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ACKNOWLEDGMENTS

This work was supported by S.A. SOPAR Company (Belgium).
The authors thank Dr. D. N. Sainsbury (Loctite, Ireland) for the generous gift of the monomers. The assistance of Mrs. Duhamel and Mr. Pels was greatly appreciated.

Toxicity of Polyalkylcyanoacrylate Nanoparticles II: Doxorubicin-Loaded Nanoparticles

P. COUVREUR *x, B. KANTE *, L. GRISLAIN *, M. ROLAND *, and P. SPEISER ‡

Received May 21, 1981, from the * *Laboratoire de Pharmacie Galénique, Université Catholique de Louvain, 1200 Bruxelles, Belgium* and the ‡ *School of Pharmacy, Federal Institute of Technology, Zürich, Switzerland.* Accepted for publication October 7, 1981.

Abstract □ The possibility of significantly reducing toxicity of an anticancer drug such as doxorubicin by fixing it on polyisobutylcyanoacrylate nanoparticles was studied. It was shown that, when the drug was adsorbed on nanoparticles, significant reduction of both mortality and weight loss of mice were recorded under various administration schedules. Furthermore, cardiotoxicity was decreased due to the poor uptake by the myocardium.

Keyphrases □ Polyalkylcyanoacrylate—toxicity of doxorubicin-loaded nanoparticles □ Doxorubicin—toxicity of polyalkylcyanoacrylate nanoparticles □ Toxicity—doxorubicin-loaded polyalkylcyanoacrylate nanoparticles □ Drug carrier systems—toxicity of doxorubicin-loaded polyalkylcyanoacrylate nanoparticles

The first toxicological data concerning the polyalkylcyanoacrylate nanoparticles did not demonstrate any distinct toxicity susceptible to hinder their use in human medicine (1). The aim of the present investigation was to reduce considerably the toxicity of an anticancer drug such as doxorubicin by fixing it on nanoparticles. The idea of using doxorubicin in association with a macromolecular carrier such as DNA, to minimize the detrimental effect of this anticancer drug on normal cells, has been previously investigated (2, 3).

Doxorubicin, an anthracycline antibiotic, has produced encouraging results in the treatment of neoplastic diseases (4). However, it is toxic, with its most severe side effects involving the heart (acute and chronic cardiomyopathy), bone marrow, and intestine (5, 6). For these reasons, and because doxorubicin is highly adsorbed on nanoparticles, this cytostatic drug was chosen as the experimental model.

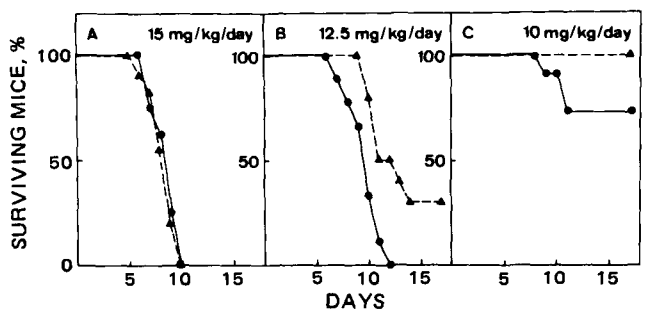

Figure 1—*Percent of surviving mice after intravenous administration on 3 consecutive days of various doses of free (●) and nanoparticle-bound doxorubicin (▲).*

EXPERIMENTAL

Polyalkylcyanoacrylate Nanoparticle Preparation—After dissolution of doxorubicin[1] (10 mg) in 10 ml of aqueous solution containing 100 mg of a polysaccharide[2], 50 mg of citric acid, and 1 mg of calcium chloride, 100 μl of isobutylcyanoacrylate monomer was dispersed under mechanical stirring.

After polymerization (3 hr), the resulting milky suspension was brought to isotonicity with 72 mg of sodium chloride. The size of the particles obtained was then estimated by measuring light scattering, arising from a laser source[3].

Measurement of Doxorubicin Linked to Nanoparticles—A 10-ml nanoparticle suspension was centrifuged[4] at 20,000 rpm for 1 hr. Sediment was then separated and dissolved in 10 ml of dioxane containing 20% water.

The content of doxorubicin was determined in both supernate and sediment by fluorimetric[5] dosage. For this purpose, 200-μl samples were diluted to 10 ml by water (for the supernate) or by dioxane (for the sediment) and measurements were performed using reference solutions of doxorubicin.

