Sustained release of papaverine for the treatment of cerebral vasospasm: *in vitro* evaluation of release kinetics and biological activity

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Cerebral vasospasm remains an unpredictable and inadequately treated complication of aneurysmal subarachnoid hemorrhage. To date, pharmacological treatment has been plagued in part by an inability to attain sufficiently high concentrations of vasodilator drug in the cerebrospinal fluid without precipitating systemic side effects such as hypotension. To circumvent this limitation of current pharmacological therapy, the authors have developed a sustained-release preparation of papaverine that can be implanted intracranially at the time of surgery for aneurysm clipping. *In vitro* evaluation of drug-release kinetics has demonstrated that reliable, sustained release of effective amounts of papaverine is possible. An *in vitro* bioassay using isolated preparations of canine basilar artery has confirmed the biological activity of this preparation. These *in vitro* studies are described.

**KEY WORDS** • cerebral vasospasm • pharmacokinetics • papaverine • sustained-release polymer

Cerebral vasospasm complicates aneurysmal subarachnoid hemorrhage (SAH) in as many as 60% to 70% of cases. When severe (that is, when vessel diameter is reduced by at least 50%), vasospasm can be associated with permanent ischemic neurological deficits. While the computerized tomography appearance may help to identify those patients at greater risk for developing symptomatic vasospasm, the problem remains essentially unpredictable. Therefore, optimum treatment must be safe enough to be administered prophylactically to all patients at risk. Presently, such treatment amounts to the use of the calcium channel blocker nimodipine, and of hypervolemia and induced hypertension. These treatment strategies have improved the long-term outcome following aneurysmal SAH, however, they have not been shown to reverse the vascular narrowing itself and have not eliminated the problem of delayed ischemic neurological injury. Furthermore, each of these strategies is associated with complications (aggravated cerebral edema, intracerebral hemorrhage, systemic hypotension) that can aggravate the existing neurological deficits.

Experimental work has repeatedly shown that vasodilator drugs can reverse established angiographically identified vasospasm when administered by the intrathecal route, despite being ineffective when administered by the intravenous or intra-arterial route. Under such circumstances, the desired pharmacological effect can be achieved with doses of drug that minimize the risk of systemic hypotension. The morbidity associated with long-term intrathecal drug administration through indwelling catheters has precluded the practical application of these experimental results to the clinical setting. We have attempted to address this issue by developing a sustained-release form of papaverine that could be implanted intracranially at the time of surgery for aneurysm clipping. Here we report the results of *in vitro* controlled release and physiological studies using this drug preparation.

**Materials and Methods**

**Preparation of the Sustained-Release Matrix**

Purified ethylene vinyl acetate copolymer (EVAc)* was dissolved in methylene chloride to give a 10% wt/vol solution. A mass of papaverine hydrochloride equal to 20%, 30%, or 40% of the weight of EVAc was added to the polymer in solution. A proportional amount of glucose was then added so that the total mass of solute (drug and glucose) equaled the weight of EVAc in solution. The resulting mixture was then agi-

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* Ethylene vinyl acetate copolymer Elvax obtained from Du Pont Co., Wilmington, Delaware.
† Papaverine was a generous gift of the former Rorer Pharmaceuticals, Horsham, Pennsylvania.
tated in a vortex mixer to yield a uniform suspension, which was immediately poured into a glass mold precooled in a bath of dry ice and acetone to \(-78^\circ\text{C}\). The suspension gelled immediately, thus minimizing drug precipitation. The solid matrix was dried for 48 hours at \(-20^\circ\text{C}\), then for an additional 48 hours in a vacuum at room temperature to evaporate the methylene chloride.\(^7\) The result was a sheet of EVAc uniformly impregnated with 20%, 30%, or 40% papaverine hydrochloride by weight (a 20%, 30%, or 40% drug load). Discs of varying diameter were cut from this sheet.

**In Vitro Drug-Release Kinetics**

A disc cut from the papaverine-impregnated sheet of EVAc was weighed, then suspended in a standard 25-ml jacketed organ bath containing gas-bubbled Krebs-Ringer buffer (120 mM NaCl, 4.5 mM KCl, 1 mM MgSO\(_4\), 27 mM NaHCO\(_3\), 10 mM Glu, 2.5 mM CaCl\(_2\), and 1 mM NaH\(_2\)PO\(_4\)), at pH 7.4 and 37\(^\circ\text{C}\). Buffer was continuously replaced at a rate of 25 ml/hr using a peristaltic pump to approximate the rate of secretion of cerebrospinal fluid (CSF) in man. Buffer overflow was collected under vacuum and assayed by ultraviolet spectrophotometry for papaverine hydrochloride. Incubations were continued uninterrupted for 10 days to determine the long-term in vitro drug-release kinetics.

