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Parenteral Toxicity Studies with Benzyl Alcohol

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Parenteral Toxicity Studies with Benzyl Alcohol. KIMURA, E. T., DARBY, T. D., KRAUSE, R. A., and BRONDYK, H. D. (1971). *Toxicol. Appl. Pharmacol.* **18**, 60–68. Benzyl alcohol was injected into mice, rats, dogs, and monkeys and compared with ethyl alcohol to delineate its limits of tolerance. Intravenously, 1 ml/kg of 0.9% benzyl alcohol did not affect, in anesthetized and unanesthetized dogs and anesthetized monkeys, the blood pressure, heart rate, respiration, ECG or hematalogic parameters. The lethal iv dose of 0.9% benzyl alcohol in anesthetized, normovolemic dogs was 88–113 ml/kg (0.83–1.06 g/kg). Intracarotid and intrarenal injections of 0.9% benzyl alcohol did not cause any significant effects on ECG, blood pressure, heart rate, respiration or EEG of anesthetized dogs. Rapid iv injections of 0.9% benzyl alcohol were nonlethal in mice at 50 ml/kg (0.48 g/kg). Both 0.9% benzyl and ethyl alcohol could be given, iv, to rats, slowly, with no fatalities at 40 ml/kg. After rapid injection, the LD50 for 0.9% benzyl was 33.4 ml/kg while 37.5 ml/kg of the ethyl alcohol was nonlethal. Volumes of 22.5 ml/kg or less of 0.9% benzyl alcohol were nonlethal in rats. There is a calculated safety factor of 38 following a rapid injection of a 30-ml vial dose of 0.9% benzyl alcohol to a 50-kg adult. Intraarterially, 0.9% benzyl and ethyl alcohol were tolerated by rats at 44.5 ml/kg. Benzyl alcohol (94%) was 23 times more toxic in rats, iv, than ethyl alcohol (95%). Again, the calculated LD50 in mice for benzyl alcohol (94%) was less than 0.5 ml/kg (0.48 g/kg), and that for ethyl alcohol (95%) was 1.9 ml/kg (1.46 g/kg).

This report is an outgrowth of a communication[2] to us that a rapid injection of saline containing 0.9% benzyl alcohol was lethal in mice. A number of parenteral solutions for intravenous therapy, such as *Sodium Chloride Injection, USP, with Preservative* and *Water for Injection, Bacteriostatic, USP*, contain this aromatic alcohol as a preservative at a concentration of 0.9%. Additionally, a fairly large number of drugs in injectable form contain benzyl alcohol as a bacteriostat. The question was also raised as to what might happen if the entire contents of the multiple-dose vials of 0.9% benzyl alcohol in saline or water (10, 20, and 30 ml) were to be given to patients during intravenous therapy.

Historically, Macht (1918a, b), Nielsen *et al.* (1921), Gruber (1923), Duncan (1939), and Duncan and Jarvis (1943) have reported on the pharmacology of benzyl alcohol

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and its esters. However, a search of the current literature showed an almost complete lack of more recent information bearing on the parenteral toxicity and pharmacology of benzyl alcohol as it relates to its clinical usage. Studies were, therefore, carried out to delineate the limits of tolerance of benzyl alcohol following intravenous and intra-arterial injections in experimental animals.

METHODS

Toxicologic studies were carried out with benzyl alcohol in mice, rats, and dogs with (1) the undiluted material (94% A.R. grade, Merck); (2) a 2.5% dilution in saline; and (3) 0.9% solutions, both in saline and in water. *Sodium Chloride Injection, USP, with Preservative* and *Water for Injection, Bacteriostatic, USP*, were used as source materials for 0.9% benzyl alcohol in saline and in water, respectively. Parallel studies were run with ethyl alcohol at concentrations of 95% and 0.9% in saline. Swiss-Webster female mice[3] (18–24 g) in groups of 10; Charles River female rats (170–220 g) in groups of 6; and beagles of both sexes (4.8–12.4 kg) in groups of 3 were used in these studies. Mice, rats, and dogs were injected intravenously at a slow rate of 1 ml/min. Mice were also injected intravenously at a rapid rate of 1 ml/5 sec; rats were also injected intra-arterially (femoral) at both 0.1 and 1 ml/min. Survivors were observed for a week. The LD50 and associated confidence limits were calculated by the method of Litchfield and Wilcoxon (1949).

