Induction of Hepatic Veno-occlusive Disease in Dogs

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The authors attempted to induce hepatic veno-occlusive disease (VOD) in 64 dogs. Preparative treatments included combinations of total-body irradiation (TBI) or localized hepatic irradiation (LI) or both and chemotherapy consisting of dimethylbusulfan (DMB), L-phenylalanine mustard (L-PAM), methotrexate, or monocrotaline. VOD occurred infrequently in those dogs given 9.2 Gy TBI and DMB (1/10), TBI and/or LI (9.2-27 Gy) with L-PAM (2/36) or high dose methotrexate and LI (0/2). Specifically, VOD occurred in the dogs with a shorter interval between TBI and DMB or in the dog that received the glutathione reductase inhibitor, buthionine sulfoximide (BSO) before L-PAM. In contrast, among 17 dogs given monocrotaline, 8 developed VOD, particularly when used with L-PAM±irradiation (7/13). The major cause of death, early gastrointestinal toxicity, was further augmented by higher doses of irradiation, by shortening the interval between LI and L-PAM administration to less than 4 weeks, and administering BSO or monocrotaline before L-PAM. Gastrointestinal toxicity was lessened by giving low dose cyclophosphamide given before L-PAM. VOD can be produced in dogs especially with monocrotaline or BSO given before and L-PAM±irradiation. However, gastrointestinal toxicity renders the study of VOD beyond the acute phase difficult. Nevertheless, this approach appears useful for the study of VOD in other animals and for developing agents aimed at preventing VOD. (Am J Pathol 1987, 126:114-125)

HEPATIC veno-occlusive disease (VOD) developing after bone marrow transplantation is a particularly common and serious problem in Seattle, with at least a 21% prevalence and a 33% mortality.[1,2] It is due to hepatotoxic injury caused by the pretransplant chemoradiation therapy, which produces postsinusoidal obstruction of hepatic venules and Zone 3 hepatocyte necrosis, resulting in ascites, jaundice, and encephalopathy.[3] Speculations on the pathogenesis of VOD include primary chemoradiation injury to the endothelium of central veins and sinusoids and/or the hepatocytes in Zone 3 of the liver acinus.[3] Deposition of coagulants in and around the central venules may also be involved.[4-6] There is no effective treatment for established VOD and no known way to prevent it other than better patient selection and lower dose chemoradiation therapy.[2]

We sought to develop an animal model similar in its genesis to the human situation following marrow transplantation in order to establish safe and effective prophylaxis and better treatment for human VOD. Our attempts to create a model of VOD were carried out in the dog because of our extensive past experience with marrow transplantation in dogs, including the unpublished observation of VOD in 5 transplanted dogs prepared with intense chemotherapy and irradiation[7] and after dimethylbusulfan (DMB) treatment.[8] We reasoned that a combination of intense chemotherapy with irradiation would give us a reliable and reproducible model of canine VOD with which to study pathogenetic events and allow prophylactic therapies to be tested. This report details the development of such a model.

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Materials and Methods

Study Design

Using dose escalation schedules, we administered various regimens of antineoplastic chemotherapy and irradiation to dogs with the intent of studying regimen-related toxicity, in particular, the clinical and histologic expression of VOD (Table 1). Autologous marrow rescue was used when the regimen was anticipated to produce fatal marrow toxicity.[9] Dose modifications, combinations of agents, and spacings between the agents were based on survival and toxicity patterns in earlier experiments. The following agents were used: DMB, L-phenylalanine mustard (L-

Table 1 — Experimental Regimens and Results

Regimen							
Prior TBI*	LI (Gy)	DMB† L-PAM (mg/sq m)	Monocrotaline	L-PAM	Number of dogs	VOD	Other toxicities‡
+					10	1	2 septicemia 2 pancreatic atrophy
+	90				4	0	
+	110				2	0	
+	110 BSO				3	1	3 GI toxicity
−	180				1	1	GI toxicity
−	90 × 2				2	0	2 GI toxicity
+	90 × 2				3	0	3 GI toxicity Pancreatic atrophy Pancreatitis
−	Cy, 90 × 2				2	0	2 GI toxicity
+	Cy, 145				3	0	1 GI toxicity Pancreatitis
−		10	−		2		1 GI toxicity
+		14 BSO	−		1	0	
1		10	+		2	0	
1		10	+		2	0	GI toxicity
−		14	+§		2	0	1 GI toxicity 1 retransplanted
−		18	+‖		3	0	
+		10–14	+¶		4	0	4 severe GI toxicity 2 localized gastric ulceration
+			+ (±BSO)		3	No	1 fatal seizure
−		14	+		1	1	Intestinal fibrosis Pancreatitis Hepatocyte atrophy
−			+	+	2	1	1 GI toxicity
−			+	Cy +	2	1	1 GI toxicity
+			+	+	6	3	5 GI toxicity 3 pancreatic fibrosis 1 hemorrhagic gastritis 1 seizure
−		14	+	+	3	2	1 wasting 1 GI toxicity 1 severe VOD
Methotrexate, LI**††				2	0		Wasting cholestasis

*TBI, 60 Co, 9.2–10 Gy.
†DMB, 7.5–15 mg/kg.
‡All dogs that did not die from complications or VOD were killed by pentothal injection.
§L-PAM given 3 weeks after LI.
‖L-PAM given 4 weeks after LI.
¶L-PAM given 1 day, 3 weeks, and 4 weeks, respectively, after LI.
**LI, liver irradiation given by linear acceleration, 10–18 Gy, in one dose.
††Methotrexate, 200 mg/kg, citrovorum rescue twice a day for 2 days, LI 14–18 Gy.
BSO given 15 minutes to 4 hours before monocrotaline or L-PAM or LI.
Monocrotaline postscript indicates dose in milligrams per kilogram.
Cy, 7.5 mg/kg, given 7 days before melphalan.

PAM), localized irradiation to the liver (LI), total-body irradiation (TBI), methotrexate (MTX) with citrovorum factor rescue, monocrotaline, and enhancement of hepatotoxicity with buthionine sulfoximide (BSO).

