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Reduction of Experimental Myocardial Infarct Size with Hyperosmolar Mannitol (39285)

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It has been shown that hyperosmotic mannitol improves ventricular function, increases total and collateral coronary blood flow, and reduces epicardial ST segment elevation during acute myocardial ischemia in anesthetized and in awake, unsedated dogs (1, 2). In addition, hyperosmolar mannitol and sucrose are capable of reducing the decline in performance of hypoxic cat papillary muscles (3) and of preventing the morphological changes of swelling in mitochondria in hypoxic cat papillary muscles and in dog hearts with acute myocardial ischemia (4). There have been no data, however, directly demonstrating that hyperosmolar mannitol can reduce infarct size measured histologically. Accordingly the present study was done to quantitate the effect of hyperosmolar mannitol on the amount of necrosis resulting from temporary circumflex coronary artery occlusion in the anesthetized dog.

Methods. Mongrel dogs of either sex weighing 21 to 61 lb were anesthetized with sodium pentobarbital (30 mg/kg) intravenously. The dogs were intubated and ventilated with a Harvard respirator (Model 1063) at approximately 300 ml of room air/kg body wt/min. Sterile surgical technique was used. The chest was opened through the fourth left intercostal space and a segment of the circumflex branch of the left coronary artery was isolated 1-2 cm from its origin. Ischemia was induced by occluding the artery with a silk snare around the artery which was pulled into a small glass tube. The development of myocardial ischemia was confirmed by the development of cyanosis in the posterolateral left ventricular wall and by the development of ST segment elevation in lead II of the ECG. Occlusions were maintained for 40 min and then released to restore coronary blood flow. Dogs that survived the occlusion and release were sacrificed 2-4 days later for determination of infarct size. Lead II of the ECG and femoral arterial blood pressure were continuously recorded on a Grass polygraph (Model 5 P1) during the acute phase of each experiment.

Dogs were randomly assigned to one of two groups. A treated group was given hyperosmolar mannitol intravenously (12.5 g/50 cc = 25% solution) by a Harvard peristaltic pump (Model 1202) at 0.4 ml/min/kg for 30 min prior to coronary occlusion and at 0.2 ml/min/kg during and for 60 min after release of the coronary occlusion. The control group of dogs received isotonic saline at a similar infusion rate. Systemic arterial osmolalities were obtained in both groups of dogs prior to infusion, just prior to occlusion, 20 min after occlusion, and 20 min after release of the occlusion. All of the mannitol-treated dogs utilized in this study had serum osmolalities of at least 330 mOsm at the time of occlusion of the circumflex coronary artery (Fig. 2). Following completion of the mannitol or saline infusion, the dogs' chests were closed and the animals were allowed to recover.

We have shown previously that there is significant variability in the amount of coronary collateral flow among dogs in this model (5). To make certain that we were not preselecting dogs with better intrinsic coronary collateral flow for the treated group, we determined the coronary collateral flow patterns in surviving dogs of *each* group in the *untreated* situation by using the thioflavin S dye technique (5). The chest was reopened 48 hr after release of the occlusion and the circumflex coronary artery reoccluded by silk ligature for 20 sec prior to excision of the heart to determine the qualitative distribution of coronary collateral flow *during* circumflex occlusion.

Thioflavin S (4% solution in normal saline) then was injected (1 cc/kg) intravenously, and 10 sec later the heart was excised. (In our laboratory, myocardium receiving flow of greater than 10–15% of normal, fluoresces yellow green when examined under uv light, while myocardium receiving less flow is nonfluorescent, as determined by radioactive microspheres.) After cooling in ice-cold isotonic KCl, the endocardial distribution of the necrosis was photographed and the posterior papillary muscle and subjacent myocardium (PP + SM) was cut into three longitudinal slices (2–3 mm thick) perpendicular to the surface of the heart. Each of these slices and one through the anterior papillary muscle (control) were photographed under white and ultraviolet light for assessment of the distribution of necrosis and thioflavin S fluorescence, respectively. The slices were fixed in 10% phosphate-buffered formalin and embedded in paraffin for histologic sectioning. Sections were cut from each slice which were stained with hematoxylin and eosin (H and E), periodic acid Schiff (PAS) for glycogen, and Heidenhain's variant of Mallory's connective tissue method (CT). The percentage of necrotic tissue in the posterior papillary muscle (PP) and in the posterior papillary muscle and subjacent myocardium was determined from the histologic slides as described previously (6). In this model, all the cells injured by severe ischemia are histologically necrotic by 24 hr, and the border between normal and necrotic cells can easily be identified. Slides were placed in a photographic enlarger, and the image of necrotic and spared areas in each slide traced on heavyweight biology paper. These areas then were cut out and the paper was weighed to permit calculation of the percentage of necrosis within the papillary muscle, defined by the longitudinal direction of muscle fibers. The percentage of nonfluorescent and of necrotic tissue in transmural sections through the PP and subjacent myocardium was similarly determined by projecting histologic sections or kodachrome slides.

