

Intraarterial Infusion of High-Concentration Papaverine Damages Cerebral Arteries in Rats

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PURPOSE: To determine the appropriate concentration of papaverine for therapeutic intraarterial infusion against cerebral vasospasm. **METHODS:** We investigated histopathologic changes in cerebral arteries and brain tissue of normal Wistar rats that had received infusions of papaverine via the carotid artery. Rats were infused with 0.20 mL papaverine (concentration, 0.4% to 4.0%) via the internal carotid artery. Injury to the vascular wall was evaluated by transmission electron microscopy; pathologic changes in cerebral tissue were studied by light microscopy. **RESULTS:** Neither brain necrosis nor brain edema was seen under light microscopy at any concentration. At 4.0% papaverine concentration, degeneration of endothelial cells and medial smooth muscle, including vacuole formation, was observed under electron microscopy. At 1.4% concentration, degeneration of endothelial cells was seen. Extravasation of Evans blue dye was noted when drug concentration exceeded 1.4%. At 0.8% concentration, no histopathologic change was noted. **CONCLUSION:** On the basis of these results, we recommend a papaverine concentration of 0.8% or less for intraarterial infusion.

Index terms: Animal studies; Drugs, effects; Drugs, intraarterial injection; Vasospasm

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The superselective intraarterial infusion of papaverine is effective in the treatment of cerebral vasospasm after subarachnoid hemorrhage (1–7). Superselective infusion of 0.2% (10^{-4} mol/L) papaverine at a site just proximal to the narrowing vessels is considered sufficient to dilate the spastic arteries in most cases. But when papaverine is infused far from the spastic site because of difficulties in catheterization, or when relatively papaverine-resistant vasospasm exists (8), a larger dose or higher concentration of papaverine may be needed to dilate the affected arteries.

We investigated the histopathologic changes in cerebral arteries and brain tissue of normal

Wistar rats who received infusions of papaverine via the carotid artery. The integrity of the blood-brain barrier was examined by intravenous injection of Evans blue dye. Pathologic changes in cerebral tissue were studied by hematoxylin-eosin staining and light microscopy. Injury to the vascular wall was examined by transmission electron microscopy.

Materials and Methods

Animal Preparation

All experimental procedures followed the guidelines set for animal experiments by the National Cardiovascular Center. Male Wistar rats weighing 250 to 300 g were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg). The animals were fixed in the supine position, intubated, and mechanically ventilated. Arterial gas and blood pressure were maintained within the normal range. The carotid artery bifurcation was exposed and the external carotid artery was ligated with a 5–0 nylon suture. After injection of 1 mL of 2% Evans blue dye into the femoral vein, 0.2 mL of papaverine hydrochloride (diluted with saline to concentrations of 0.4 wt/vol% [2×10^{-4} mol/L], 0.8 wt/vol% [4×10^{-4} mol/L], 1.4 wt/vol% [7×10^{-4} mol/L], 2.0 wt/vol% [1×10^{-3} mol/L], and 4.0 wt/vol% [2×10^{-3} mol/L]) or 0.2 mL of saline was injected into the

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left common carotid artery via a 27-gauge needle. The concentrations of papaverine were determined on the basis of clinical use. We used 0.4% to 4.0% concentrations to evaluate the upper concentration limits of papaverine for safe therapeutic intraarterial infusion, although the generally accepted concentration of papaverine for clinical use is 0.2% to 0.4%. The 0.2 mL volume was injected at different infusion rates (0.02, 0.20, or 2.00 mL/min) using a microinjection pump (CMA/100, Carnegie Medicine, Stockholm, Sweden). The hole in the common carotid artery made by the needle was repaired with 10-0 nylon. Six hours after the infusion, the rats were reanesthetized and transcardially perfused with ice-cold saline followed by 10% buffered formalin.

Extravasation of Evans Blue Dye

The brain was removed and cut into 2-mm-thick coronal sections. Extravasation of Evans blue dye was identified in a section including the caudoputamen nucleus; the area of staining was measured with an image analyzer (Video Micro Meter VM-30, Olympus, Tokyo, Japan). The percentage of the area of extravasation was calculated as $100 \times \text{area of Evans blue dye extravasation} / \text{area of the ipsilateral hemisphere}$.