Dogs

Of the 64 dogs studied, 59 were beagles (1/2–2 1/2 years old), weighing 6.5–12 kg, and 5 were other breeds weighing 13–17 kg. The dogs were housed in single cages and fed commercial food and tap water *ad libitum.*

Procedures

Prior to chemotherapy or irradiation, dogs received sedation with Inovar-Vet (fentanyl, 4 mg/ml, and droperidol, 20 mg/ml, 1 ml/10 kg intramuscularly). Food was withheld on the day of conditioning with antineoplastic agents or irradiation. Fifty-five dogs received 56 autologous marrow transplants (1 dog had 2) as rescue from marrow toxicity of the VOD induction regimens. Bone marrow was aspirated from the iliac bones prior to the administration of TBI, L-PAM, or DMB; stored at 4 C in the refrigerator; and given as an autologous transplant 24 hours after the chemotherapy. After treatment, the dogs were supported with intravenous and subcutaneous Ringer's solution and broad spectrum antibiotics, generally ampicillin and gentamicin or amikacin and were weighed daily. Following marrow transplant or the induction regimen, peripheral blood leukocytes and platelet counts were checked daily until they returned to normal or until the dog's death. Liver function tests (total and direct bilirubin, alkaline phosphatase, lactic dehydrogenase, and glutamic oxaloacetic transaminase) were monitored, usually on Days 3, 7, 10, 14, and 21.

Histologic Studies

Autopsies were performed on all dogs. Most survivors were sacrificed on Day 28 by sodium pentobarbital injection. Multiple sections of liver and gastrointestinal tract were fixed in formalin and Carnoy's fixative. We stained permanent sections of liver with hematoxylin and eosin (H&E) and trichrome to assess the anatomy of terminal venules and sublobular veins. Other formalin-fixed H&E-stained histologic sections examined included tissue from the lungs, apex of left-ventricular myocardium, pancreas, lymph node, kidneys, skin, bone marrow, spleen, thymus, and brain in 2 dogs that developed seizures. A definitive histologic diagnosis of VOD required these characteristics, subendothelial fibrous narrowing of central venules on trichrome-stained sections. A diagnosis of early VOD required subendothelial narrowing of central venules by entrapped red cells, debris, and edema (Figure 1). Sinusoidal widening and central congestion of Zone 3 of the liver acinus, while frequent, was not sufficient for a diagnosis of VOD.

Toxic Agents and Dosages Studied

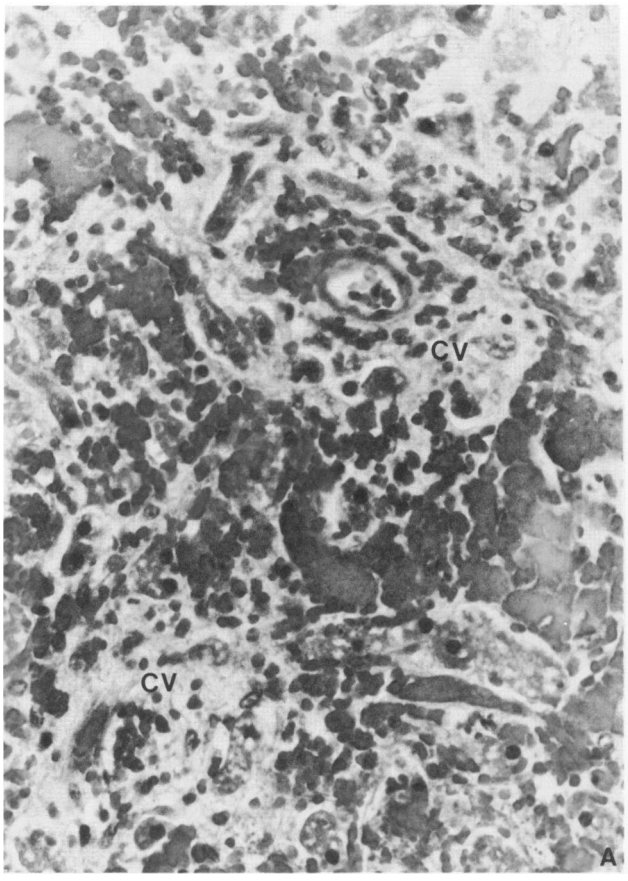
Total-Body Irradiation

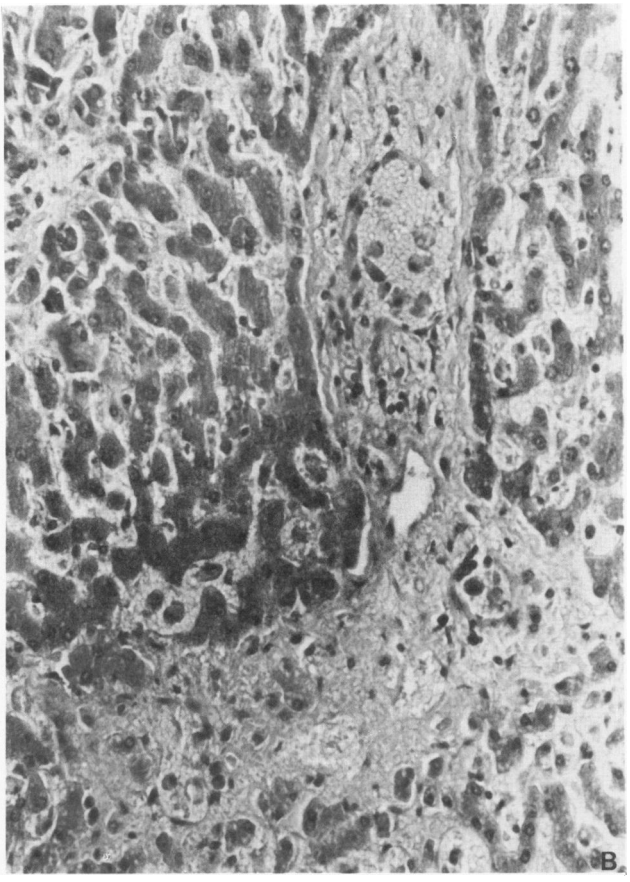
Forty-one beagles had been given TBI 6 weeks to 4 years preceding the present experiments in conjunction with allogeneic (n = 9) or autologous (n = 32) marrow transplants. The TBI was given from two opposing 60 Co sources at exposure rates of 2–7 cGy/min for total dose of 9.2-16 Gy.[9]