Results. Papillary muscle infarct size was quantitated histologically in nine mannitol and 10 saline control hearts from dogs which survived a 40-min circumflex coronary occlusion and 48 hr of reperfusion. The extent of necrosis was significantly reduced in mannitol-treated dogs. In all of the dogs in both groups myocardial necrosis was observed in the posterior papillary muscle and in the surrounding posterolateral left ventricular wall. In saline-treated dogs there were generally large confluent areas of necrosis (Fig. 1). In contrast, mannitol-treated dogs usually had only focal areas of necrosis, and in a few dogs the lesions were barely visible (Fig. 1). The distribution of the yellow necrotic lesions seen grossly corresponded closely to the distribution of necrosis observed in H and E, PAS, and CT stained sections. Quantitative comparisons (Table I) demonstrated that saline-treated dogs had 62% necrosis of the posterior papillary muscle and mannitol-treated dogs had 27% ($P < .01$). When both the posterior papillary muscle and subjacent myocardium were considered, saline-treated dogs had a mean of 33% necrosis and mannitol-treated dogs had 13% (Table I). Thus, mannitol treatment was associated with approximately a 50% reduction in the amount of myocardial necrosis observed in these animals.

ST segment elevation was observed in lead II in all of the dogs of both groups within 20 to 30 sec following circumflex coronary artery occlusion and reached peak values within 5 to 10 min after occlusion (Table I). Mannitol administration did not affect these ECG changes; peak limb lead II ST segment elevation was 0.36 and 0.38 mV in the saline and mannitol-treated groups of survivors, respectively.

The serum osmolalities and hemodynamic parameters associated with mannitol administration are shown in Fig. 2. Serum osmolality in the saline group of animals averaged 301 mOsm prior to saline infusion and remained constant throughout the experiment. The mannitol-treated group of animals had a similar mean serum osmolality of 304 mOsm prior to mannitol infusion, and this increased to 341 mOsm just prior to occlusion and rose more slowly to achieve a level of 358 mOsm by 20 min after the release of the 40-min occlusion.

