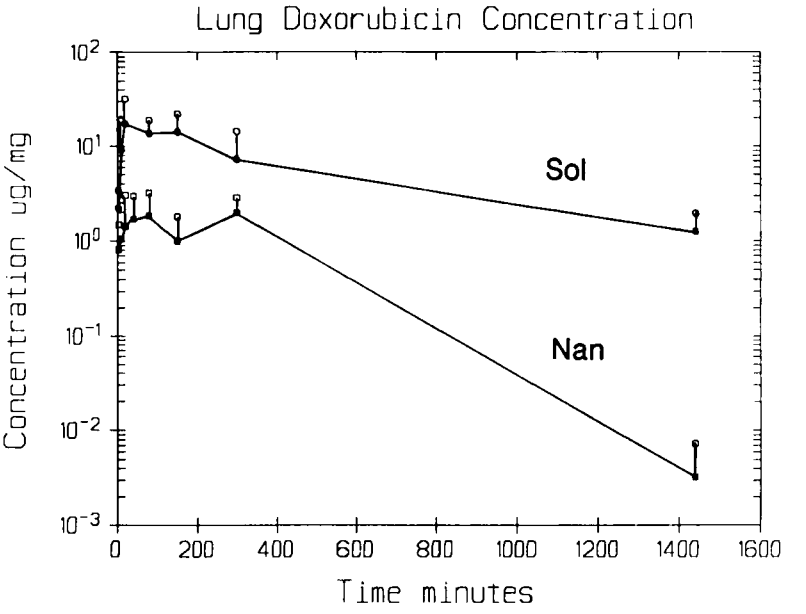

Figure 6

Concentration-time profile of doxorubicin in the lung after the intravenous administration of doxorubicin in normal saline (o) and the nanoparticles (□).

nanoparticles serve as slow release compartments. Consequently, a comparable dose of doxorubicin in normal saline generates much higher levels of the free drug in the serum over a short interval.

In conclusion, the incorporation of doxorubicin in the matrix of nanoparticles may overcome some of the main drawbacks of the drug at high concentrations. Considering the sustained release of the drug from the nanoparticles in the blood and lower concentrations of the free drug in the heart, such a drug delivery system could probably minimize the cardiotoxicity of doxorubicin.

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