Reduction of Doxorubicin (Adriamycin) Bone Marrow Toxicity

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Abstract □ Doxorubicin (adriamycin), an antineoplastic antibiotic, is a potent suppressant of bone marrow. Previous studies on doxorubicin disposition indicated that its diversion from bone marrow in the first few minutes after administration should result in a marked decrease in total exposure to the drug (concentration × time) with a concomitant reduction in concentration–time-dependent toxicity. To test this hypothesis, the descending aorta of rabbits was occluded just proximal to the iliac bifurcation for 30 min to deprive bone marrow of blood flow. Both these rabbits and the control rabbits were given 5 mg/kg of doxorubicin intravenously, and the total white cell count in peripheral blood was monitored periodically for 15 days. The decrease in toxicity produced by the occlusion was quite evident by comparison of white cell counts and deaths in all groups. A possible mechanism of this effect was shown to be a decreased doxorubicin exposure of bone marrow tissue in the occluded animals as judged by relative doxorubicin concentration–time curves in rabbits with and without the aortic occlusion.

Keyphrases □ Doxorubicin (adriamycin)—reduction of bone marrow toxicity, aortic occlusion, rabbits □ Adriamycin—reduction of bone marrow toxicity, aortic occlusion, rabbits □ Toxicity, bone marrow—doxorubicin, reduction by aortic occlusion technique, rabbits □ Antineoplastic agents—reduction of doxorubicin bone marrow toxicity, aortic occlusion, rabbits

Doxorubicin (adriamycin) is a valuable chemotherapeutic drug in the treatment of solid neoplasms (1, 2). The serious toxicity of doxorubicin includes depression of proliferative elements of bone marrow as well as a mucositis and a cumulative dose-dependent delayed myocardiopathy (1–3). In short-term therapy with doxorubicin, the bone marrow suppression with the resultant leukopenia is the major dose-limiting toxicity. Recent pharmacokinetic investigations in these laboratories[1] led to the conclusion that the bone marrow toxicity of doxorubicin may be decreased by a clinically feasible method, albeit not yet proven acceptable or desirable.

This paper concerns the reduction of doxorubicin bone marrow toxicity and its probable mechanism by a temporary restriction of bone marrow blood flow.

EXPERIMENTAL

Female New Zealand white rabbits, 2.0–3.0 kg, were anesthetized with pentobarbital sodium USP[2], 25 mg/kg iv. A lower midline laparotomy was performed, and the aorta was occluded just proximal to the iliac bifurcation with a bulldog clamp. Doxorubicin hydrochloride[3] was administered in a dose of 5 mg/kg into the marginal ear vein immediately after the aorta was occluded. The clamp was removed after 30 min, and the incision was closed. Three hundred thousand units of penicillin G benzathine suspension[4] was administered intramuscularly to prevent possible infection due to surgery.

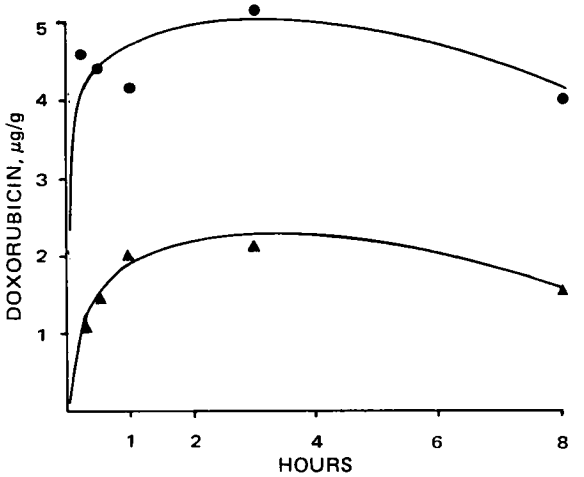

Figure 1—*Femur bone marrow concentrations after doxorubicin given intravenously, 3 mg/kg, in rabbits with (▲) and without (●) aortic occlusion. Each point represents the mean of duplicate determinations in one rabbit.*

Two control groups of animals were treated in the following manner. One group underwent surgery as described with aortic occlusion and penicillin therapy, but doxorubicin was not administered. The second group underwent the surgery, identical handling, trauma, and penicillin therapy as before and received 5 mg/kg of doxorubicin but the aorta was not occluded.