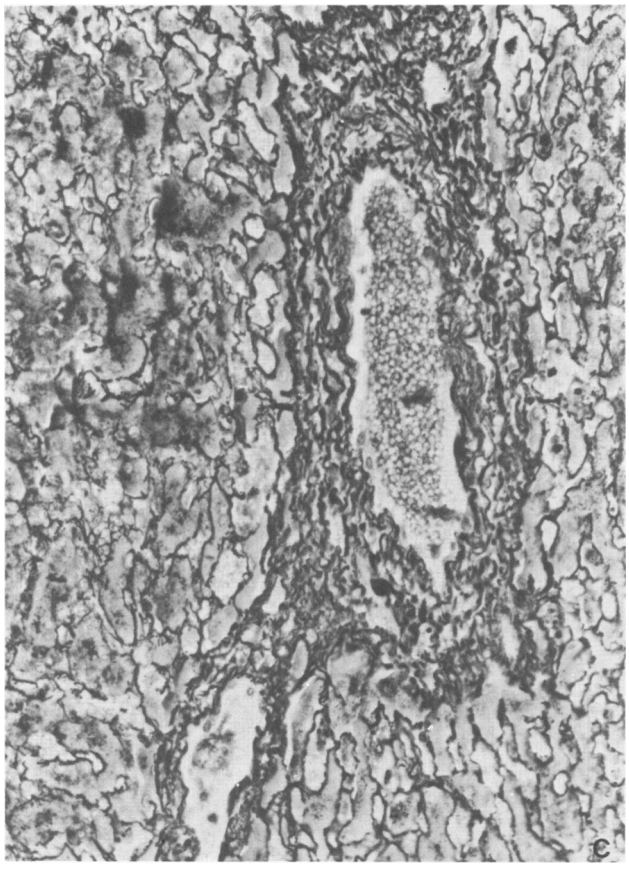
Localized Hepatic Irradiation

LI was given to 16 dogs (6 of which had received previous TBI) at the University of Washington with a Varian Clinac 6-100 6MEV photon linear accelerator at a dose rate of 220 cGy/min. Radiation was given through opposing anterior—posterior and posterior—anterior fields while the dogs were under sedation and placed in a prone position. The initial irradiation field, to the entire liver, was determined by scout flat plate x-ray films of the abdomen. The field included the area between the dome of the diaphragm and the lower anterior costal margin. In subsequent experiments, the liver was localized with computed tomographic (CT) scan of the abdomen to better define relationships between liver and the extent of the intestines included in the field (see Figure 2 A and B). A computer program was used to draw the liver outline on a CT scout image using operator generated information from the cross-sectional CT slices; this liver outline could be used for more accurately establishing the margins of the irradiation field. The inferior margin of the radiation field was moved cephalad 2–3 cm from the lower edge of the anterior costal margin. This excluded only a small (20%) portion of the right anterior liver while at the same time spared most of the upper gastrointestinal tract except for the cephalad half of the stomach and duodenum. The land-

Figure 1—Hepatic veno-occlusive disease in dogs (see Table 2 for details). **A**—C67. Severe early VOD with central hemorrhagic necrosis, and subtotal obliteration of the lumens of central venules *(CV)* by cellular debris and red cells trapped within the subendothelial space. (Trichrome, medium magnification) **B**—B779. Fibrous obliteration of subendothelial space. (H&E, medium magnification) **C**—Proliferation of reticulum fibers with narrowing of the sublobular vein. (Wilder's reticulin, medium magnification)







mark for the superior margin, the seventh vertebral process, corresponded to the anterior dome of the liver and the diaphragm.

Dimethylbusulfan

DMB (Burroughs-Wellcome Laboratories, Research Triangle Park, NC), a polyfunctional alkylating agent, is a potent homologe of busulfan.[10] This poorly soluble agent was reconstituted and given intravenously in the solvent dimethylsulfoxide to 8 dogs and in ethanol to 2 dogs.

L-Phenylalanine Mustard

L-PAM (melphalan), an alkylating agent, was purchased from Burroughs-Wellcome Laboratories. Doses were prepared freshly according to the manufacturer's directions and given intravenously to 42 dogs at dosages of 90–180 mg/sq m.

Monocrotaline

(Trans-World Chemical, Chevy Chase, Md) is a pyrrolizidine alkaloid which causes VOD in several animal species, though it has not been well studied in dogs.[11] Doses were prepared fresh by dissolving the crystals in 0.2 normal HCl and then adjusted to pH 6.5 with 0.2 normal NaOH and then diluted with