An aliquot of buffer was assayed for papaverine by measuring the ultraviolet absorbance at 237 nm using an ultraviolet spectrophotometer.\(^6\) Drug concentration was determined by comparing the optical density of a sample with that of known standard concentrations of papaverine prepared daily in Krebs-Ringer buffer. The amount of drug released per hour can be determined from the volume of buffer collected and the concentration of papaverine in that volume.

**In Vitro Physiological Studies**

The basilar artery was harvested from mongrel dogs sacrificed by exsanguination. A vessel ring, 4 mm in length and cut from the parent artery, was immobilized over rigid stainless-steel wire prongs in a standard 25-ml jacketed organ bath containing gas-bubbled Krebs-Ringer buffer, at pH 7.4 and 37\(^\circ\text{C}\). The vessel was stretched to a predetermined optimum length and allowed to equilibrate for 1 hour.

Vessel rings were contracted using serotonin (10\(^{-5}\) M) or KCl (20 mM). Isometric tension was measured using a force transducer and recorded with a strip-chart polygraph.\(^6\) A papaverine-impregnated disc, 30% drug-loaded and 20 mm in diameter, was suspended in the experimental chamber in such a way that it did not interfere with the physiological recording. A blank disc was placed in the control chamber. Isometric tension was recorded continuously for 10 to 40 minutes and was expressed as a percentage of the maximum force induced by serotonin or KCl.

**Results**

**In Vitro Drug-Release Kinetics**

The sustained release of papaverine from drug-loaded discs, 10 mm in diameter and incubated in Krebs-Ringer buffer for 220 hours, is shown in Fig. 1 *upper left*. There was a typical initial burst of drug release.\(^5\)\(^6\)\(^7\) A sustained linear rate of drug release was achieved after 4 hours of incubation. Discs that were 30% or 40% loaded with papaverine released drug at approximately 150 \(\mu\text{g/hr}\), resulting in a steady-state concentration of drug in the organ bath equal to 1.6 \(\times\) 10\(^{-5}\) M. At this concentration, papaverine is an effective vasodilator *in vitro*.\(^3\) A 20% drug-loaded disc released papaverine at 50 \(\mu\text{g/hr}\).

The total amount of drug released during the course of the incubation is shown in Fig. 1 *upper right*. This was determined by the release kinetics of the drug/polymer matrix, the percentage of drug loading, and the size of the disc in question.\(^4\)\(^5\)\(^6\) When these data are expressed as a cumulative percentage of the total drug load (dry weight of the disc \(\times\) % drug loading) released during the incubation (Fig. 1 *lower*), it becomes clear that, while the 30% and 40% drug-loaded discs released comparable amounts of papaverine, the rate of drug release was greatest from a disc loaded with 30% papaverine and 20% glucose. By 220 hours, approximately 33% of the papaverine had been released from the 30% drug-loaded disc. During the same interval, approximately 19% of the drug had been released from the 20% and 40% drug-loaded discs.

**In Vitro Physiological Studies**

When placed in an organ bath containing a single dog basilar artery ring, a disc 20 mm in diameter and containing a 30% load of papaverine effected a prompt and dramatic reduction in the isometric tension induced by 10\(^{-5}\) M serotonin. The disc had been prewashed to eliminate the burst effect of drug release. After 40 minutes of incubation, isometric tension was reduced to 10% of the baseline maximum force induced by serotonin. In contrast, the isometric tension of the single control vessel ring incubated with a blank disc was equal to 85% of the baseline maximum tension (Fig. 2 *left*). In a second pair of arterial rings constricted using serotonin as described, a similar result was observed. The isometric tension of the vessel ring incubated in the presence of a papaverine-loaded disc was reduced to 16% of the baseline maximum force by 10 minutes. In contrast, the isometric tension of the control vessel ring remained at 70% of the maximum force induced by serotonin. Statistical analysis was not possible because of small sample size.

The discs were removed from the baths. Vessel rings
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were washed with 100 ml of Krebs-Ringer buffer, allowed to equilibrate, and constricted with 20 mM KCl. When stable isometric tension was achieved, drug-loaded or blank discs were placed in the organ baths. As shown by the example illustrated in Fig. 2 right, the drug-loaded disc caused a prompt reduction in isometric tension. By 20 minutes, the isometric tension had been reduced to less than 50% of the maximum force generated. Isometric tension of the control artery remained at greater than 90% of the baseline value during the same interval. In a total of five vesseltings constricted with KCl and incubated for 10 minutes with papaverine-loaded discs (30% drug load), the mean isometric tension (+_ standard deviation) was 56.8% _+ 15.86% of the maximum force induced. In the five control vesselings incubated with blank discs, the mean isometric tension was 97.4% _+ 15.05% of the baseline value. This difference is statistically significant (p < 0.05, one-tailed Student t-test for the difference between means).

