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Does Adriamycin Undergo an Enterohepatic Circulation? (41304)

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Abstract. To determine whether adriamycin (ADR) and/or its metabolites undergo an enterohepatic circulation following an iv administration, anesthetized rats with biliary and urinary fistulae were intraduodenally perfused with bile flowing from bile duct-cannulated rats which had been injected iv with 20 mg/kg [^{14}C]ADR. During a 6-hr duodenal perfusion period, 27.3% of the ADR injected to bile donor rats was recovered as total radioactivity in the intestinal perfusate of bile recipient rats and approximately 1.6 and 0.18% were excreted in their bile and urine, respectively. The intestinal tissue of bile recipient rats contained concentrations of the total drug equivalents ranging from 1.98 μg/g in the initial portions of the duodenum to 12.21 μg/g in the distal parts of the ileum. Minimal levels were observed in the heart, lung, kidney, and liver (0.25–0.86 μg/g tissue). In order to estimate the amount of the total [^{14}C]ADR equivalents perfused into the small intestine of the bile recipient rats, [^{14}C]ADR 20 (mg/kg) was injected iv to a separate group of anesthetized rats in which bile was continuously collected for a 6-hr period. In these experiments, total radioactivity excreted through the biliary route accounted for 30.6% of the injected dose. These combined results indicate that approximately 10% of the total [^{14}C]ADR equivalents eliminated in bile over a 6-hr period are reabsorbed from the lumen of the small intestine of the anesthetized rat, an amount which roughly represents 3% of the injected ADR.

Adriamycin (NSC 123127, ADR) is an antineoplastic antibiotic effective against a variety of solid tumors and hematologic malignancies (1, 2). Despite the abundant literature on ADR's pharmacokinetics, few studies have examined the intestinal absorption of the drug. Arena *et al.* (3) reported no fluorescence products in the plasma and bile of rabbits treated orally with ADR (20 mg/kg) and more recently, Harris and Gross (4) demonstrated less than 5% absorption of the drug by a segment of the rabbit small intestine with an intact blood supply.

Because of the wide clinical use of ADR and because of its extensive biliary excretion (5–8), present information on the intestinal absorption of the drug appears incomplete. We felt, therefore, that the possibility of an enterohepatic circulation of ADR and/or metabolites should be explored further. Accordingly, in the present report we studied the absorption of ADR from the small intestine of anesthetized rats. In order to closely reproduce the *in vivo* conditions under which the drug and/or metabolites may undergo an enterohepatic circulation, the intestinal absorption of the total ADR equivalents was examined in rats receiving intraduodenally bile from bile duct-cannulated donor rats which had been injected iv with 20 mg/kg [^{14}C]ADR. The results have demonstrated that approximately 10% of the total drug equivalents eliminated through the bile over a 6-hr period are reabsorbed from the lumen of the small intestine, an amount which roughly represents 3% of the intravenously administered ADR.

Methods. Albino male Sprague–Dawley rats weighing 280–320 g (Taconic Farms, Inc., Germantown, N.Y.) were used in these studies. The animals were housed in a temperature controlled room (22°C) with alternating 12-hr light/dark cycles and maintained on Purina lab chow and water *ad libitum* for at least a week before being

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used. Experimental rats designated to receive ADR intraduodenally were fasted for 48 hr prior to being studied.

The intestinal absorption of ADR and/or metabolites was studied in anesthetized rats receiving intraduodenally bile from donor rats injected iv with 20 mg/kg [^{14}C]ADR. Figure 1 illustrates the experimental model used in these studies. Both bile donor (A) and bile recipient (B) rats were anesthetized with pentobarbital sodium (50 mg/kg, ip) and their trachea and external branch of the left jugular vein were cannulated with PE 240 and PE 50 cannulae, respectively. In both rats A and B the abdomen was opened and the bile duct (PE 50) and the urinary bladder (PE 240) were cannulated following ligation of the penis. The bladder cannula was progressively reduced to an internal diameter of 0.58 mm (PE 50) to avoid lag in urine flow due to excessive cannula dead space. Subsequently, the bile duct cannula of rat A was inserted into the distal end of the bile duct of rat B, passed through the