Blood pressure, heart rate, and respiration studies were carried out with 1 ml/kg of 0.9% benzyl alcohol in saline, intravenously, in 6 unanesthetized male dogs (8–20 kg) and in 7 anesthetized rhesus monkeys (2.0–4.5 kg) of both sexes. These intravenous injections were made into either the cannulated femoral or saphenous vein over a 5-, 10-, or 20-min interval. Eighteen additional anesthetized dogs (9–19 kg) of both sexes were given a rapid 10 ml intra-carotid or renal artery injection of 0.9% benzyl alcohol in saline and in water. Sodium pentobarbital at 30 mg/kg, intravenously, was used as an anesthetic in both dog and monkey studies here and in subsequent experiments. Femoral blood pressure was recorded either via direct arterial puncture or via implanted plastic catheters; renal artery injections were made via catheter from the carotid artery. Respiration was recorded either via a pneumograph or a cuffed endotracheal tube. Standard Lead II ECG's as well as other recordings were made on a Grass polygraph. Blood samples were taken for complete blood count and for chemical analyses 1 and 10 min after injection and 24 hr later. Gross and microscopic pathology of the kidneys of dogs receiving renal injections and the brains of dogs receiving carotid artery injections were determined.

EEG studies, in conjunction with blood pressure and Lead II ECG studies, were carried out with 0.9% benzyl alcohol in saline and in water in 4 decamethonium-immobilized beagles of both sexes (8–10 kg) under nitrous oxide anesthesia and on artificial respiration. The solutions were injected into the carotid artery at doses of 0.5, 1, 2, 4, and 8 ml over at least a 2-min period.

Minimally effective and minimally lethal doses were determined for 0.9% benzyl alcohol in saline and in water in 8 anesthetized dogs (11–15.5 kg) of both sexes. Minimally effective doses were determined by giving intracarotid injections in increasing

[3] A. R. Schmidt & Company, Madison, Wisconsin.

amounts until effects were noted. Minimally lethal doses were determined following intravenous infusions into catheterized femoral veins beginning at a rate of 0.2 ml/kg/min for 30 min, followed by 0.4 ml/kg/min for 30 min, then 1 ml/kg/min for 20 min and, finally, at 2 ml/kg/min until death. Circulatory, respiratory, and ECG studies were also carried out concurrently. Blood samples for erythrocyte fragility studies were taken from the femoral or jugular veins by direct puncture before drug administration and after 15, 75, and 105 ml had been infused.

Plasma half-life studies were determined in two unanesthetized dogs given 52 and 104 mg/kg of benzyl alcohol in saline from a 2.5% solution, intravenously, over a 1 min period. Heparinized blood samples were withdrawn by jugular puncture prior to, 0.5, 1, 2, 3, 4, and 6 hr after medication. The plasma was assayed for benzyl alcohol, using the assay procedure of Cummins and Perry (1969).

RESULTS

The comparative LD50 findings for benzyl alcohol and ethyl alcohol in mice, rats, and dogs are shown in Tables 1, 2, and 3, respectively.

TABLE 1

INTRAVENOUS LD50 VALUES IN MICE FOR BENZYL ALCOHOL AND ETHYL ALCOHOL

Speed of injection	Solution	LD50 (95% C.L.) (ml/kg)
Rapid (1 ml/5 sec)	94% Benzyl alcohol	<0.5[a]
	95% Ethyl alcohol	1.9 (1.7–2.1)
	0.9% Benzyl alcohol	>50[b]
	0.9% Ethyl alcohol	>50[b]
Slow (1 ml/min)	0.9% Benzyl alcohol	>50[b]
	0.9% Ethyl alcohol	>50[b]

[a] 10/10 fatalities from 0.5 to 5 ml/kg. Volumes less than 0.5 ml/kg not precise and not attempted.
[b] Nonlethal at a maximum volume of 1 ml/20 g weight.

Mice (Table 1) were able to tolerate rapid intravenous injections of either 0.9% benzyl alcohol or ethyl alcohol in saline at a maximum volume of 50 ml/kg, equivalent to 0.48 g/kg of benzyl alcohol and 0.37 g/kg of ethyl alcohol. There were no fatalities. The only signs of drug action were transient respiratory arrest immediately after injection, followed by recovery within less than half a minute. As expected, responses following a slow rate of injection of either 0.9% benzyl or ethyl alcohol were less pronounced than those seen following rapid injection. Saline controls in both mice and rats were asymptomatic at 50 ml/kg, intravenously.