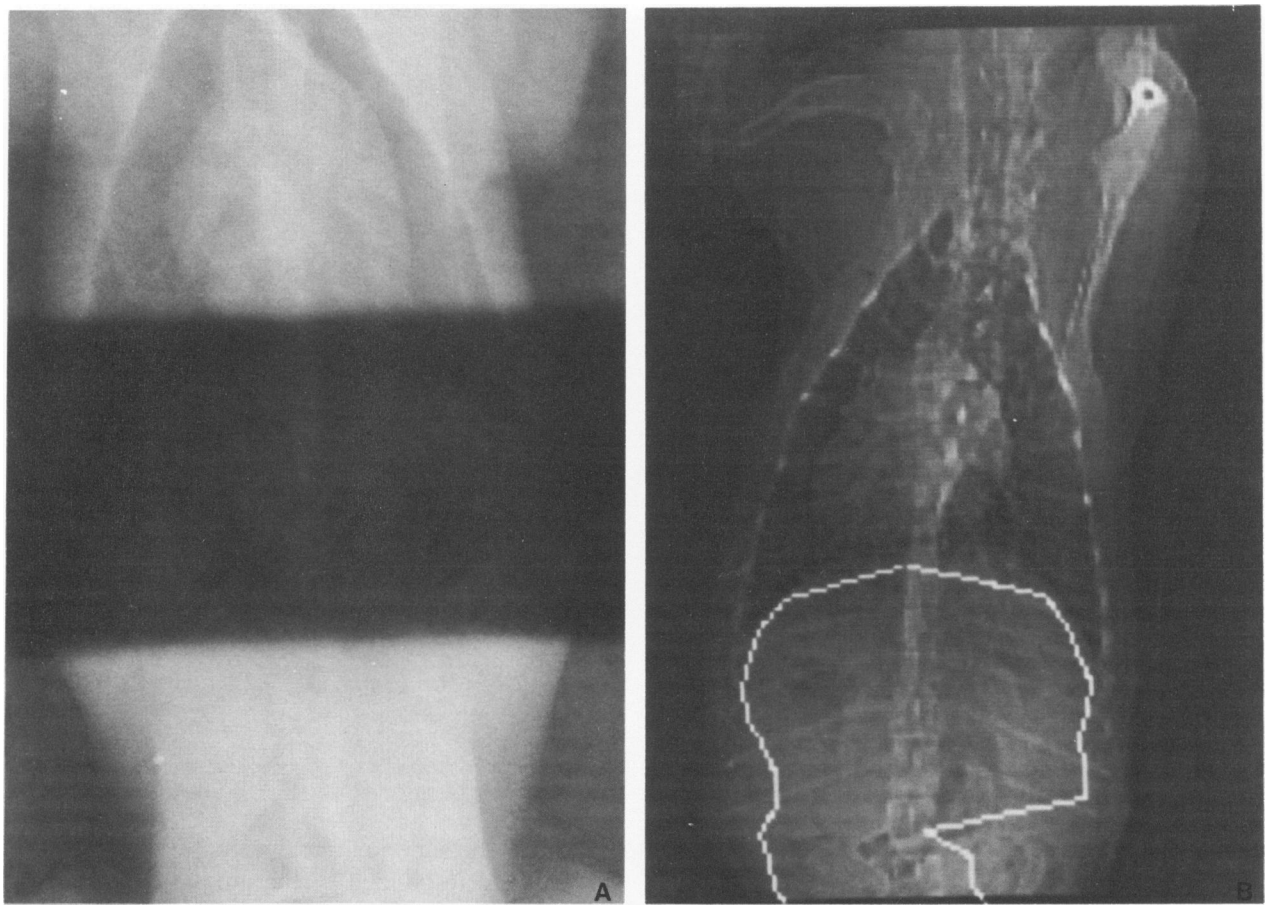


Figure 2—Localized hepatic irradiation. **A**—CT scout image of dog's abdomen used to delimit hepatic irradiation field. Outline of liver is computer-generated with margins of liver marked by the CT operator or transverse (cross-sectional) CT slides. Raising up lower margin to Level 13, we missed only a small portion of liver while sparing most of the intestine. **B**—PA simulator film of dog showing irradiation field for linear accelerator. Field margins are established with liver outline from CT scout image.

isotonic saline to a concentration of 5 mg/ml. Monocrotaline was administered intravenously to the first 2 dogs and intraperitoneally in all subsequent dogs.

Methotrexate

MTX is a tetrahydrofolate reductase inhibitor with well-known hepatotoxic properties.[12] In addition, high-dose MTX is associated with stomatitis, gastroenteritis, and marrow suppression. The toxicity to the marrow and probably some other organs can be offset by "rescue" with citrovorum factor,[13] thereby obviating the need for autologous marrow transplantion. In this protocol, MTX, 200 mg/kg, was given intravenously on Day 0. On the following 2 days, dogs received subcutaneous fluids and two doses of citrovorum factor intravenously (3 mg/kg, 8 hours apart). Both MTX and citrovorum factor were generously provided by Lederle Laboratories, Pearl River, NY).

Hepatotoxic Enhancement

In an attempt to enhance hepatotoxicity, 6 dogs were given BSO (Chemical Dynamics Corp., S. Plainfield, NJ), a potent inhibitor of glutathione synthesis. Glutathionine serves as an endogenous defense against hypoxic and free radical mediated injury to endothelial cells, hepatocytes, and other tissues.[15] BSO was given intravenously before administration of either monocrotaline, irradiation, or L-PAM at 0.2 mmol/kg. This was a dose previously shown to completely inhibit the endogenous generation of glutathione reductase in mouse kidney.[14]

Gastrointestinal Protectants

Cyclophosphamide (Cy; Adria Laboratories, Columbus, Ohio), 7.5 mg/kg was given intravenously 7 days before L-PAM to lessen gastrointestinal (GI) toxicity. Studies have shown greater intestinal epithelial tolerance to high dose L-PAM by such pretreatment with Cy in sheep.[16]

Results

Experimental regimens and results are summarized in Table 1. Specific details of the dogs developing VOD are in Table 2. Observations of particular interest are given within each subsection dealing with specific toxic agent(s). More complete details are available in the Appendix.

Dimethylbusulfan

Ten dogs received DMB. 1 at 7.5 mg/kg, 6 at 10 mg/kg, and 3 at 15 mg/kg. All dogs had been given TBI 84–1624 (median 175) days earlier. The only dog developing VOD after DMB (B779, Figure 1 B and C) was the one with the shortest interval (84 days) following TBI.

L-Phenylalanine Mustard (Melphalan)

Forty-four dogs received L-PAM either alone or combined with other agents. Doses of L-PAM ranged from 90 to 180 mg/sq mm given as split or single doses (Table 2). Twenty-nine of the dogs had previously received TBI range 49–1368 (median 125) days. Dog T1029 received 180 mg/sq m and died on Day 8 with severe gastrointestinal (GI) toxicity and extensive early hepatic VOD. None of the 12 dogs (10 with previous TBI) treated subsequently had histologic evidence of VOD. All 7 dogs receiving 180 mg/sq m, either as a single (2) or split dose (5), died approximately 1 week after treatment with severe GI toxicity (Figure 3A), hemorrhage, and septicemia. Dogs receiving 110 mg/sq m of L-PAM survived without sequelae. Thus, in subsequent studies, using combinations of L-PAM with other agents, a dose of 110 mg/sq m was used along with autologous marrow rescue.