Blood samples were taken from all rabbits prior to and periodically after surgery, and the total white blood cell count was monitored until death or up to 15 days after surgery. The blood counts were performed[5] on a blind basis.

For studies of doxorubicin uptake into bone marrow, the aorta of each of five rabbits was occluded for 30 min as already described and doxorubicin was given intravenously into the marginal ear vein in a dose of 3 mg/kg immediately after the clamp was applied. Rabbits were killed at 15, 30, 60, 180, and 480 min after injection; bone marrow was removed from the femur and analyzed for doxorubicin levels according to a previously published method (4). Five control rabbits were treated similarly, except the aorta was not occluded.

RESULTS AND DISCUSSION

The effect of temporary reduction of blood flow to a major blood cell-producing tissue on doxorubicin toxicity was tested. Occlusion of all blood flow through the aorta at the level of the iliac bifurcation for 30 min was performed to accomplish a reduction or a near complete halt in blood flow to the pelvis and hindlimbs.

A group of five rabbits received 5 mg/kg of doxorubicin immediately after the aorta was occluded. For controls, three rabbits underwent aortic occlusion without administration of doxorubicin and another five rabbits that had no aortic occlusion received 5 mg/kg of doxorubicin. The total white cell count was monitored in all animals (Table I).

Aortic occlusion with no doxorubicin administration had no significant effect on the white blood cell count of the rabbits. When doxorubicin was administered in a dose of 5 mg/kg with no aortic occlusion, four out of the five rabbits died. As can be seen from the

[1] *Cancer Chemother. Rep.*, in press.
[2] Obtained from Western Medical Supply, Inc., Arcadia, Calif.
[3] Supplied by Dr. Harry Wood, Drug Development Branch, National Cancer Institute, Bethesda, Md.
[4] Bicillin L-A, Wyeth Laboratories, Philadelphia, Pa.
[5] By the clinical laboratory of the University of Southern California Medical School Vivarium.

Table I—Total White Cell Counts in Rabbits

Day Post-surgery	Rabbit Number 1	2	3	4	5	6	7	8	9	10	11	12	13
	5 mg/kg Doxorubicin with Aortic Occlusion					5 mg/kg Doxorubicin without Aortic Occlusion					No Doxorubicin with Aortic Occlusion		
0	7,800	7,800	8,400	10,800	11,500	10,100	8,900	9,200	10,500	9,300	9,600	8,400	8,300
1	11,400	7,800	8,400	10,700	11,300	7,800	7,400	8,100	9,600	12,500	—	—	—
2	6,400	5,500	7,700	10,300	11,300	4,600	3,600	6,700	6,600	5,200	11,900	8,500	7,700
3	6,300	4,800	6,800	9,900	9,500	2,700	2,900	3,300	3,200	3,000	—	—	—
4	5,200	4,600	4,500	9,500	7,800	1,600	2,100	2,200	1,800	2,200	—	—	—
5	4,900	3,800	2,600	8,800	6,000	500	900	3,800	Died	1,200	8,000	7,900	6,800
6	5,200	4,200	3,200	8,200	6,200	Died	Died	3,900	—	Died	—	—	—
7	5,200	5,400	4,200	8,200	6,200	—	—	4,400	—	—	—	—	—
8	4,800	5,600	3,800	7,800	6,400	—	—	3,400	—	—	9,400	6,600	8,400
9	5,400	5,700	5,400	8,100	6,600	—	—	4,800	—	—	—	—	—
10	6,200	6,400	6,200	8,200	6,600	—	—	5,600	—	—	—	—	—
11	—	—	6,000	8,000	6,600	—	—	—	—	—	8,800	7,000	9,200
12	6,800	6,900	6,600	8,300	7,900	—	—	6,500	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—	—	—	—
15	7,600	7,400	7,800	9,200	9,200	—	—	6,800	—	—	8,900	7,500	9,200

total white cell count, death was probably due to severe bone marrow depression. The fifth rabbit experienced a moderate depression in white cell count. The rabbits that underwent aortic occlusion and received 5 mg/kg of doxorubicin had only mild or moderate bone marrow depression. None of these animals died.