Rapid intravenous injections of the undiluted alcohols showed that benzyl alcohol was more toxic than ethyl alcohol. The calculated LD50 for 95% ethyl alcohol was 1.9 ml/kg (1.46 g/kg), while the smallest volume of benzyl alcohol which could be injected with any precision (0.01 ml/20 g weight, equivalent to 0.5 ml/kg or 0.48 g/kg) was uniformly fatal to mice.

Experiments using 94% benzyl alcohol and 95% ethyl alcohol, injected at a slow rate,

TABLE 2

INTRAVENOUS AND INTRA-ARTERIAL LD50 VALUES IN RATS FOR BENZYL ALCOHOL AND ETHYL ALCOHOL

Speed of injection	Solution	LD50 (95% C.L.) (ml/kg)
	A. *Intravenous*	
Rapid (1 ml/5 sec)	0.9% Benzyl alcohol in saline	33.4 (30.3–36.8)
	0.9% Ethyl alcohol in saline	>40[a]
Slow (1 ml/min)	0.9% Benzyl alcohol in saline	>40[a]
	0.9% Ethyl alcohol in saline	>40[a]
	94% Benzyl alcohol	Between 0.05 and 0.08
	95% Ethyl alcohol	1.87 (1.63–2.34)
	B. *Intra-Arterial*	
Rapid (1 ml/5 sec)	0.9% Benzyl alcohol in saline	>44.5[a]
	0.9% Ethyl alcohol in saline	>44.5[a]
	94% Benzyl alcohol	0.42 (0.31–0.58)
	95% Ethyl alcohol	About 11

[a] Maximum volume, equivalent to about 8 ml/200 g weight.

TABLE 3

INTRAVENOUS TOXICITY OF 94% BENZYL ALCOHOL AND 95% ETHYL ALCOHOL IN DOGS

Solution	Dose (ml/kg)	Mortality ratios
94% Benzyl alcohol	0.0056	0/3
	0.028	0/3
	0.044	0/3
	0.05	1/3
	0.0525	0/3
	0.053	0/3
	0.055	3/3
95% Ethyl alcohol	1.33	0/3
	1.6	1/3
	1.86	3/3

were not run since relatively minute volumes were used in our rapid injection studies (i.e., 0.01–0.1 ml), and these could, in essence, be viewed as slow rates.

Rats (Table 2) were also able to tolerate slow intravenous injections of either 0.9% benzyl alcohol or ethyl alcohol in saline with no fatalities even at a rather high dose of 40 ml/kg, equivalent to 0.378 g/kg of benzyl alcohol and 0.293 g/kg of ethyl alcohol.

After rapid intravenous injections, 0.9% benzyl alcohol in saline was somewhat more toxic than 0.9% ethyl alcohol when results were based on LD50. The LD50 for 0.9% benzyl alcohol in saline was 33.4 ml/kg (0.314 g/kg) with deaths due to respiratory arrest. However, at doses of 22.5 ml/kg and lower, 0.9% benzyl alcohol in saline did not induce any fatalities. A volume of 37.5 ml/kg of 0.9% ethyl alcohol in saline was non-lethal, while a maximum volume of 40 ml/kg (0.293 g/kg) induced one fatality out of 6 rats. Death was due to respiratory paralysis. Ethyl alcohol, 95%, was about 23 times less

toxic than 94% benzyl alcohol, after slow intravenous injections. The LD50 for the former was 1.87 ml/kg (1.44 g/kg) while that for benzyl alcohol lay between 0.05 and 0.08 ml/kg (0.064 g/kg).

Intra-arterially, 0.9% dilutions of both alcohols in saline were nonlethal and well tolerated by rats. As was seen with our prior intravenous studies in rats, 95% ethyl alcohol was about 20 times less toxic than 94% benzyl alcohol in rats, intra-arterially. The LD50 for benzyl alcohol was 0.42 ml/kg (0.41 g/kg) while that for ethyl alcohol was estimated to be about 11 ml/kg (8.53 g/kg). Death was either immediate via respiratory arrest or delayed due to pulmonary edema.

Necrosis of the injected legs, following femoral artery injections, occurred at 0.4 ml/kg (0.39 g/kg) and higher with 94% benzyl alcohol and at 10.5 ml/kg (8.08 g/kg) and higher with 95% ethyl alcohol. The onset time for these necrotic changes was 10 days for benzyl alcohol and 1–3 days for ethyl alcohol. Doses which did not induce these changes were 0.1 ml/kg and lower for benzyl alcohol and 5.6 ml/kg and lower for ethyl alcohol. These studies in mice and rats, using the undiluted alcohols, were carried out primarily to obtain their comparative LD50 values.