Electron Microscopic Examinations

Six male Wistar rats weighing 250 to 300 g were used in each group. Six hours after the intraarterial infusion of papaverine at 2.0 mL/min, the brain was perfused for 2 hours at 4°C with cold normal saline, or with saline that included 2% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4). The intracranial internal carotid and middle cerebral arteries on the left side were dissected under a microscope and cut into small pieces. Subsequently, the specimens were rinsed in the same buffer, postfixed with 2% osmium oxide (OsO_4) in the same buffer, stained overnight with 1% uranyl acetate (pH 5.0), dehydrated by a graded-ethanol series, and embedded in Epon 812 (Taab, Berkshire, England). Ultrathin sections were examined with transmission electron microscopy at accelerating voltage of 75 kV.

TABLE 1: Extravasation of Evans blue dye

Area Rate of Extravasation, %*	Injection Rate, mL/min		
	0.02	0.20	2.00
Saline	0	0	0
Papaverine wt/vol% (mol/L)	0	0	0
0.4 (2×10^{-4})	0	0	0
0.8 (4×10^{-4})	0	0	0
1.4 (7×10^{-4})	0	0	15.2 ± 6.0
2.0 (1×10^{-3})	0	0	23.6 ± 4.8
4.0 (2×10^{-3})	0	7.2 ± 1.2	31.9 ± 5.1

* $100 \times \text{area of extravasation} / \text{area of ipsilateral hemisphere}$.

Note.—n = five in each group; values are expressed in mean \pm SEM.

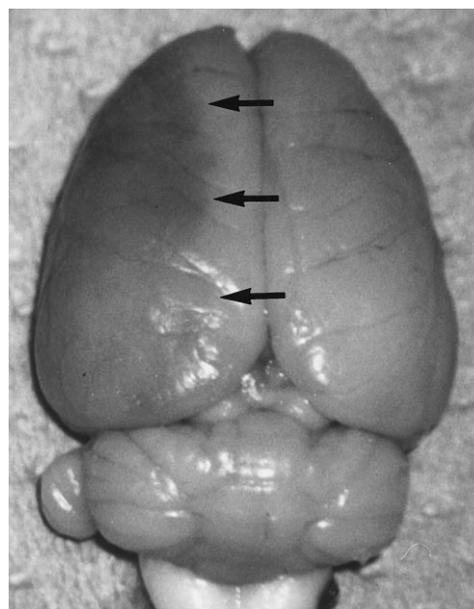


Fig 1. Photograph of rat brain surface after intraarterial infusion of the highest concentration (4.0%) of papaverine shows severe extravasation of Evans blue dye in the ipsilateral cerebral hemisphere (arrows).

TABLE 2: Change in the arterial wall on transmission electron microscopy

Concentration	Vacuole in the Endothelium (n = 6)	Vacuole in the Media (n = 6)
Saline	0	0
Papaverine, %		
0.4	0	0
1.4	4	0
4.0	6	4

Note.—Samples were obtained from the middle cerebral artery.

Results

Extravasation of Evans Blue Dye

Areas of staining with Evans blue dye after infusion of saline or the various concentrations of papaverine are shown in Table 1. No extravasation was noted at papaverine concentrations up to 0.8%, irrespective of the infusion rate. Extravasation was noted in rats who had been infused with 1.4% or higher concentrations of papaverine at the highest infusion rate (2.0 mL/min). The area of staining that received the highest concentrations of papaverine is shown in Figure 1. At the highest concentration (4.0%), extravasation was seen even when the 0.2 mL/min infusion rate was used.