Heart rate and mean systemic arterial blood pressure in the mannitol-treated

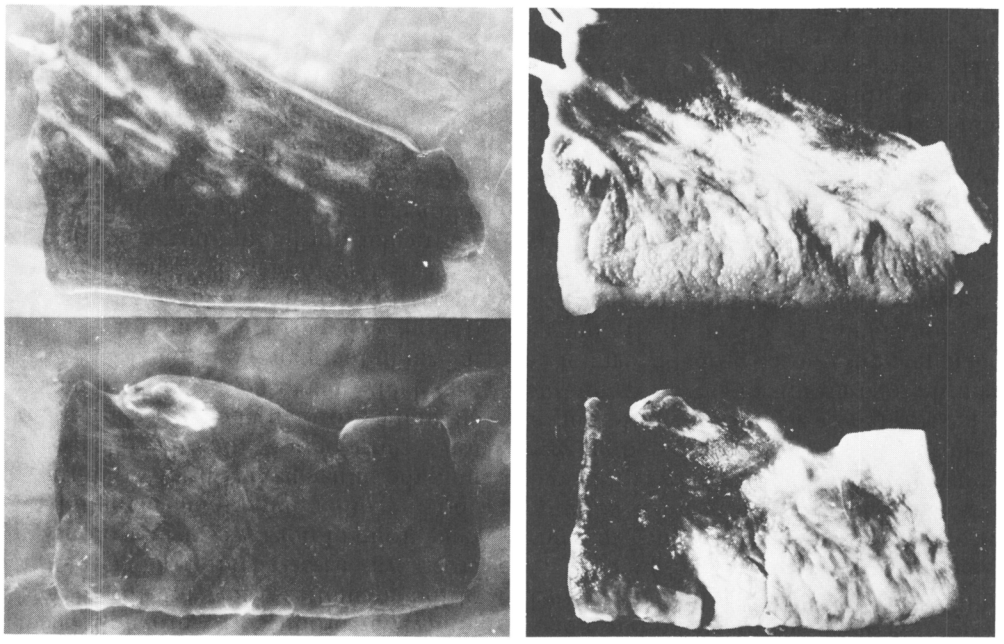

FIG. 1. Transmural slices of left ventricle cut longitudinally through the posterior papillary muscle of a representative saline control dog (top) and a mannitol-treated dog (bottom). Both slices were photographed under white (left) and ultraviolet (right) light. The control slice shows necrosis (light areas) involving much of the posterior papillary muscle and subendocardial myocardium. The mannitol-treated slice shows a small focus of necrosis in the posterior papillary muscle. The uv photographs demonstrate the distribution of areas with significant collateral flow (light areas with yellow-green fluorescence) vs areas of severe ischemia (dark nonfluorescent areas) which were observed at the time of sacrifice when serum osmolalities had returned to control levels in both groups. In the control dog, the distribution of necrosis is similar to the distribution of severe ischemia. Conversely, in the mannitol-treated dog, the area of necrosis is much smaller than the area of severe ischemia.

group did not differ significantly from the saline-treated group either before, during, or after circumflex coronary artery occlusion. There was a significant decrease following occlusion in systolic arterial blood pressure by 26 and 21 mmHg, respectively, in the saline and mannitol-treated groups ($P < .001$); these pressures remained at slightly lower levels throughout the occlusion and following its release.

Survival was not favorably improved by mannitol infusion. Nine of 29 (31%) mannitol-treated dogs and 10 of 17 (59%) controls survived occlusion and 48 hr of reflow (Table II). The difference between groups was not statistically significant (chi-square analysis corrected for small sample size, $P > 0.1$), and considerable variability in mortality is expected with this model and has been reported in previous studies (7). As is apparent from this table, most of the dogs in both groups that died did so either very soon after acute circumflex coronary artery occlusion or during the immediate reflow period.

The higher mortality in the mannitol-treated group of dogs raises the possibility that the differences in infarct size were due to preselection of mannitol survivors with smaller areas of ischemia. This possibility was assessed at the time of sacrifice (when both groups had returned to control osmolalities) by reoccluding the circumflex artery and injecting the fluorescent dye, thioflavin S, intravenously to outline the areas of severe ischemia.

The posterior papillary muscles and subendocardium in both groups of animals had large areas of nonfluorescence, while the subepicardial third of myocardium was usually fluorescent with patchy extensions of fluorescence into the midmyocardium. The percentage of nonfluorescent tissue in the posterior papillary muscles and subjacent myocardium was similar in saline and man-

TABLE I. NECROSIS, ST ELEVATION, AND THIOFLAVIN S NONFLUORESCENCE IN UNTREATED AND MANNITOL-TREATED DOGS SURVIVING 40 MIN OF TEMPORARY CORONARY OCCLUSION.

	Necrotic tissue (%)			
	Posterior papillary muscle (PP) alone	PP + subjacent myocardium	Peak ST, lead II (mV)	Nonfluorescent tissue in PP and subjacent myocardium (%)
Untreated dogs				
2039	72	32	.45	72
2045	17	9	.05	41
2079	69	33	.40	79
2087	11	3	.00	39
2101	67	26	.80	85
2114	88	57	.30	87
2121	60	33	.40	64
2134	62	30	.30	46
2141	88	55	.50	80
2154	84	56	.35	65
Mean ± SE	62 ± 9	33 ± 6	.36 ± .07	66 ± 6
Mannitol treated dogs				
2019[a]	39	19	.20	–
2044[a]	19	6	.10	27
2071	16	5	.60	55
2089	7	3	.05	46
2109	43	25	.50	73
2119	37	18	.60	50
2125	0.4	0.2	.20	76
2161	17	7	.60	88
2164	64	36	.60	–
Mean ± SE	27 ± 7	13 ± 4	.38 ± .08	59 ± 8
P (untreated vs mannitol)	<.01	<.025	N.S.	N.S.

[a] Two dogs from a preliminary study which received somewhat lower doses of mannitol but which attained preocclusion osmolalities of 330 or greater.