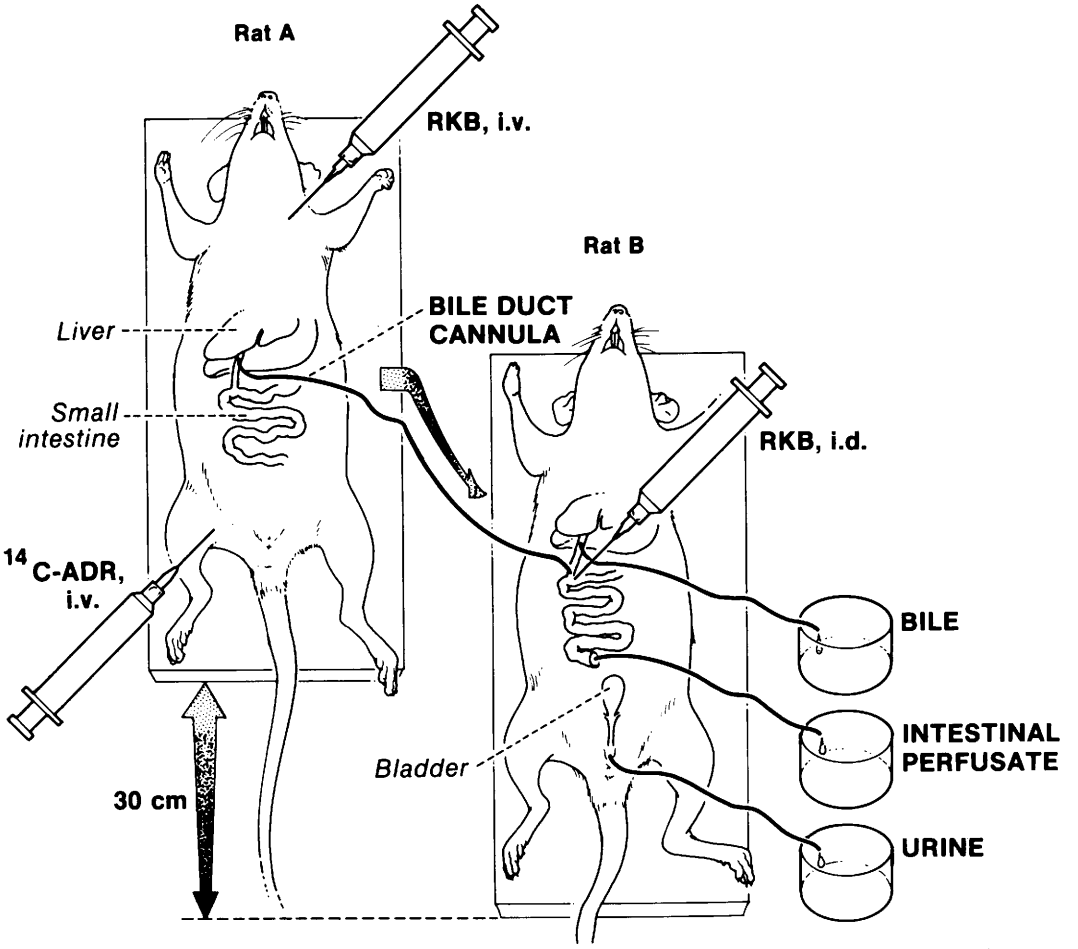

FIG. 1. Schematic representation of the experimental rat model used for studying the enterohepatic circulation of ADR. ADR (20 mg/kg, containing 3 μCi/kg [^{14}C]ADR) was injected iv to rat A through one femoral vein. The bile duct of rat A was cannulated (PE 50) and the cannula was inserted into the distal end of the bile duct of rat B and passed through the sphincter of Oddi until the tip reached the duodenal lumen. Bile, urine, and intestinal perfusate were collected from rat B for a 6-hr period following the ADR injection. Rat A was placed at a height of approximately 30 cm from rat B to allow free flowing of bile into the lumen of the small intestine of rat B. Ringer–Krebs bicarbonate solution (RKB) was infused intrajugularly to rat A (2.5 ml/hr) and intraduodenally (id) to rat B (6 ml/hr). See Methods for more details.