In an attempt to increase the tolerated dose of L-PAM, 5 dogs were given Cy 7.5 mg/kg, 7 days before receiving L-PAM. Two of the 3 dogs survived a dose of 145 mg/sq m, and only 1 dog died, on Day 7 of GI toxicity. However, both dogs given 180 mg/sq m died of GI toxicity.

Localized Liver Irradiation

Twenty-one dogs received a single dose of LI. Variables included the dose, 10–18 Gy, the inferior margin of the irradiation field, the presence or absence of previous TBI, and the interval between LI and subsequent treatments with L-PAM or monocrotaline. By raising the inferior margin of the irradiation field 2–3 cm, based on the CT scan of the abdomen (see Mate-

Table 2—Induction of VOD in Dogs

Dog	Irradiation (Gy)	Induction regimen	Survival and findings
B779	9.2 TBI	DMB 10	d. 95/11
T1029	—	L-PAM 180	d. 7, early VOD
C124	9.2 TBI	BSO 15 min L-PAM d. 0	d. 137/7
C49	14 LI d.36 14 LI d.56	Mcrl 30 d. 0 125 d. 56 L-PAM d. 80	d. 88, wasting and anasarca
C67	14 LI d.0	L-PAM d. 21 Mcrl 60 d. 148 L-PAM d. 160	d. 167/7, severe VOD
B922	10 TBI	Mcrl 60 d. 0 L-PAM d. 12	d. 386/20
C84	—	Mcrl 60 d. 0 L-PAM d. 13	d. 21
C51	14 LI d.75	Mcrl 125 d. 0 and 24	VOD on d.66 liver Bx, necropsy, local intestinal fibrosis, chronic pancreatitis, Zone 3, hepatocellular atrophy
C46	9.2 TBI	Mcrl 60 d. 0 L-PAM d. 1	d. 63/7, chronic pancreatitis, severe GI toxicity
C101	9.2 TBI	Mcrl 125 d. 0 L-PAM d. 1	d. 66/8, severe GI toxicity, chronic pancreatitis
B868	16 TBI	Cy d. 0 Mcrl 60 d. 0 L-PAM d. 7	d. 355/5, GI toxicity

Gy, Gray, equivalent to 100 rads.
DMB, 10 mg/kg intravenously in DMSO.
L-PAM, all at 100 mg/sq m except T1029, 180 mg/sq m.
Mcrl, monocrotaline. Number refers to dose in mg/kg. C49 and C51 received intravenously and all others received intraperitoneal administration.
BSO, 0.2 Mmol/kg Postscript refers to time interval between BSO and ensuing treatment.
Survival calculated from time of TBI and from beginning of induction regimen on day (d)0.