Acute toxicity studies in dogs (Table 3) with the undiluted alcohols showed ethyl alcohol to be roughly 30 times less toxic than benzyl alcohol following intravenous injection. Dyspnea, diarrhea, ataxia, mydriasis, nystagmus, urination, respiratory arrest, collapse, and cardiac arrest were seen following injection with both alcohols. Delayed deaths occurred, accompanied by hemorrhagic and edematous lungs. As noted above, these studies with the undiluted material were carried out primarily to determine the acute toxicity of these materials for comparative purposes.

Intravenous injections of 0.9% benzyl alcohol in saline at 1 ml/kg in 6 untrained, unanesthetized, catheter-implanted beagles showed no significant drug related changes of either blood pressure, heart rate, respiration, or ECG. The hematology and blood chemistries of these animals showed no remarkable changes except for blood sugar changes in several dogs. These changes are possibly due to the fasting condition as well as to normal intermittent periods of excitement in unanesthetized animals.

Similar studies in 7 rhesus monkeys infused with 1 ml/kg of 0.9% benzyl alcohol in saline did not show any changes in blood pressure, heart rate, respiration, or blood chemistry.

Rapid intracarotid or renal artery injections in 8 anesthetized dogs of 10 ml of 0.9% benzyl alcohol in saline or water did not induce any gross changes in either blood pressure, heart rate, respiration, or ECG. Blood samples taken prior to and at 1 and 10 min after the injections were essentially normal. Blood glucose levels were low in some dogs. These changes occurred sporadically with no apparent relationship to the solution injected or to the route of injection. Some inflammatory changes were seen histologically in the tubular epithelium of the kidneys of several of these animals and were considered to be due to the mechanical trauma of catheterization. Additional experiments were, therefore, performed in 10 beagles given rapid injections via radiopaque catheter into the renal arteries. Either the carotid or femoral arteries were isolated for passage of the catheter to the renal arteries. Four of these 10 dogs were given 10 ml of 0.9% benzyl alcohol in saline; 4 received 0.9% benzyl alcohol in water; and 2 received plain 0.9% sodium chloride. Three days later, the dogs were sacrificed for gross and microscopic pathology studies of the injected and noninjected kidneys.

TABLE 4

PLASMA CONCENTRATIONS OF BENZYL ALCOHOL IN UNANESTHETIZED DOGS AFTER INTRAVENOUS INJECTION

Dog No.	Sex	Weight (kg)	Dose (mg/kg)	Plasma concentration (μg/ml)						
				0.5 Hr	1 Hr	2 Hr	3 Hr	4 Hr	6 Hr	Half-life (hr)
8167	F	12.5	52	6.3	2.9	2.1	1.3	0.8	0.3	1.5
7964	M	11.8	104	12.3	5.0	3.3	2.2	1.3	0.1	1.5

Histologic examination showed no morphologic differences between the injected kidneys and the controls in 7 of the 10 dogs. In 2 dogs injected with 0.9% benzyl alcohol in water, and in 1 dog injected with plain 0.9% sodium chloride, large areas of ischemic necrosis with reparative connective tissue proliferation and tubular cast formation were seen in the treated kidney. The ischemic necrosis, with attendant early repair, was considered a result of the experimental catheterization of the renal artery and kidney and the benzyl alcohol was not considered to be a contributing factor.

EEG studies in decamethonium immobilized dogs, given intracarotid injections of 0.9% benzyl alcohol in saline and in water showed no obvious alterations of the background EEG pattern, heart rate, or blood pressure.

Studies to determine the minimum effect dose in anesthetized dogs showed that rapid intracarotid artery injections of 0.9% benzyl alcohol in saline and in water induced only minimal effects on the hematologic, respiratory, and cardiovascular parameters at volumes of 30 and 40 ml of 0.9% benzyl alcohol in water and 20 and 30 ml of 0.9% benzyl alcohol in saline. Increasing the volumes to 40 and 50 ml did not increase the effect to any great degree. Red blood cell fragility studies showed that no changes occurred after injections of 0.9% benzyl alcohol in either saline or water.

Studies to determine the minimum lethal dose in anesthetized dogs showed the lethal dose to be between 88 and 113 ml/kg (0.83–1.06 g/kg), intravenously. A slow decline in blood pressure, increase in pulse width and an increase in heart rate occurred after half of the lethal dose had been infused. These changes appeared to be more related to fluid overload than to the benzyl alcohol. There was no significant difference noted between 0.9% benzyl alcohol in saline and 0.9% benzyl alcohol in water. No changes in red blood cell fragility were seen with either test solution after infusion of 75 ml of fluid.