[1] Adriablastina, Montedison Farmaceutica Benelux, Bruxelles, Belgium.
[2] Dextran 70, Fison Laboratories, S.K.-RIT, Belgium.
[3] Nano-Sizer, Coulter Electronics, Harpenden, England.
[4] Beckman Centrifuge, model J-21C, Beckman Instruments, Palo Alto, Calif.
[5] Vitatron Fluorimeter, type U.F.D., Vitatron, Holland.

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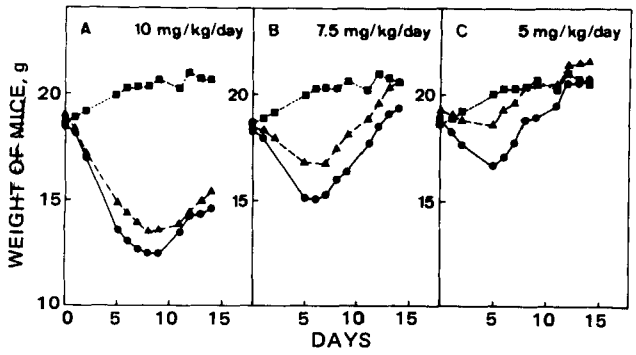

Figure 2—*Comparative loss of weight of the mice after intravenous administration on 3 consecutive days of various doses of free (●) and nanoparticle-bound doxorubicin (▲); (■) represents the weight of control mice.*

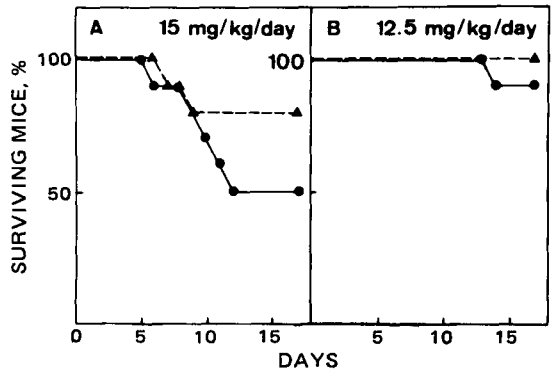

Figure 3—*Percent of surviving mice after intravenous administration on 2 consecutive days of various doses of free (●) and nanoparticle-bound doxorubicin (▲).*

Toxicity Studies—Toxicity of free and nanoparticle bound doxorubicin was determined using female mice[6]. Bound and free drug were given intravenously using various dosages for 5, 3, or 2 consecutive days of administration. During 5 consecutive days, 3, 4, 5, 6, and 8 mg/kg were given; 5, 7.5, 10, 12.5, and 15 mg/kg were given during 3 consecutive days; and 12.5 and 15 mg/kg during 2 consecutive days, respectively.

If the density of the polymer was assumed to be one, the cumulative dose of injected nanoparticles varied between 150 and 450 mg/kg of body weight.

An average of at least 10 mice were used per drug form in each dose series. The lethality was recorded 30 days after administration of the drug, and the weight of the mice was controlled daily.

Tape Sectioning Technique—After ether anesthesia, mice were killed by immersion in liquid nitrogen and embedded in a solution of carboxymethylcellulose. Sagittal sections measuring between 15 and 40

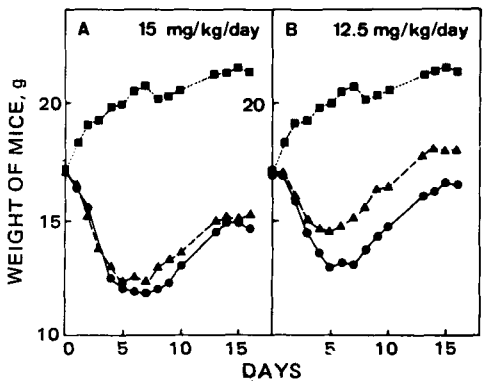

Figure 4—*Comparative loss of weight of the mice after intravenous administration on 2 consecutive days of various doses of free (●) and nanoparticle-bound doxorubicin (▲); (■) represents the weight of control mice.*

[6] NMRI mice, Animal House K.U.L., Heverlee, Belgium.