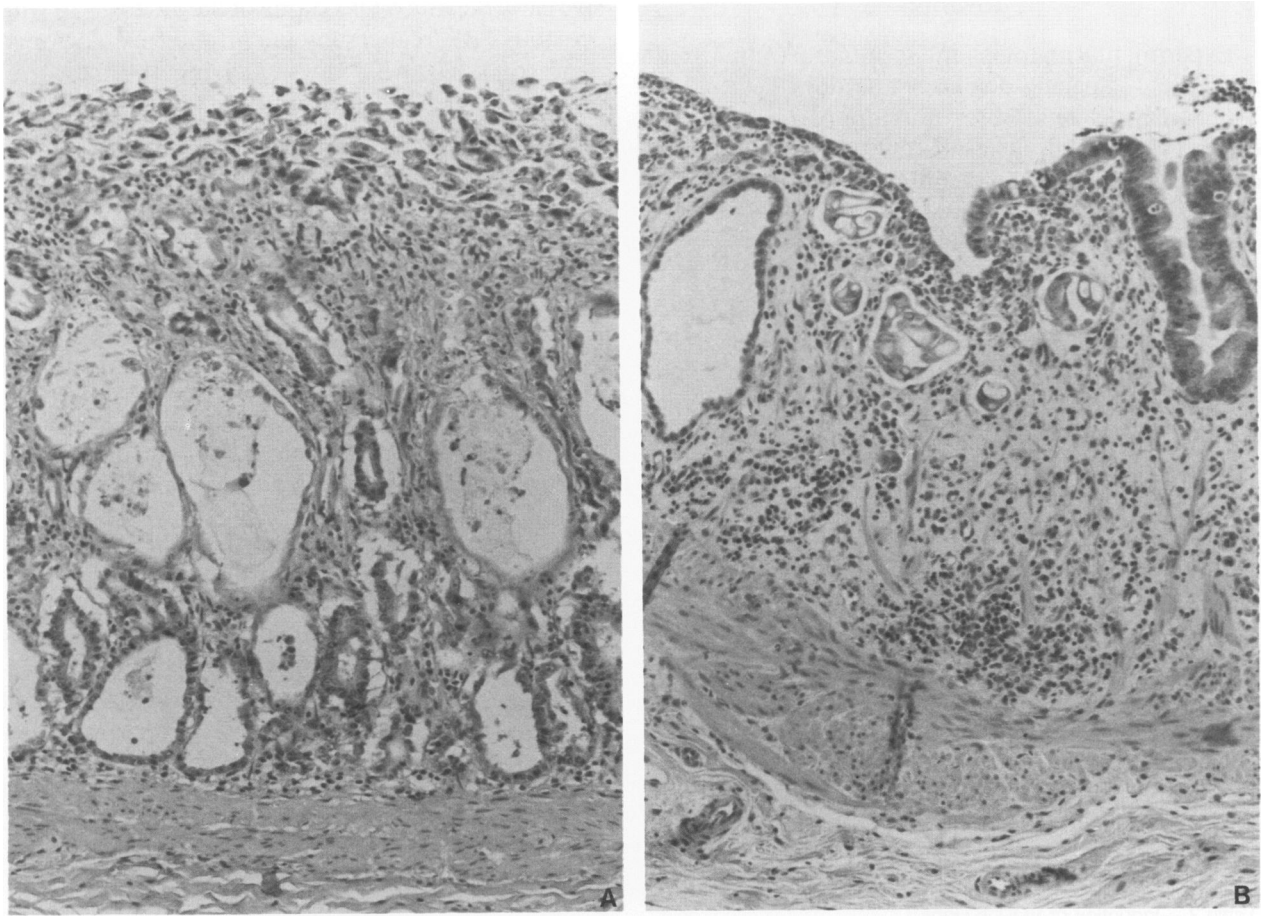


Figure 3 — GI toxicity. **A** — C67. Representative example of severe acute gastrointestinal toxicity with extensively damaged crypts, cytologic atypia, and ulceration. (H&E, medium magnification) **B** — C51. Severe localized chronic GI toxicity with ulceration, chronic inflammation, and loss of intestinal crypts, crypt dilution, and some early regeneration of epithelium budding down from surface. (H&E, medium magnification)

rials and Methods and Figure 2), fatal acute GI toxicity was reduced, and the dose of LI tolerated increased from 10–14 Gy (Table 1). At intervals of 3 or 4 weeks later, the LI was followed by treatment with L-PAM. These experiments indicated a poor survival with a 14 Gy LI - L-PAM interval of 3 weeks (1 of 3 survived).

Three dogs received higher total doses of irradiation. Two previously given 9.2 Gy TBI received 10 or 14 Gy LI, respectively. The latter dog also received L-PAM 4 weeks after LI. The third dog received 18 Gy LI and L-PAM 4 weeks later. All 3 died early of severe GI toxicity with a picture resembling late GI toxicity: gastric and small intestinal ulceration and fibrosis within the LI irradiation portal (Figure 3B). Thus, the survivable dose of LI followed by L-PAM was less than 20 Gy. Finally, though the combination of liver irradiation plus L-PAM produced high mortality from GI toxicity, none of the dogs developed VOD with only these two agents.

Methotrexate and Liver Irradiation

Two dogs received MTX, citrovorum factor rescue, and LI, 14 or 18 Gy, respectively, on Day 2 after MTX. Neither dog developed VOD, although Dog C59 had elevated liver function tests (alkaline phosphatase, lactate dehydrogenase) and icterus associated with histologic findings of bile ductule proliferation and localized peliosis. Both dogs had atrophic and ulcerated gastric mucosa, and Dog C59 had loss of villi and crypts in the small bowel.

Monocrotaline

Seventeen dogs received monocrotaline in total doses ranging from 30 to 250 mg/kg: 3 received 30 mg/kg, 10 received 60 mg/kg, and 4 received 125–250 mg/kg. Of 11 dogs given TBI previously, 2 that later received only monocrotaline 30 or 60 mg/kg had no hepatic lesions at autopsy.

Two dogs (C49 and C51) received higher doses of monocrotaline (155–250 mg/kg) in split doses and irradiation with or without melphalan. Dog C49 had infrequent obliterative VOD lesions in terminal hepatic venules at necropsy. Dog C51, given 125 mg/kg monocrotaline on Days 0 and 24, had percutaneous liver biopsies at 24 and 66 days. The Day 24 biopsy was unremarkable; the Day 66 biopsy 42 days after the second dose of monocrotaline, showed focal VOD lesions, hemosiderin admixed with fibrous tissue within lumens of few small central veins. At necropsy, C51 had atrophic Zone 3 hepatocytes.

Ten dogs received regimens utilizing monocrotaline at 30–125 mg/kg followed by L-PAM 1–15 days later. Seven dogs had previously received TBI, 9.2–10 Gy, and 1 each had received LI or LI + L-PAM, respectively. Dogs B922 and C67 had unequivocable VOD and widespread hemorrhagic obliteration of central venules (Figure 1A). Three others, C46, C101, and C84, had changes highly consistent with early VOD, subendothelial widening with entrapped red blood cells. The remaining 5 dogs had no VOD. All dogs had severe GI toxicity, particularly of the small intestine, with cytologic atypia, mucosal ulceration, bacterial superinfection, and hemorrhagic infarction. Three of the dogs also had chronic pancreatic fibrosis, presumably related to previous TBI.[17] Thus, the combination of monocrotaline plus L-PAM ± prior irradiation was successful in producing VOD in 5/10 dogs, but also produced severe GI toxicity not previously encountered in similar regimens without monocrotaline.

Buthionine Sulfoximide

Five dogs, all previously given TBI, received BSO in 15 minutes to 4 hours prior to the administration of LT (1), L-PAM (3), or monocrotaline (1). Those dogs given BSO before either monocrotaline or LI had no evidence of veno-occlusive disease or enhanced GI toxicity. In contrast, the 3 dogs given BSO before melphalan, 110 mg/sqm, had increased GI toxicity, and 1, C124, had lesions consistent with early VOD.

Cy, Monocrotaline, and L-PAM

Two dogs with previous TBI were first given oral antibiotics and Cy, 7.5 mg/kg, in an attempt to protect against the enhanced gut toxicity of monocrotaline, 60 mg/kg, followed by L-PAM given 7 days later. Dog 868 died 5 days after L-PAM with early VOD as well as intestinal injury with cytologic atypia of the mucosa, ulceration, and bacterial super-infection. Dog 61 died 8 days after L-PAM having neither clear-cut VOD nor GI toxicity.

Discussion

VOD has been observed in patients after high doses of several alkylating agents, such as DMB, busulfan, BCNU, DTIC, azathioprine Indocine-N-oxide, mitomycin C, and high-dose irradiation (reviewed by McDonald et al[18]). Since the canine intestinal tract is extremely sensitive to Cy, we could not use the regimen of 120 mg/kg Cy plus TBI, which commonly produces VOD in patients who received marrow transplantation in Seattle.[1] Instead, in an algorhythmic series of dose escalation studies we investigated the effect of high-dose DMB, L-PAM, LI, and monocrotaline on the development of VOD and GI toxicity. Despite the small number of dogs treated with various regimens, some patterns were evident. No combination of chemotherapy using DMB, L-PAM, or MTX with irradiation was very successful in producing VOD. On the other hand, the administration of monocrotaline before L-PAM and LI resulted in histologic lesions of VOD in 8 dogs. In 1 dog (C-67, Table 2) a prolonged treatment utilizing two transplant regimens produced pronounced acute VOD lesions. The development of VOD in 1 dog simply by giving BSO before L-PAM was a particularly promising finding.