The rabbits were all in excellent health when experiments were commenced. A transient body weight loss of 100–200 g was observed after surgery. No other changes in their clinical condition were observed except for the sudden death of four out of the five rabbits without aortic occlusion. Although the exact cause of death was not determined, a fatal septicemia was strongly suspected because of the leukopenic pattern noted before death.

The results of experiments to determine the effect of 30 min of occlusion on femur marrow uptake of doxorubicin are shown in Fig. 1. Data from rabbits without occlusion that received 3 mg/kg of doxorubicin are also shown. The rabbits with occlusion had an area under the concentration–time curve up to 8 hr of 923 μg min/ml compared to 2235 μg min/ml for those without occlusion. Both areas correlate to the respective smooth curves that were estimated by sight.

Doxorubicin uptake into tissues occurs rapidly (5–8), and the binding to tissues is extremely tenacious[1]. The pelvic and hindlimb bone marrow which normally receive blood flow distal to the occlusion do not receive a normal supply of doxorubicin for 30 min after injection because of blood flow reduction. During this time the doxorubicin becomes "fixed" to other tissues.

When the clamp is released and more normal blood flow resumes, the doxorubicin plasma concentration is very low. Hence, the marrow concentrations of doxorubicin do not reach values encountered without occlusion up to 8 hr after drug administration. The exposure of the marrow based on relative concentration–time areas is only 41% that without occlusion. There is little reason to expect this relationship to change significantly after 8 hr since the marrow concentrations are well past their maximums and the drug should decay from this tissue at similar rates in both cases.

Close examination of marrow levels in the occluded animals indicates that blood flow to the femur marrow was not completely halted during occlusion. The 15- and 30-min points had significant doxorubicin concentrations. There would not, of course, be any doxorubicin in this tissue at these times if flow were stopped completely. Perhaps the clamp on the aorta was not well placed, thus allowing some aortic flow to proceed distally to the femur marrow. It is also possible that some collateral blood vessels supply the femur marrow and receive flow from a point higher than the clamp was placed.

The dose levels selected were based on experiments in this laboratory. A 3-mg/kg doxorubicin dose in rabbits may be given every 7–10 days generally without fatality. The white cell counts are variably depressed at this dose. This level and frequency of doxorubicin would be a likely treatment regimen in rabbits. Therefore, this dose was selected for the uptake studies.

A single 5-mg/kg dose produces a much more profound and less variable drop in white cell count than does a 3-mg/kg dose. For this reason, a 5-mg/kg dose was used for studying the effect of aortic occlusion on white cell count.

Occlusion of the abdominal aorta in humans was used previously to increase tumor exposure to nitrogen mustard (mechlorethamine hydrochloride) (9, 10). The bone marrow of the lower part of the body of rats and rabbits was demonstrated to be protected by temporary occlusions of the abdominal aorta after nitrogen mustard administration (11). Nitrogen mustard has a very short $T_{1/2}$ in the body since it is so reactive. A significant proportion of nitrogen mustard was irreversibly eliminated from the blood during the period the occlusion was applied. Hence, the tissues distal to the occlusion receive protection.

Temporary occlusion of the abdominal aorta is used to control hemorrhage in emergency treatment or during elective aortic surgery. The occlusion may be accomplished by directly clamping the aorta after entering the abdominal cavity or by using external abdominal tourniquets or compression. The use of catheters equipped with inflatable sleeves or balloons for obstructing the aorta was described and evaluated (12–14). These catheters may be introduced by peripheral arterial catheterization.