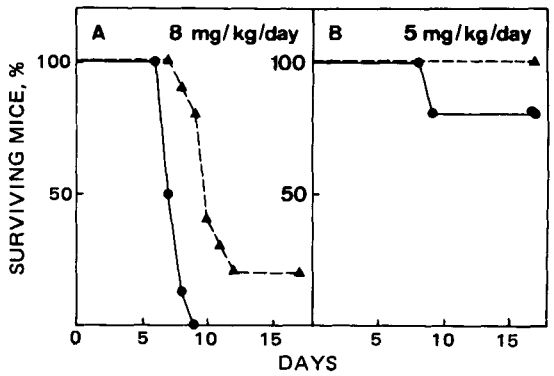

Figure 5—*Percent of surviving mice after intravenous administration on 5 consecutive days of various doses of free (●) and nanoparticle-bound doxorubicin (▲).*

μm were cut through the animals according to a previously described technique of tape-sectioning (7).

Fluorescence Technique—After drying, the sections were examined with an optic microscope[7] under UV light. A yellow filter[8] was used for the examination of doxorubicin fluorescence. Registration on film was taken on a daylight color film[9]. The exposure times were automatically determined using a photoelectric cell incorporated in the camera[10].

RESULTS AND DISCUSSION

The fluorimetric dosage showed that 94% of the doxorubicin employed was fixed by the nanoparticles. That corresponded to 94 mg of drug/g of polymer (confirmed by preliminary, unpublished data obtained by HPLC dosage).

Figure 1 shows the mortality, 15 days after injection, of either free or bound doxorubicin at doses of 15, 12.5, and 10 mg/kg/day. No mortality was recorded when free or bound doxorubicin was given at a dose less than or equal to 7.5 mg/kg/day. Except for a dose of 15 mg/kg/day, which appeared highly toxic in both forms tested (confirming that doxorubicin fixed to nanoparticles remained active), the mortality noted for nanoparticle-treated groups was always less than that recorded for the free drug injected groups. For example, after 12 days at the 12.5-mg/kg/day dose, a 100% mortality rate was observed in the free doxorubicin group, as compared to the 50% mortality rate in the nanoparticle-injected group (Fig. 1B).

These results were completed by controlling the weight of the treated animals (Fig. 2). At the doses of 10, 7.5, and 5 mg/kg/day, the loss of weight in the free doxorubicin group was significantly higher than that noted in the nanoparticles group ($p \geq 0.975$). At the dose of 5 mg/kg/day, the weight loss in the free doxorubicin group was twofold higher than for the groups injected with the bound drug (Fig. 2A). The mortality observed

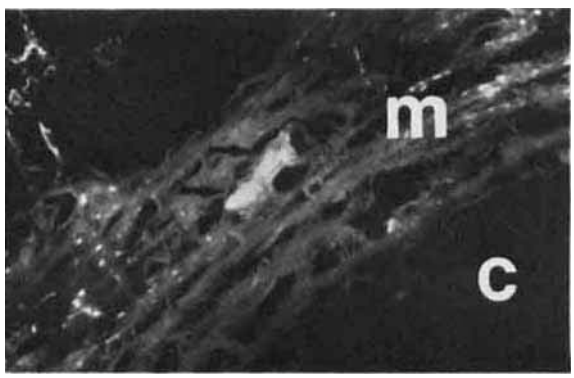

Figure 6—*Detail of a whole body fluorogram showing the heart of a mouse sacrificed 3 hr after the last of three injections of 10 mg/kg of free doxorubicin. There is a high accumulation of doxorubicin in the muscular fibers of the heart (→) (m = muscular myocardic fibers; c = myocardic cavity).*

[7] Fluorescent Microscope, Model Dialux 20, Leitz, Wetzlar, Germany.
[8] I_2 513-418, Leitz, Wetzlar, Germany.
[9] Kodak 400 ASA, Brussel, Belgium.
[10] Leitz Vario Orthomat System, Leitz, Wetzlar, Germany.

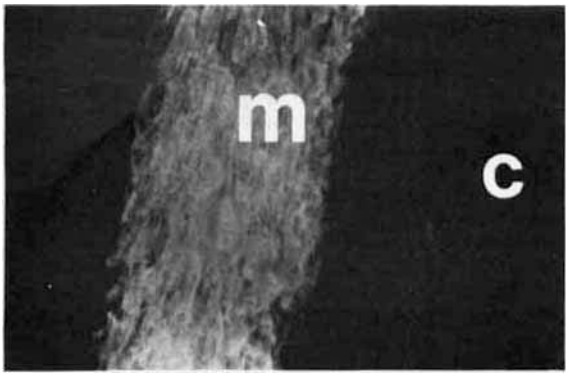

Figure 7—*Whole body fluorogram showing the heart of a mouse sacrificed 3 hr after the last of three injections of 10 mg/kg of doxorubicin bound to nanoparticles. In comparison with Fig. 6, no fluorescence appears in the myocardium (m = muscular myocardic fibers; c = myocardic cavity).*