Unfortunately, the same regimens that produced VOD tended to be asymptomatic or overshadowed by severe GI toxicity. The latter may not be surprising, because previous toxicity studies with various alkylating agents have shown that the GI tract of the dog is much more susceptible to these agents than that of other animals, such as the rhesus monkey or the mouse.[19] Our studies did confirm the previous observations that pretreatment with low-dose Cy 1 week before L-PAM administration raised the threshold for L-PAM-associated GI toxicity. However, this pretreatment was not successful in preventing the enhancement of gut toxicity seen when monocrotaline preceded L-PAM.

We anticipated that DMB might induce VOD because of the strong statistical association found between busulfan[20] and its closely related analog, DMB[3], with the development of VOD in man after marrow transplantation. Furthermore, a review of earlier canine studies using DMB[7,8] also had shown several cases of VOD. Somewhat to our surprise, the dogs had a higher tolerance and survival with this alkylating agent. Only 1 of 10 dogs receiving DMB developed VOD (Figure 1a, Table 2). The survival of all 3 dogs after receiving 15 mg/kg of DMB was unexpected in view of the higher mortality reported earlier[8] and may indicate a lessening of activity.

In man, VOD has been reported after fractionated hepatic irradiation in excess of 35 Gy[5] without the

addition of chemotherapeutic agents. In contrast, numerous investigators have failed to produce an animal model of VOD using irradiation.[21] Zook et al[23] and Bradley et al[22] studied the effects of fractionated irradiation to the right hemithorax and the right half of the liver of dogs with either fast high-energy neutrons (mean energy 15 MeV) at 10–33.75 Gy or photons at 30 to 67.5 Gy. All dogs given a dose of 22.5 Gy or greater of fast neutrons and 1 of 5 dogs given 67.5 Gy photon irradiation developed abnormal hepatic signs or elevated serum liver chemistries, or died of hepatic failure; yet the histologic studies showed hepatic atrophy and portal fibrosis rather than VOD. In our earlier dose escalation studies with TBI in the dog,[24] we likewise found that neither single dose nor fractionated TBI at dose rates <20 cGy/min produced VOD. In the present study we were also unable to create VOD using combination of TBI/LI, though the GI tract had lesions resembling late irradiation damage with localized ulceration and fibrosis.

Among the different irradiation variables we studied, the interval between liver irradiation and subsequent chemotherapy or treatment had the greatest influence on hepatic and GI toxicity. Results from several different regimens suggested a greater toxicity when TBI was given more recently than 100 days before treatment with monocrotaline or alkylating agents. It is of interest that the only dog developing VOD after DMB had been irradiated only 84 days earlier.

Monocrotaline, a pyrrolizidine alkaloid (PA), has a well-known propensity to cause VOD and other toxic lesions in a variety of animal species.[11] We found only a single report describing PA-induced VOD in dogs with oral lasiocarpine.[25] Unfortunately, the toxicity of PAs is variable and dependent on the specific PA used, the dose schedule, and route administration. In our studies, the dogs proved much more resistant to the doses of monocrotaline which produce clinical or histologic VOD in the monkey and other animals.[26,11] We also encountered considerable variation in response to doses of monocrotaline in the dogs. Finally, in the dogs, the histologic effects of monocrotaline given as a single agent in large doses were subclinical.

The active metabolites of PA, pyrroles[11] and alkenals[27] produce a variety of cellular injuries by inhibiting membrane-bound enzymes, inhibiting protein synthesis, and reacting with low-molecular-weight SH groups such as glutathione. Hence, the enhanced toxicity of the combination of monocrotaline and L-PAM is understandable, particularly since both agents function as alkylating agents to form DNA–DNA and DNA–protein crosslinks which interfere with DNA replication.[28,29]

In conclusion, we have shown that regimens using BSO and alkylating agents (L-PAM and monocrotaline) with or without irradiation can produce a good acute phase model of VOD in dogs, which may be useful in studying agents directed at prevention of VOD. In order to achieve longer survival, however, new approaches need to be explored. Such approaches might include different timing of the localized liver irradiation before subsequent chemotherapy, alternative methods of lessening GI toxicity such as the instillation of chemotherapy directly into the hepatic artery, and/or finding an animal less susceptible to GI toxicity.

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Appendix

Table 1—Studies With DMB

Dog	Liver X-ray or TBI (Gy)	DMB (mg/kg)*	Outcome
T1085	9.2, d. 1 TBI	5, d. 0	d. 19, euthanized, acute cholecystitis
B779	9.2 auto TBI	10	d. 95/11, VOD, septicemia, pancreatic atrophy
B854	9.2 auto TBI	10	d. 258/53, euthanasia
B855	9.2 auto TBI	10	d. 248/10, sepsis, pancreatic atrophy
B650	10 auto TBI	7.5*	d. 303/28, euthanasia
B772E	9.2 auto TBI	10*†	d. 175/64, euthanasia
B773	9.2 auto TBI	10*†	d. 183/26, euthanasia
B435	9.2 allo TBI	10*	d. 1624/40, euthanasia
B633	9.2 allo TBI	15	d. 1230/36, euthanasia
B667	9.2 auto TBI	15	d. 933/43, euthanasia
B766	9.2 auto TBI	15	d. 914/48, euthanasia

*†CCl_4, 0.3 cc given by mouth 6 hours before DMB.
†DMB was dissolved in hot ethanol before intravenous administration.

