Acute Renal Toxicity of Doxorubicin (Adriamycin)-Loaded Cyanoacrylate Nanoparticles

Luc Manil,[1,3] Patrick Couvreur,[2] and Philippe Mahieu[1]

Received March 25, 1994; accepted August 9, 1994

Acute doxorubicin-loaded nanoparticle (DXNP) renal toxicity was explored in both normal rats and rats with experimental glomerulonephritis. In *normal* rats, 2/6 rats given free doxorubicin (DX) (5 mg / kg) died within one week, whereas all control animals and all rats having received free NP or DXNP survived. A 3 times higher proteinuria appeared in animals treated with DXNP than in those treated with DX. Free NP did not provoke any proteinuria. Two hr post-injection, DXNP was 2.7 times more concentrated in kidneys than free DX ($p<0.025$). In rats with *immune experimental glomerulonephritis*, 5/6 rats given DX died within 7 days, in contrast to animals treated by DXNP, NP, or untreated, which all survived. Proteinuria appeared in all series, but was 2-5 times more intense ($p>0.001$) and prolonged after doxorubicin treatment (400-700 mg / day), without significant difference between DXNP and DX. Rats treated by unloaded NP behaved as controls. These results demonstrate that, in these experimental conditions, DXNP killed less animals than free DX, despite of an enhanced renal toxicity of the former. Both effects (better survival and nephrosis) are most probably related to an enhanced capture of DXNP by cells of the mononuclear phagocyte system, including mesangial cells.

KEY WORDS: nanoparticles; polyalkylcyanoacrylate; doxorubicin; targeting; kidney; toxicity.

INTRODUCTION

Doxorubicin (DX) is a widely used anticancer anthracyclin drug (Adriamycin°, Adriblastina°), acting as an intercalating agent into DNA strains. Its cardiac toxicity is a serious limitation for clinical use: besides an acute and reversible toxicity, causing arrythmia and conduction disorders, chronic and irreversible damages frequently occur after administration of a cumulative dose of 450-500 mg / m^2. Moreover, potentially severe haematological and gastrointestinal disorders are frequently observed.

Injection of DX using drug targeting aims at increasing the tumor uptake while lowering adverse cardiac effects. For this purpose, polyalkylcyanoacrylate nanoparticles (NP) loaded with doxorubicin were successfully tested in experimental and clinical conditions (1-7). However, these particles also accumulate in reticuloendothelial cells, and in other cells with endocytosis capabilities, such as glomerular mesangial cells (8). By contrast, they do not accumulate in tubules (8). Chronic renal (mainly glomerular) toxicity has been previously described by Okuda et al. (9), after i.v. administration of two sequential DX injections (2 mg / kg) at 20-day interval. Under these conditions, no general acute toxicity was observed, but an increased and long-lasting (> 28 weeks) proteinuria was noted shortly after the second injection.

The aim of the present study was to test the acute renal toxicity of DX nanoparticles in normal rats and in animals with glomerular inflammation, and to compare it with that of free DX and of unloaded NP.

MATERIALS AND METHODS

Nanoparticles

Polyisobutylcyanoacrylate drug-unloaded ("nude") nanoparticles (NP) were prepared as previously described (2). Briefly, the monomer (Ethnor, Paris, France) was added at room temperature to an aqueous solution (100 µl / 10 ml) of 10^{-3} M H_3PO_4 containing 1% dextran 70 and 5% glucose, under continuous mechanical stirring. After 30 min of polymerization, the resulting NP formed a stable and homogeneous milky suspension (mean diameter of the particles measured by laser light scattering (Nanosizer, Coulter): 150 ± 16 nm).

Doxorubicin loaded-NP (DXNP) were provided under lyophilized form by Sopar Biochem (Belgium) (1 mg DX / 10 mg monomer). An homogeneous and red suspension was obtained by the addition of a pH 7.4, 0.1 M phosphate buffer. The particle mean diameter was 150 ± 18 nm.

Solutions of *free doxorubicin (DX)* (Farmitalia, Italy), containing all the same reagents except the cyanoacrylic monomer were also used for comparison.

Control solutions consisted of pH 7.4, 0.1 M phosphate buffer.

Animal Experiments

Control Rats. Eight groups of 6 normal Sprague-Dawley rats each (220 ± 20 g) received i.v. DXNP (group 1: 5 mg DX / kg), free DX (group 2: 5 mg / kg), unloaded NP (50 mg / kg, group 3), or phosphate buffer (group 4, control).

Rats with Glomerulonephritis. An acute proliferative glomerulonephritis was induced in 4 groups of 6 Sprague-Dawley rats (240 ± 20 g), according to a previously described protocol (10); briefly,

- on day −7, all rats received i.p. 2 mg of rabbit IgG mixed with Complete Freund's adjuvant.
- on day 0, all the animals were injected i.v. with 1 mg of rabbit anti-rat glomerular basement membrane IgG antibodies, together with 5 mg of free DX / kg (group 1), DXNP containing 5 mg of DX / kg and 50 mg of NP / kg (group 2), 50 mg / kg of NP (group 3), or phosphate buffer (group 4, control).
- between days + 2 and + 5, many mononuclear phagocytes infiltrated the glomeruli and, simultaneously, a pathological proteinuria occurred.