The metabolic and hemodynamic sequel to abdominal aortic occlusion and declamping also was described (15–17). The main problems appear to be the frequent occurrence of acidosis and severe hypotension and occasionally ventricular fibrillation or cardiac arrest after release of the aortic blood flow. The control and prevention of the shock may be accomplished by fluid therapy (17, 18) or use of vasopressor drugs regionally (19).

Clearly, the results of the total white cell count and doxorubicin bone marrow uptake experiments reported here demonstrate that a significant reduction in the drug exposure of bone marrow and in leukopenic response after doxorubicin administration may be accomplished by only 30 min of aortic occlusion at the iliac bifurcation. These results may have clinical significance in the use of doxorubicin since leukopenia often delays doxorubicin therapy. Also, combination therapy with other marrow toxic drugs or radiation may be more flexible using this approach.

A more complete study should be performed to determine the clinical feasibility, safety, and mechanism of the reduction of doxorubicin toxicity by temporary blood flow restriction.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 24, 1974, from the *Schools of Medicine and Pharmacy, University of Southern California, Los Angeles, CA 90033*

Accepted for publication January 31, 1975.

Supported by Contract NO1-CM-23241 from the National Cancer Institute, U.S. Public Health Service, Bethesda, MD 20014

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COMMUNICATIONS

Relative Bioavailability of Cyclobarbital Calcium from Aqueous Solution Compared to Tablets

Keyphrases □ Cyclobarbital calcium—relative bioavailability from aqueous solution and tablets □ Bioavailability—cyclobarbital calcium, aqueous solution and tablets

To the Editor:

For biopharmaceutical studies, the aqueous drug solution is generally recommended as the reference dosage form if no comparison can be made with intravenous data (1). The highest relative bioavailability of a drug is expected from the solution since dissolution of the drug in the GI tract seems to be bypassed. The present report on cyclobarbital calcium shows that this supposition is not always true.

The pharmacokinetics and relative bioavailability of cyclobarbital calcium were studied in six healthy volunteers (21–26 years of age). Three preparations were used:

1. Tablets[1], containing 200 mg of cyclobarbital calcium, 60 mg of potato starch, 27 mg of lactose, 7 mg of talc, 3 mg of magnesium stearate, and gelatin *q.s.*
2. Tablets[2], containing 200 mg of cyclobarbital calcium.
3. Aqueous solution of cyclobarbital calcium (300 mg/150 ml), which was prepared within 15 min prior to administration.

After fasting overnight, at 9 am the volunteers were given 300 mg of cyclobarbital calcium incorporated in one of the preparations together with 150 ml of water. Additional water was not supplied when cyclobarbital calcium was given in solution. The volunteers remained in an upright position for 15 min after intake of the drug and then rested supine for at least 3.5 hr.

Blood samples were taken at 0.15 or 0.33, 0.66, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 8.0, 12, 24, and 32 hr following drug administration. Each volunteer received the three preparations in a random sequence, with an interval of at least 1 week. Cyclobarbital plasma concentrations were determined after a single extraction step by GC with nitrogen selective detection, following on-column methylation with trimethylanilinium hydroxide. The use of a nitrogen detector for the sensitive assay of barbiturates in biological fluids was described previously for hexobarbital (3).

In Table I the cyclobarbital plasma peak concentrations and times (mean values) are given, as well as the bioavailability relative to Preparation 1. Relative bioavailability was estimated for each individual by comparing the areas under the experimental plasma concentration curves and correcting for the undetermined area to infinity. The elimination of cyclobarbital followed first-order kinetics with an average half-life of 11.6 hr (range of 8–17 hr). The half-lives were fairly constant within the individuals during the three trials.

[1] Prepared by the Dutch Pharmacist's Laboratory, The Hague (2).
[2] Commercially obtained as Phanodorm, Merck/Bayer.