Table 2—Melphalan

Dog	Previous TBI	Dose (mg/kg)	Outcome
T1029	—	180	d. 7, VOD, GI toxicity
B840	9.2 allo	180	d. 127/7, GI toxicity
B601	—	90 × 2d	d. 4, GI toxicity
B602	—	90 × 2d	d. 4, GI toxicity
B879	9.2 auto	90 × 2d	d. 120/5, GI toxicity, pancreatitis
B965	9.2 auto	90 × 2d	d. 125/7, GI toxicity, pancreatic atrophy
B917	9.2 allo	90 × 2d	d. 147/5, GI toxicity, pancreatic atrophy
B641	9.2 allo	90	d. 21, euthanasia
B642	9.2 allo	90	d. 1393/25, euthanasia
B645	9.2 allo	90	d. 1428/26, euthanasia
B646	9.2 allo	90	d. 1044/27, euthanasia
B835	9.2 allo	110	d. 224/34, euthanasia
B836	9.2 allo	110	d. 429/28, euthanasia
Pretreatment With Cyclophosphamide and Melphalan D.7			
B955	9.2 auto	145	d. 130/28, euthanasia
B957	9.2 auto	145	d. 120/6, GI toxicity, chronic pancreatitis
B969	9.2 auto	145	d. 150/29, euthanasia
C132	—	90 × 2d	d. 7, GI toxicity
C133	—	90 × 2d	d. 7, GI toxicity

Table 3—Liver X-Irradiation

Dog	Previous TBI	Liver X-ray (Gy)	Interval (weeks)	Melphalan (mg/sqm)	Outcome
B991	—	10 Gy whole	—	—	d. 24, euthanasia
C37	—	10 Gy whole	—	—	d. 6, GI toxicity
B990	—	10 Gy whole	4	110	d. 29, euthanasia
B993	—	10 Gy whole	4	110	d. 5, GI toxicity, septicemia
C64	—	10 Gy whole	4	110	d. 35, euthanasia
C37	—	14 Gy*	4	—	d. 5, GI toxicity
C28	—	14 Gy*	4	110	d. 29, euthanasia
C57	—	14 Gy*	4	110	d. 23, pneumonia
C50	—	14 Gy*	3	110	d. 5, GI toxicity
B995		14 Gy*	3	110	d. 5, segmental GI toxicity
C67	—	14 Gy*	3	110	d. —, survived, retransplanted
Variable High-Dose Combination TBI Localized Melphalan (110 mg/sqm)					
				Irradiation	
C47	9.2 auto	14 Gy*	4 weeks	+	d. 7, GI, toxicity, localized intestinal ulceration and fibrosis
B942		18 Gy*	4 weeks	+	d. 5, severe localized GI toxicity
C76	9.2 auto	10 Gy*	d. 1	−	d. 159/6, severe GI toxicity

*Lower field margin moved 2–3 cm cephalad.

Table 4—Methotrexate, Liver X-Irradiation

Dog	Previous TBI	Liver irradiation*	Outcome
C59	—	14 Gy*	Day 41, euthanasia
C89	—	18 Gy*	Day 38, euthanasia

*Lower field margin 3 m above anterior costal margin.

Table 5—Monocrotaline

Dog	Previous TBI	Monocrotaline mg/kg	Monocrotaline Date	Liver X-ray	Melphalan (110 mg/sqm)	Outcome
B943	9.2 allo	30	Day 0	—	—	d. 920/21, euthanized
C107	9.2 auto	60	Day 0	—	—	d. 51/6, died of seizures
C49	—	30	Day 0	14 Gy*	Day 80	d. 88, wasting anasarca, VOD
		125	Day 56	day 56		
C51	—	125	Day 0	4 Gy*	—	d. 96, euthanized ascites, chronic intestinal fibrosis, chronic pancreatitis,
		125	Day 24	day 75		central hepatocyte atrophy
C131	—	125	Day 20	14 Gy*	Day 29	d. 34, Severe GI toxicity
				day 29		
C67	—	60	Day 148	14 Gy*	Day 21	d. 167, severe VOD
				day 0	Day 160	
C106	9.2 auto	60	Day 0	—	Day 12	d. 66/17, GI toxicity
B918	(2 × 5) auto	60	—	—	Day 15	d. 415/21, pancreatic fibrosis
B922	(2 × 5) auto	60	—	—	Day 12	d. 386/20, VOD, hemorrhagic gastritis
C84	—	60	—	—	Day 13	d. 21, VOD
C140	—	30	—	—	Day 14	d. 20, severe GI toxicity
C141	9.2 auto	30†	—		Day 14	d. 103/6, severe enteritis
C46	9.2 auto	60	Day 0	—	Day 1	d. 63/7, early VOD, chronic pancreatitis, severe GI toxicity
C101	9.2 auto	125	Day 0	—	Day 1	d. 66/8, VOD, severe GI toxicity, chronic pancreatitis

*Lower field margin 3 cm above anterior costal margin.
†Sodium solicylate, 325 mg/day, by mouth, given on Days 1 and 2.

Table 6—Buthionine Sulfoximide

Dog	Previous TBI	Monocrotaline (60 mg/kg)	BSO*	Liver X-ray	Melphalan (110 mg/sqm)	Outcome
C117	9.2 auto	–	−3 hr	14 Gy	—	d. 146/32, euthanized
C120	9.2 auto	+	−15 min	—	—	d. 94/25, euthanized
C116	9.2 auto	–	−15 min	—	Day 0	d. 178/7, severe enteritis
C124	9.2 auto	–	−15 min	—	Day 0	d. 137/7, early VOD, septicemia, bowel toxicity
C142	9.2 auto	–	−4 hr	—	Day 0	d. 123/6, possible VOD, non-dx, intestinal ulceration, septicemia

*BSO given 15 minutes to 4 hours before LI, monocrotaline, or L-PAM.

Table 7—Cyclophosphamide Gut Protection

Dog	Previous TBI	Cy	Monocrotaline (60 mg/kg)	Liver X-ray	Melphalan	Outcome
C61	16 auto	Day 0	Day 0	—	Day 6	d. 376/14, no gut toxicity possible VOD, non-dx
B868	16.0 auto	Day 0	Day 0	—	Day 7	d. 509/12 early, VOD, gut toxicity with bacterial superinfection