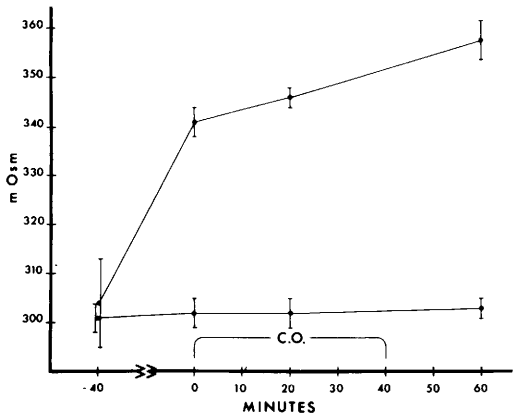

FIG. 2. Serum osmolalities vs time in mannitol-treated and saline control groups. Points are mean values of dogs which survived 40 min of coronary occlusion (C.O.) and reflow and were used for quantitation of infarct size. Brackets indicate ± 1.0 SEM.

nitol-treated dogs (Table I), suggesting that the difference in necrosis in the two groups was not related to an intrinsic difference in coronary blood flow distribution. There was a significant positive linear correlation between the gross percent of necrosis and nonfluorescent tissue in posterior papillary muscle and subjacent myocardium in saline-treated dogs ($r = 0.86$ $P < 0.005$). Mannitol-treated dogs had less necrosis relative to nonfluorescent areas, and the correlation between necrosis and nonfluorescence was not significant.

Discussion. Previous studies have demonstrated that in the anesthesized dog and in isolated dog hearts pretreatment with hypertonic mannitol improves ventricular function, reduces myocardial injury as assessed by epicardial ST segment mapping and increases both total and collateral coronary blood flow (1). Other more recent studies have also shown that mannitol improves ventricular function and produces general increases in regional myocardial blood flow in awake, unsedated dogs with either acute or chronic myocardial ischemia (2); also, recent studies have shown that mannitol increases coronary blood flow sig-

TABLE II. TIME OF DEATH VS SURVIVAL AFTER TEMPORARY CORONARY OCCLUSION IN UNTREATED AND MANNITOL PRETREATED DOGS.[a]

	Total number of dogs	V.F. during occlusion	V.F. at reflow or later[b]	48-hr survivors
Mannitol	29	14 (48%)	6 (20%)	9 (31%)
Saline control	17	4 (24%)	3 (18%)	10 (59%)

[a] None of the differences between groups were significant by chi-square analysis corrected for small sample sizes.

[b] One dog in each group died later than 2 min postrelease, i.e., without reflow V.F.

nificantly in patients with coronary artery disease (8). In hypoxic isolated, isometrically contracting cat papillary muscles mannitol has been demonstrated to be capable of reducing the decline in performance (3) and in well-oxygenated isolated papillary muscles mannitol's direct inotropic effect appears to be related to the regulation of intracellular calcium concentration (9). Studies done in anesthetized dogs have previously demonstrated that mannitol has the ability to influence the "no flow" phenomenon (10) by acting to prevent the increase in coronary vascular resistance and modifying the reduction in reflow to the subendocardial portion of the ischemic area following prolonged coronary artery occlusion (11). However, previously there has been no direct morphological evidence that mannitol has the ability to reduce the amount of necrosis following ischemic injury. Thus, the present study was done to determine directly by histologic quantitation whether or not hyperosmolar mannitol has the ability to decrease necrosis in the anesthetized dog with temporary circumflex coronary artery occlusion. The data obtained in this study demonstrate that although mannitol does not prevent all cell death in acute ischemia, it does reduce the amount of necrosis by about 50% when given prior to and during circumflex coronary artery occlusion.

Although mannitol did not affect peak limb lead II ST segment elevation in this study, it has been shown to decrease epicardial ST segment elevation (1). Most likely, the present results reflect a relative insensitivity to changes in the severity of ischemia of limb lead compared with epicardial lead ST segment elevation.