sphincter of Oddi and tied *in situ* as the tip could be felt in the lumen of the duodenal curvature. An additional PE 50 cannula was inserted into the duodenal lumen of rat B through a small incision of the duodenal wall. The cannula was tied and used for fluid perfusion. The pyloric sphincter was then ligated to prevent possible reflux of the duodenal content into the stomach. Finally, the end portion of the ileum next to the ileocecal valve was transected and cannulated (PE 340) to collect perfusate. After the abdomen was sutured in both animals, rat A was placed at a height of approximately 30 cm from rat B to allow free flowing of bile into the lumen of the small intestine of rat B. Thereafter, collection of bile, urine, and intestinal perfusate in rat B was resumed. Bile and urine were collected in preweighed vials over 60 min intervals and volumes were determined by difference between full and empty weight of vials. Perfusate from the ileal cannula was collected in four samples of approximately 8 ml each. To avoid photooxidative reactions of ADR and fluorescent metabolites (9), all cannulae and respective vials were thoroughly covered with aluminum foil. Body temperature of both rats A and B was monitored continuously with a rectal probe and maintained at 37.5°C throughout the study by means of infrared heating lamps connected to an automatic temperature controller device (Yellow Springs Instruments, Yellow Springs, Ohio). Immediately after surgery, rat A was given a constant infusion of Ringer–Krebs bicarbonate solution (2.5 ml/hr) through the jugular vein cannula to replace water and electrolyte losses with bile and urine. In rat B, perfusion of the duodenum was also initiated with Ringer–Krebs bicarbonate solution at a rate of 6 ml/hr.

Following a 30-min equilibration period, allowed to establish basal rates of bile and urine flow, [^{14}C]ADR was injected at 20 mg/kg to rat A through a femoral vein. Six hours later, rat A was sacrificed, the bile duct cannula cut, and the portion of the cannula connected to the bile duct of rat B was clamped. At the same time, the duodenal perfusion rate of rat B was increased to 12 ml/hr to insure that all rat A's bile had been flushed thoroughly into the recipient rat intestine, and experiment B was continued for an additional 60-min period. At the end of the 7-hr period, rat B was sacrificed, its liver, heart, kidneys, spleen, and lung were removed, and their weights were determined. At the same time, the small intestine was also removed after each end was ligated and the perfusate collected. The small intestine was then cut in four parts of similar length and their weights were determined.

To establish the amount of the total [^{14}C]ADR equivalents perfused into the duodenum of rat B, the biliary excretion of the total drug equivalents was examined in a separate group of bile duct-cannulated rats (control experiments) where bile flow was collected for 6 hr following administration of [^{14}C]ADR at 20 mg/kg through a femoral vein. These control animals were treated exactly as the bile donor rats and received intrajugularly Ringer–Krebs bicarbonate solution at the rate of 2.5 ml/hr throughout the entire bile collection period.

ADR and [^{14}C]ADR (labeled at the C-9 side chain) were obtained from the Division of Cancer Treatment, National Cancer Institute, NIH. ADR was dissolved in distilled water (15 mg/ml) since it is not readily soluble in saline at this concentration. [^{14}C]ADR was purified chromatographically as previously described (8) and added in traces to the nonradioactive ADR solution on the day of the experiment. An average of 3 μCi/kg was used in each study. ADR was determined radioisotopically as total drug equivalents in fluids and tissues as previously described (8).

To ascertain that the radioactivity recovered in bile recipient rats was associated with the anthracycline moiety, selective bile samples (those obtained during the 3-hr collection period when excretion of radioactivity was maximal) were analyzed by thin-layer chromatography as previously described (10). In these samples most of the radioactivity (50–60%) was recovered in an area with an R_f of 0.57–0.63 which corresponds to that of the parent molecule (10). The remainder (20–30%) could be accounted for by polar anthracycline products as their R_f's ranged from 0.10 to 0.50. This

distribution pattern of ADR and fluorescent metabolites is entirely in agreement with that found previously in the bile of ADR-treated rats (10).

Results and Discussion. While carrying out preliminary studies we found two conditions were essential to obtain reproducible results in the present experimental model. First, bile recipient rats had to be fasted 48 hr prior to being used and second, the rate of perfusion of the duodenal lumen had to be at least 5–6 ml/hr. Under these conditions, the perfusate flowed continuously from the ileum cannula (after an initial delay) and no swelling of the intestinal lumen was observed. Moreover, no evidence of mechanically induced biliary obstruction was obtained in the bile donor rats. Thus, in the control group of bile duct-cannulated rats, biliary excretion of the total [^{14}C]ADR equivalents accounted for 30.6% of the injected dose (20 mg/kg) at the end of the 6-hr collection period (Table II). This is quite comparable to the total radioactivity recovered (approximately 30%) from fluids and tissues of bile recipient rats at the end of the perfusion experiment.