Proteinuria was measured daily for 10 days by the method of Kingsbury and Clarcke (11). Protein assays were

[1] Internal Medicine Department, University of LIEGE, Sart-Tilman, B-4000 Liége, Belgium.
[2] Laboratoire de Pharmacie Galénique et de Biopharmacie, URA CNRS 1218, Université de PARIS-Sud, F-92296 Chatenay-Malabry, France.
[3] To whom correspondence should be addressed at 6, Avenue des Tilleuls, F-91440 Bures-sur-Yvette, France.

0724-8741/95/0100-0085$07.50/0 © 1995 Plenum Publishing Corporation

performed as previously described (12). Rats were then sacrificed and their kidneys were examined by light microscopy and by immunofluorescence using anti-rabbit IgG, anti-rat IgG and anti-rat C3 antisera conjugated to fluorescein (10, 13). DX content in kidney tissue was analyzed by HPLC, according to Bertani et al. (13) (samples extracted in isopropanol; solvent: acetonitrile: water: 0.1M phosphoric acid (34: 40: 26); flow rate 1 ml/min; 25 cm Li Chrosorb C-18 column; sensitivity 10 ng / g).

Statistical Analysis

All parameters were expressed by mean ± standard deviation. Student's t test was used to compare each point of the proteinuria curves.

RESULTS

Normal Rats (Table 1)

No animal of the groups 1,3 and 4 died during the 10 day observation period. Conversely, 2 / 6 rats given free DX (group 2) had died by the end of the experiment. Creatinin and urea serum concentrations on the 7th day post-injection were normal in the 4 series.

Proteinuria was more significantly enhanced ($p < 0.001$) in rats having received DXNP (460 ± 110 mg / day on day 7, for 5 mg DX / kg) than in animals injected with free DX (115 ± 47 mg /day) NP did not induce any proteinuria.

Kidneys contained 2.7 times higher DX concentration 2 h after i.v. injection of 5 mg DXNP / kg than after administration of the same dose of free DX ($p < 0.025$). Immunofluorescence microscopy did not display any significant lesion in any series.

Rats with Proliferative Glomerulonephritis (Table 2)

Five out of 6 rats having received 5 mg of free DX died between the second and the sixth day post-injection. As compared to control rats, serum creatinin and urea concentration were not modified by unloaded NP administration; these parameters were not significantly enhanced in rats injected with DXNP but they strongly increased in the unique survivor of the second series (DX)

As expected (10), proteinuria appeared in control animals, reaching a maximum on day 3, slowly decreasing thereafter. Proteinuria clearly worsened when using DXNP or DX ($p < 0.001$), but not with unloaded NP. There was no significant difference between DX and DXNP series. After immunostaining (10), linear glomerular deposits of rabbit IgG, rat IgG and rat C3 were observed by optical microscopy in all groups of animals, without marked differences in the glomerular infiltration by mononuclear phagocytes.

DISCUSSION

As Bertani et al. (13), we confirm that free DX provokes adverse effects on normal rat kidneys at relatively high dose (5 mg / kg) but we also demonstrate that this effect is strongly amplified when the drug is associated to NP. According to one of our previous studies (8), it is likely that this toxicity, never observed with free NP, has to be related to a modification of the drug distribution, leading to a strong uptake by glomerular mesangial cells as well as by scattered resident monocytes. It is unlikely that such a targeting could amplify tubular toxicity, since it has been previously demonstrated that NP did not accumulate in the tubules (8).

In animals with acute proliferative glomerulonephritis, severity of renal disease was enhanced in the presence of doxorubicin, without significant difference between the two galenic forms. These results suggest that an increase of DX concentration in activated macrophages is not an essential factor for proteinuria induced by immune complexes. However, in spite of the severe proteinuria, DXNP never impaired but rather improved slightly urea and creatinin blood balance, as compared to free DX. Moreover, for the same DX dose that, under free form, killed 5 / 6 animals within a week, no one rat injected with DXNP died. This better survival rate with DXNP is probably due to the relative protection of myocardial and gastrointestinal cells, that all display low endocytosis capabilities for nanoparticles (3-6).

In summary, doxorubicin delivery by means of nanoparticles can induce acute renal damage (proteinuria), that has to be related to a higher uptake of the drug by certain renal glomerular cells with endocytosis capabilities. Chronic toxicity data with DXNP are not available but deserve to be investigated later on. Since renal chronic toxicity occurs after repeated administrations of free drug (9), it could be important to assess whether association of DX to NP could also modify this long term nephrotoxicity. However, these data on acute renal toxicity of DXNP does not seem to be sufficiently severe to prevent one from taking advantage of the benefits of doxorubicin targetting, for both tumour destruction and myocardium protection.