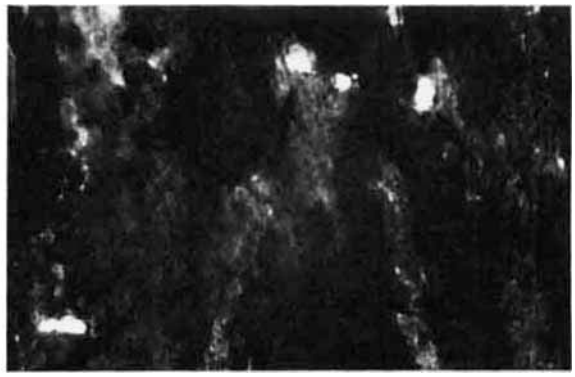

Figure 8—*Detail of whole body fluorogram of a mouse sacrificed 3 hr after the last of three injections of 10 mg/kg of doxorubicin bound to nanoparticles. Inversely to Fig. 6, fluorescence is retained in the lung (→).*

was primarily related to the intestinal toxicity of doxorubicin; the same mechanism was also responsible for the observed weight loss.

Therefore, at a dose of 10 mg/kg/day, the weight difference between mice treated with free doxorubicin and those treated with the bound drug was likely to be underestimated (Fig. 2C). No dead mice were recorded in the latter group, whereas significant mortality was noted in the former group. Dead mice were deleted from the weight loss charts.

When the administration schedules of both free and bound doxorubicin were limited to two successive injections of 12.5 and 15 mg/kg/day similar results were obtained. When the drug was linked to nanoparticles, significant differences in both mortality (Fig. 3) and weight loss (Fig. 4) were again observed. However, at the dose of 15 mg/kg/day, the weight loss difference (Fig. 3B) between the two experimental groups was underestimated for the same reasons mentioned previously.

Figure 5 shows the variance in time of the survival rate of the animals after five consecutive injections of 5 and 8 mg/kg/day of both free and bound doxorubicin, respectively. Three hours after the last of three daily injections of 10 mg/kg of body weight of both free and bound doxorubicin, mice were killed and sectioned. After drying, the sections were examined under a fluorescent microscope to determine which organs presented an orange-red fluorescence due to the presence of doxorubicin. Because a side effect of doxorubicin involved the heart, the investigations concentrated on the cardiac tissue. Figure 6 shows a high amount of cytostatic behavior in the cardiac muscle when free drug was injected into the animal. By contrast, no fluorescence was found in the myocardium after administration of the same dose of doxorubicin bound to the nanoparticles (Fig. 7). Examination of other organs confirmed the high uptake capacity of the nanoparticle adsorbed doxorubicin by the lung (Fig. 8) and liver, while the free drug seemed to show less fluorescence in these tissues. It appears that nanoparticle bound doxorubicin has a preferential distribution for organs such as the liver and lung, with significantly less distribution in the heart.

CONCLUSIONS

These results show a significant decrease of doxorubicin's toxicity when fixed to nanoparticles. This decrease corresponds to both a diminution in weight loss and to a higher survival rate in mice after administration of bound doxorubicin for various doses and administration schedules. In addition, the absence of doxorubicin fluorescence in the cardiac muscle when the drug was adsorbed on the nanoparticles can be of interest, considering the important cardiotoxicity of the drug. However, these observations were made on a limited number of animals and should be completed with a larger number of specimens injected under various administration schedules, dose, and time before sacrifice. It should be noted that the use of polyalkylcyanoacrylate nanoparticles as the drug carrier can reduce considerably the inherent toxicity and side effects of a cytotoxic drug and could be useful in cancer chemotherapy.

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ACKNOWLEDGMENTS

This work was supported by S. A. SOPAR Company and by IRSIA (Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture).

The authors thank Professor Trouet, Dr. Tulkens and Dr. Deprez-De Campeneere for helpful discussions and valuable comments. The authors also thank Dr. D. N. Sainsbury (Loctite, Ireland) for the generous gift of the monomers. The excellent technical assistance of Mr. Bulckens and Mrs. Duhamel was greatly appreciated.