The plasma half-life of benzyl alcohol (2.5% in saline) was found to be approximately 1.5 hr in unanesthetized dogs injected at a dose level of 52 and 105 mg/kg, intravenously (Table 4). The apparent volume of distribution averaged 11 l/kg (calculated as $V_d^1 = a_0/c_0$ where a_0 is the dose and c_0 is the extrapolated plasma concentration at zero time), suggesting that most of the drug is distributed in the tissues. Some effects were noted upon injection of 52 mg/kg, namely: coarse head tremors of several minutes duration; defecation immediately upon injection; and emesis within 10 min after injection. No drug effects were seen in the dog receiving the larger dose of 104 mg/kg. Histologic examination of the kidneys of the 2 dogs revealed no alterations attributable to the 2.5% test solutions.

DISCUSSION

The relative safety of 0.9% benzyl alcohol was evident, first, from our studies in unanesthetized and anesthetized dogs and in anesthetized monkeys. One ml/kg of 0.9% benzyl alcohol, intravenously, in saline did not affect either the circulatory or respiratory system, the ECG, or the CBC and blood chemistries.

Second, studies to determine the lethal intravenous dose of 0.9% benzyl alcohol in anesthetized, normovolemic dogs again showed the relative safety of this solution. The lethal dose lay between 88 and 113 ml/kg. Circulatory changes seen after one-half of the lethal dose had been infused were believed to be due more to fluid overload than to the alcohol.

Finally, ancillary experiments using the intracarotid and intrarenal routes of injection showed that anesthetized dogs were able to tolerate 0.9% benzyl alcohol with no significant effects on the ECG, blood pressure, heart rate, respiration, or EEG. Rapid intracarotid injections induced only minimal effects on the above parameters at volumes of 30 and 40 ml of the alcohol in water and 20 and 30 ml of the alcohol in saline. Increasing the volume to 40–50 ml failed to increase the responses to any great degree.

The above data, therefore, suggest that the contents of a 30-ml vial of 0.9% benzyl alcohol could presumptively be given to healthy adult humans without any problems of toxicity. However, sufficient data are not available to permit the suggestion that this dosage be given to a small child. It is felt that, if a physician wishes to inject more than 30 ml of saline to an adult, he should probably use material that does not contain a preservative.

The comparative acute toxicity studies in mice, rats, and dogs with benzyl and ethyl alcohols further amplified the demonstration of the relative safety of this aromatic alcohol.

The acute toxicity studies in mice showed that rapid intravenous injections of 0.9% benzyl alcohol could be given safely at a maximum volume of 50 ml/kg. At present, no explanations are offered concerning the lethal effects seen in mice, as reported previously.[2] A chemical analysis of that particular lot in question might have offered some leads.

Both 0.9% benzyl and ethyl alcohol could be given intravenously at a slow rate of injection to rats with no fatalities at 40 ml/kg. Following rapid intravenous injection, 0.9% benzyl alcohol was somewhat more toxic than 0.9% ethyl alcohol. The LD50 for benzyl alcohol in saline was 33.4 ml/kg; a volume of 37.5 ml/kg of 0.9% ethyl alcohol was nonlethal. However, at doses of 22.5 ml/kg and lower, 0.9% benzyl alcohol did not induce any fatalities in rats. There is thus a safety factor of about 38, assuming that the entire contents of a 30 ml vial of 0.9% benzyl alcohol were to be given rapidly to a 50 kg adult.

Intra-arterially, 0.9% dilutions of both alcohols in saline were nonlethal and well tolerated by rats at a high dose of 44.5 ml/kg.

The undiluted benzyl alcohol in the acute intravenous toxicity studies in rats was found to be roughly 23 times more toxic than the undiluted ethyl alcohol. Studies in mice showed similar results. The calculated LD50 for the aromatic alcohol was less than 0.5 ml/kg, while that for ethyl alcohol was calculated to be about 1.9 ml/kg.

Similar results showing the less toxic nature of ethyl alcohol over benzyl alcohol were seen following both intra-arterial injections into rats and intravenous injections in dogs. These results must be viewed in the light of their actual clinical usage wherein more than 100-fold dilutions (0.9%) are used as preservatives. These exercises with the undiluted material were carried out primarily to assess their relative acute parenteral toxicities.

The effects of benzyl alcohol administered in conjunction with some commonly used pharmacologic agents are under current investigation.

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