The present results demonstrate that ADR and/or metabolites are not extensively reabsorbed from the lumen of the small intestine of the anesthetized rat, after being eliminated through the biliary route. Thus, as illustrated in Table I, 27.3% of the dose of [^{14}C]ADR injected to bile donor rats was recovered as total drug equivalents in the perfusate of bile receiver rats. On the assumption that a mean value of 30.6% of the injected ADR was excreted in the bile of bile donor rats over the 6-hr period, we calculate that approximately 10% of the total [^{14}C]ADR equivalents eliminated in bile was reabsorbed. This indicates that only 3% of the intravenously injected drug underwent one enterohepatic circulation within 6 hr from its administration. If we then consider that approximately one-half of the reabsorbed radioactivity was reexcreted in bile over the 6-hr period (Table II), it can be estimated that less than 1.5% of the injected ADR returned to the systemic circulation as parent drug and/or metabolites.

The fate that this reabsorbed ADR and/or metabolites followed after escaping hepatic extraction, reflected entirely the distribution pattern already demonstrated for ADR following its iv administration to bile duct- and bladder-cannulated rats (10). A minimal fraction was reexcreted in urine (Table II) and the remainder was distributed in tissues (Table III). The spleen contained the high-

TABLE I. RECOVERY OF TOTAL [^{14}C]ADR EQUIVALENTS FROM THE INTESTINAL PERFUSATE OF RATS INTRADUODENALLY RECEIVING BILE FROM [^{14}C]ADR-TREATED RATS (20 mg/kg iv)

	ml	μg/ml	%[a]	%[b]
Perfused	54.2 ± 0.7[c]		30.6 ± 2.8	100
Perfusate 1[d]	7.9 ± 1.2	56.7 ± 9.4	8.3 ± 1.5	27.1 ± 4.2
Perfusate 2	8.4 ± 0.9	52.1 ± 8.7	8.1 ± 1.2	26.5 ± 3.5
Perfusate 3	7.2 ± 0.7	39.1 ± 5.5	5.2 ± 0.7	17.0 ± 2.4
Perfusate 4	6.6 ± 0.8	35.2 ± 4.8	4.3 ± 0.6	14.1 ± 1.8
Perfusate 5[e]	11.7 ± 0.9	6.9 ± 0.8	1.5 ± 0.3	4.9 ± 0.6
Total recovery	41.8 ± 2.1		27.3 ± 2.3	89.5 ± 7.6

Note. Values are means ± SD of five experiments.

[a] Recovery of total radioactivity when expressed as percentage of the dose of ADR injected to bile donor rats.

[b] Recovery of total radioactivity when expressed as percentage of the total [^{14}C]ADR equivalents entering the small intestine of bile recipient rats. The amount of total [^{14}C]ADR equivalents perfused through the small intestine was established by quantitating the biliary excretion of the total radioactivity in a separate group of bile duct-cannulated rats injected iv with 20 mg/kg [^{14}C]ADR (see Methods for details and Table II for the results).

[c] Total volume perfused (Ringer–Krebs plus estimated bile).

[d] Intestinal perfusate samples (1–4) collected during the perfusion period.

[e] Perfusate left in the intestine and collected at the end of the perfusion experiment, after the bile recipient rat was sacrificed.

TABLE II. BILIARY AND URINARY EXCRETION OF TOTAL [^{14}C]ADR EQUIVALENTS IN BILE RECIPIENT AND BILE DONOR RATS FOLLOWING AN iv ADMINISTRATION OF 20 mg/kg [^{14}C]ADR TO BILE DONOR RATS

Time (min)	Bile Recipients				Bile donors
	Bile		Urine		Bile
	%[a]	%[b]	%[a]	%[b]	%[c]
60	0.07 ± 0.03	0.38 ± 0.06	0.00	0.00	17.8 ± 1.9
120	0.21 ± 0.05	0.88 ± 0.11	0.00	0.01 ± 0.01	23.4 ± 2.3
180	0.61 ± 0.09	2.21 ± 0.33	0.02 ± 0.01	0.06 ± 0.02	27.5 ± 2.5
240	0.89 ± 0.12	3.10 ± 0.43	0.05 ± 0.01	0.19 ± 0.05	28.8 ± 2.6
300	1.15 ± 0.18	3.86 ± 0.48	0.11 ± 0.03	0.37 ± 0.07	29.8 ± 2.7
360	1.39 ± 0.21	4.54 ± 0.55	0.15 ± 0.04	0.49 ± 0.09	30.6 ± 2.8
420[d]	1.60 ± 0.29	5.22 ± 0.67	0.18 ± 0.04	0.59 ± 0.11	

Note. Values are means ± SD of five experiments.