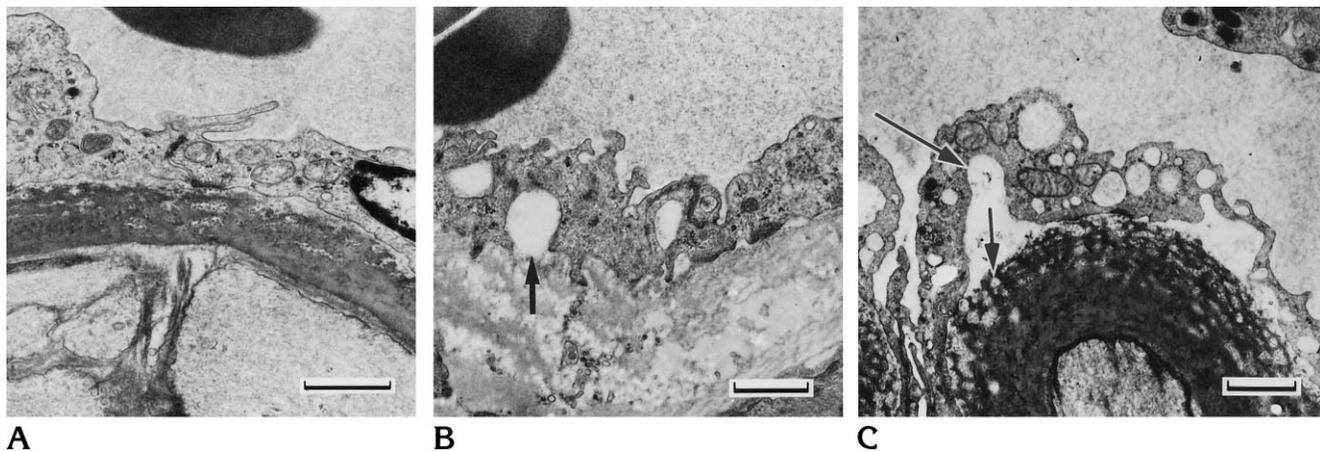


Fig 2. Transmission electron micrographs of the ipsilateral middle cerebral artery in rats after infusion of varying concentrations of papaverine (bar represents 1 μm ; original magnification $\times 8000$).

A, After infusion of 0.8% papaverine, no change is seen in the endothelium or media.

B, After infusion of 1.4% papaverine, vacuole formation is seen in the endothelium (arrow).

C, After infusion of 4.0% papaverine, vacuole formation is seen in the endothelium, which peeled off from the internal elastic lamina (long arrow). Vacuoles are also seen in the media (short arrow).

Light Microscopic Findings

No apparent difference in cerebral tissue was noted among the groups that received different concentrations of papaverine at different infusion rates. Even among the rats who received the highest concentration of the drug (4.0%) at the highest infusion rate (2.0 mL/min), no histologic difference was noted from the rats who were infused with saline.

Electron Microscopic Findings

The transmission electron microscopic findings are summarized in Table 2. No abnormal findings were seen after infusion of saline or after infusion of the lowest concentration (0.4%) of papaverine (Fig 2A). Vacuole formation in the endothelium was seen in rats infused with 1.4% papaverine (Fig 2B). At 4.0% concentration, papaverine induced the formation of many vacuoles in the endothelium of all six rats. At this concentration, injured endothelial cells peeled off from the internal elastic lamina. Vacuole formation in the internal elastic lamina was seen in four of six rats (Fig 2C).

Discussion

Intraarterial infusion of papaverine for cerebral vasospasm has been considered safer than percutaneous transluminal angioplasty (9–11), because it does not carry the risk of vessel rupture; however, transient brain stem depression,

mydriasis, change in mental status, and hemiparesis have been reported in patients treated with papaverine infusions for cerebral vasospasms (12–14). These side effects may be due to a microembolism (15), the neurodepressive effect of papaverine, or the constriction of microvessels (16). Furthermore, papaverine hydrochloride is an acidic mixture, and its high acidity may cause injury to the arterial wall as well as relaxation of the spastic artery.

Neither brain necrosis nor brain edema was seen under light microscopy at any concentration. At 4.0% concentration, degeneration of endothelial and smooth muscle cells, including vacuole formation, was observed under electron microscopy. At 1.4% concentration, degeneration of endothelial cells was seen. Extravasation of Evans blue dye was noted when the drug concentration exceeded 1.4%. At 0.8%, no histologic change was observed. Our findings indicate that at concentrations less than 0.8%, papaverine does not cause injury to the infused arteries in rats.

In our previous *in vitro* experiment, we found that 0.2% (10^{-4} mol/L) papaverine induces maximum dilatation in both normal and spastic arteries after subarachnoid hemorrhage in humans (5). In the case of intraarterial infusion, the drug may be diluted by blood, and luminal surfaces at different sites may be exposed to different drug concentrations. Intraluminal drug concentration depends on the injection site, the caliber of the vessel, the speed of injection, and

other factors. If dilution of the drug is expected, one may use higher concentrations when the catheter is placed at a site far from the narrowed segment. It is possible that unexpectedly high concentrations may reach perforators or distal arteries, resulting in adverse effects. The use of lower concentrations is also recommended to avoid formation of microembolisms (14). To prevent the accidental delivery of insufficiently diluted papaverine, the concentration should not exceed 0.8%. Although healthy rats were used in this study, it may be worthwhile to investigate the histologic changes of spastic arteries and brain tissue in a model of subarachnoid hemorrhage after infusion of papaverine in order to obtain more information about the relationship between papaverine concentration and clinical vasospasm.

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