Table 1: Renal toxicity studies and doxorubicin concentration in normal rat kidneys

	DXNP	DX	NP	Control
Number of rats	6	6	6	6
Dead (till day 7)	0	2	0	0
Blood creatinin (mg / l on day 7)	< 10 (NI)	< 10 (NI)	< 10 (NI)	< 10 (NI)
Blood urea (mg / l on day 7)	< 500 (NI)	< 500 (NI)	< 500 (NI)	< 500 (NI)
Proteinuria (mg / day on day 7)	460 ± 110	115 ± 47	<10	<10
	$p < 0.001$			
DX concentration in kidneys (μg / g at 2 hr)	70 ± 21	26 ± 10	/	/
	$p < 0.025$			

Injected doses: DXNP: 5 mg DX / kg; 50 mg NP / kg, DX: 5 mg / kg, NP: 50 mg / kg.

Table 2: Renal toxicity studies in rats with experimental proliferative glomerulonephritis

	DXNP	DX	NP	Control
Number of rats	6	6	6	6
Dead (till day 7)	0	5	0	0
Blood creatinin (mg / l on day 7)	9 ± 3	17	6 ± 3	7 ± 2
Blood urea (mg / l on day 7)	780 ± 200	1500	< 500	570 ± 150
Proteinuria (on day 3)	540 ± 192	515 ± 132	352 ± 142	342 ± 33
(mg/day) (on day 5)	459 ± 111	708 ± 556	160 ± 56	171 ± 53
(on day 7)	461 ± 81	265	70 ± 22	66 ± 13

Student t test for proteinuria: DXNP - DX: p = NS on days 3, 5 and 7; DXNP - NP: $p < 0.001$ on days 3, 5 and 7; DX - NP: $p < 0.001$ on days 3 and 5; NS on day 7; NP - control: p = NS on days 3, 5 and 7.
Injected doses: DXNP: 5 mg DX / kg; 50 mg NP / kg, DX: 5 mg / kg, NP: 50 mg / kg.

REFERENCES

1. P. Couvreur, B. Kante, M. Roland and P. Speiser. Adsorption of antineoplastic drugs to polyalkylcyanoacrylate nanoparticles and their release characteristics in a calf serum medium. *J. Pharm. Sci.* **68**:1521–24 (1979a).
2. P. Couvreur, B. Kante, M. Roland, P. Guiot, P. Baudhuin and P. Speiser. Polycyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and sorptive properties. *J. Pharm. Pharmacol.* **31**: 331–332 (1979b).
3. P. Couvreur, B. Kante, L. Grislain, M. Roland and P. Speiser. Toxicity of polyalkylcyanoacrylate nanoparticles. II: Doxorubicin-loaded nanoparticles. *J. Pharm. Sci.* **71**: 790–792 (1982).
4. P. Couvreur, L. Roblot-Treupel, M. F. Poupon, F. Brasseur and F. Puisieux, Nanoparticles as microcarriers for anticancer drugs. *Adv. Drug Deliv. Rev.* **5**:209–230 (1990).
5. P. Couvreur and C. Vauthier. Polyalkylcyanoacrylate as drug carrier: present state and perspectives. *J. Control. Rel.* **17**:187–188 (1991).
6. N. Chiannilkulchai, N. Ammoury, B. Caillou, J. P. Devissaguet and P. Couvreur. Hepatic tissue distribution of doxorubicin-loaded nanoparticles after i.v. administration in reticulosarcoma M5076 metastases-bearing mice. *Cancer Chemother. Pharmacol.* **26**:122–126 (1990).
7. C. Cuvier, L. Roblot-Treupel, S. Chevillard, J. M. Millot, M. Manfait, G. Bastian, P. Couvreur and M. F. Poupon. Doxorubicin-loaded nanospheres bypass tumour cell multidrug resistance. *Biochim. Pharmacol.* 44:509–517 (1992).
8. L. Manil, J. C. Davin, C. Dechenne, C. Kubiak, J. Foidart, P. Couvreur, P. Mahieu. Uptake of nanoparticles by rat glomerular mesangial cells in vitro and in vivo. *Pharm. Res.* **11**:(8):1160–1165 (1994).
9. S. Okuda, Y. Oh, H. Tsuruda, K. Onoyama, S. Fujimi and M. Fujishima. Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. *Kidney Int.* **29**:502–510 (1986).
10. G. T. Schreiner, R. S. Cotran, V. Pardo and E. R. Unanue. A mononuclear cell component in experimental immunological glomerulonephritis. *J. Exp. Med.* 147:369–384 (1978).
11. F. B. Kingsbury and C. P. Clarck. The rapid determination of albumin in urine. *J. Lab. Clin. Med* **11**:981–986 (1926).
12. O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**:265–272 (1951).
13. T. Bertani, F. Cutillo, C. Zoja, M. Broggini, G. Remuzzi. Tubulo-interstitial lesions mediate damage in adriamycin glomerulopathy. *Kidney Int.* **30**:488–496 (1986).