[a] Cumulative percentage of the dose injected to bile donor rats.

[b] Cumulative percentage of the total [^{14}C]ADR equivalents perfused through the small intestine of bile recipient rats (see Table I and Methods for details).

[c] Biliary excretion of the total radioactivity in a separate group of bile duct-cannulated rats injected iv with 20 mg/kg [^{14}C]ADR. The mean total radioactivity eliminated in the bile of these rats is assumed to be the same as that perfused through the small intestine of bile recipient rats.

[d] During the seventh hour of intestinal perfusion, bile recipient rats received intraduodenally Ringer–Krebs bicarbonate solution only at a rate of 12 ml/hr (see Methods for details).

est levels of the total ADR equivalents. Liver, kidney, and lung showed concentrations similar to each other and approximately two times those seen in the heart.

The minimal intestinal reabsorption of the total [^{14}C]ADR equivalents observed in the present studies is, at present, difficult to interpret. Bachur (11) reported that ADR and its polar metabolites are transformed to aglycone products while passing through the intestinal tract. Although formation of these water-insoluble metabolites could explain the minimal enterohepatic circulation of the drug observed in the present studies, a low absorption of the parent molecule, which has amphipathic characteristics, has also been demonstrated from the lumen of a 25-cm-long segment of the rabbit small intestine (4), thus suggesting that aglycone formation may not be the primary cause for this low reabsorption of ADR.

Irrespective of the mechanism, however,

TABLE III. TISSUE LEVELS OF TOTAL [^{14}C]ADR EQUIVALENTS IN RATS RECEIVING INTRADUODENALLY BILE FROM [^{14}C]ADR-TREATED RATS

Tissue	Weight (g)	Concentration (μg/g)	%[a]	%[b]
Liver	9.39 ± 1.14	0.64 ± 0.10	0.10	0.40
Heart	0.79 ± 0.17	0.25 ± 0.06	0.00	0.01
Kidneys	2.18 ± 0.35	0.54 ± 0.09	0.02	0.07
Spleen	0.66 ± 0.11	0.86 ± 0.14	0.01	0.04
Lung	1.37 ± 0.16	0.49 ± 0.09	0.01	0.04
Small intestine 1[c]	1.45 ± 0.18	1.98 ± 0.23	0.06	0.20
Small intestine 2	1.09 ± 0.40	7.39 ± 0.86	0.16	0.55
Small intestine 3	1.32 ± 0.16	12.21 ± 1.42	0.30	1.09
Small intestine 4	1.56 ± 0.17	5.98 ± 0.17	0.18	0.63

[a] Values are percentages of the dose of ADR injected to bile donor rats.

[b] Values are percentages of the total [^{14}C]ADR equivalents perfused intraduodenally to bile recipient rats (see Tables I and II and Methods for details).

[c] Segments (1–4) of the small intestine were numbered in the sequence: duodenum–ileum.

the present results have demonstrated that ADR and/or metabolites, following excretion through the bile, are not extensively reabsorbed from the lumen of the small intestine of the anesthetized rat. This finding is in agreement with previous observations by Arena *et al.* (3) and Harris and Gross (4) in rabbits, and emphasizes the negligible contribution that the enterohepatic circulation of the drug has to its biological disposition in these species.

At present, the relevance of these animal findings to an enterohepatic circulation of the drug in man is not known. It has been reported that fecal excretion of the total radioactivity in patients injected iv with [^{14}C]ADR accounts for more than 40% of the administered dose over a 7-day period (12). This amount is quite comparable to the total ADR equivalents recovered over the same time period in the bile of a patient with an intradwelling T tube treated with ADR (6), thus suggesting that, in humans as well, ADR and/or metabolites are very poorly reabsorbed. In this case, the clinical effectiveness and/or toxicity of ADR could not be related to an enterohepatic circulation of the drug and/or metabolites.

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