Although the data suggest that the incidence of VF during occlusion was greater in animals treated with mannitol, the incidence, in fact, is not significantly different in the two groups (chi-square analysis corrected for small sample size). Other investigators have not noted an increased incidence of VF secondary to mannitol (1, 2, 4). However, since more animals died in the mannitol-treated group, one must consider whether the reduced necrosis after mannitol administration was related to removal, by death, of some dogs with larger areas of ischemia. We believe this is unlikely for two reasons: (1) The distribution of collateral flow, revealed by Thioflavin S at the time of sacrifice (Table 1), was similar in the treated and untreated groups. This indicates that the severity of the ischemic injury in the survivors, on which we made our quantitative comparison, was qualitatively similar. (2) Three of the mannitol-treated dogs that fibrillated were defibrillated and infarct size was subsequently determined and found to be 21% of PP.[2] Thus, infarct size was comparable to that found in nonfibrillating survivors treated with this drug.

The mechanism by which hyperosmolar mannitol reduces necrosis following ischemic myocardial injury in the dog is not known but several possibilities deserve mention. One possibility is that hyperosmolar mannitol by remaining predominantly extracellular provides an osmotic force to prevent or reduce myocardial cell swelling which occurs after severe ischemic injury. Myocardial cell swelling has been described during both permanent and temporary ischemia but it is histologically most prominent in irreversibly injured cells after two or more minutes of arterial reperfusion (12–15). Swelling might be deleterious to cells because it might inhibit myocardial metabolism by displacing enzyme systems which require

[2] These animals were not included in the mannitol-treated series analyzed statistically because they had developed ventricular fibrillation.

a close spatial relationship. Furthermore, swelling could result in stretching of the myocardial cell membrane with permeability changes and subsequent loss of important cofactors, enzymes, and/or electrolytes. Previous studies have shown that mannitol prevents mitochondrial swelling in hypoxic and ischemic myocardial cells (4) and cell swelling in hypoxic, isolated Langendorf rat hearts (16). Whether mannitol prevents cell swelling during ischemia *in vivo* or the explosive cell swelling and electrolyte abnormalities which occur at the time of reflow in this model remains to be determined.

A second mechanism by which mannitol could reduce myocardial necrosis is by improving the relationship between oxygen supply and demand. Neither blood pressure nor heart rate was significantly different between the two groups of survivors, and hemodynamic differences probably cannot account for mannitol's effect. Willerson and his associates have shown that mannitol can acutely increase coronary collateral flow to ischemic myocardium (1, 2). The mechanism by which mannitol increases coronary blood flow is unknown but could be related to (i) decreased blood viscosity and red cell sludging, (ii) reduced ischemic cell swelling (2), and/or (iii), a direct effect on smooth muscle vascular resistance (1, 2). Despite acute improvement in collateral flow, the similarity of thioflavin S penetrance in the two groups during brief reocclusion at the time of sacrifice indicates that mannitol does not stimulate any sustained growth of collateral vessels after a 40-min temporary occlusion.

Several previous studies in brain, kidney, and heart have shown that following varying periods of ischemia reflow is at least partially inhibited when blood flow is restored (10, 11, 17–23). Hypertonic mannitol has been shown to be capable of at least partially correcting the reflow defect in all three organs (11, 21, 23). However, in the heart we have not found significant reperfusion defects until after at least 90 min of myocardial ischemia (10, 11). Since there rarely is a "no-reflow" phenomenon after only 40 min of ischemia (10), it seems unlikely that correction of reflow defect after 40 min of myocardial ischemia is the correct explanation for mannitol's ability to decrease infarct size in the model used in these experiments.

Summary. Hypertonic mannitol previously has been shown to improve cardiac function, increase collateral flow, and decrease epicardial ST segment elevation following coronary occlusion in anesthetized or awake dogs. The present study quantitates by morphologic techniques, the effect of hypertonic mannitol on infarct size. Ischemic injury was produced by proximal occlusion of the circumflex artery for 40 min and necrosis was assessed after 48 hr of reflow. One group of dogs was given isotonic saline and the other hypertonic mannitol beginning the infusions just prior to, during, and for a short period after the release of the circumflex coronary artery occlusion. Serum osmolality increased by approximately 40 mOsm in the mannitol group. The administration of hypertonic mannitol was associated with a 40–50% reduction in infarct size measured histologically. The incidence of ventricular fibrillation during occlusion and following release of the circumflex coronary artery occlusion was greater in mannitol-treated dogs although the difference was not statistically significant. Thus, the data obtained in this study extend previous observations and provide direct evidence that hypertonic mannitol can reduce infarct size in dogs with temporary circumflex artery occlusion and reflow.

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