Discussion

While the incidence and severity of delayed ischemic neurological deficits following SAH have been reduced by the combined use of calcium channel blockers, induced hypertension, and hypervolemia, intracranial arterial spasm remains a principal cause of morbidity and mortality in patients following the rupture of a berry aneurysm. Furthermore, the incidence of vasospasm itself has not been diminished by current treatments, which are assumed to be effective by improving cerebral perfusion despite the presence of angiographically detectable vasospasm or by a direct neuronal protective effect. It is reasonable to suggest that, if the vascular constriction itself could be prevented or minimized, morbidity due to vasospasm would be further reduced.

One possible explanation for the failure of systemically administered vasodilator drugs to reverse cerebral vasospasm may be a failure to achieve therapeutic drug concentrations in the CSF without using doses that also cause systemic hypotension. There is experimental evidence indicating that these drugs can be effective cerebral vasodilators when administered directly into the subarachnoid space while being ineffective when delivered via the parenteral or oral route. Voldby, et al., demonstrated the reversal of acute cerebral vasospasm in dogs by injecting 100 mg of nimodipine directly into the lateral ventricle. Systemic hypotension was avoided because only nanomolar concentrations of nimodipine were achieved in plasma. Gioia, et al., demonstrated a similar effect of nimodipine on chronic vasospasm when the drug was administered into the cisterna magna of dogs. Systemic hypotension was insignificant when 4 ml of 10^-7 M nimodipine was injected into the cisterna magna. Oral and sublingual drug administration resulted in protracted hypotension without a salutory effect on vasospasm.

Kuwayama, et al., studied the effect of papaverine on experimental cerebral vasospasm in dogs. Papaver-
ine administered by the intravenous route caused transient hypotension and mild hyperpnea without reversing the vasospasm. When papaverine was administered by the intra-arterial route, there was a prompt but transient reversal of vasospasm complicated by severe hypotension and hyperpnea. Papaverine, 15 mg, injected directly into the cisterna magna produced a prompt and less transient vasodilatation but was also associated with hypotension. However, the local concentration of papaverine achieved by intracisternal drug administration greatly exceeded the maximum concentration required to dilate the basilar artery. If one assumes that the 15 mg papaverine administered was immediately dispersed in a volume equal to the entire volume of CSF in man (a volume much greater than that in the cisterna magna of dogs), then the concentration of papaverine achieved (0.3 mM) would greatly exceed the maximum effective vasodilating concentration of this drug (0.03 mM). One can assume that the concentration of papaverine achieved in the posterior fossa of dogs likely exceeded 0.3 mM. The risk of systemic side effects from the intrathecal administration of papaverine might therefore be lessened without reducing effectiveness by using a lower dose of the drug.

Ogata, et al., first evaluated the efficacy of papaverine administered via the intrathecal route in the treatment of experimental vasospasm. These investigators induced vasospasm of the basilar artery by instilling autologous blood into the basal cisterns of monkeys by an open surgical procedure. Those monkeys demonstrating vasospasm on angiography on Day 8 were treated with papaverine administered directly into the sylvian fissure through externalized polyethylene tubes. The investigators were able to demonstrate a dose-dependent reversal of vasospasm. Drug concentrations ranging from $8 \times 10^{-3}$ to $8 \times 10^{-5}$ M reversed vasospasm without toxic side effects. The vasodilating effect was transient in all cases, never lasting more than 24 hours beyond termination of the infusion. Therefore, long-term drug delivery in this manner would be required for effective treatment of vasospasm following SAH. The risk of central nervous system infection associated with chronic, externalized indwelling catheters, however, has precluded this form of therapy on a prophylactic basis. The importance of prophylactic treatment is emphasized by pathological studies of cerebral arteries that have been in a state of chronic spasm, which have shown morphological changes in the intima and media; at this point, vasodilator therapy may be ineffective. A sustained-release preparation of papaverine small enough to be implanted in the subarachnoid space at the time of surgery for aneurysm clipping could continuously deliver drug into the subarachnoid space without the risk of infection attributed to externalized catheters and, therefore, might be used prophylactically.

In this paper, we report the results of in vitro testing of just such a sustained-release preparation of papaverine. We have demonstrated a continuous steady rate of drug release into Krebs-Ringer buffer over a 10-day period. We selected Krebs-Ringer as the incubation buffer because it resembles CSF in ionic strength and composition. Papaverine could be expected to have a similar solubility and to demonstrate similar release kinetics in CSF. The stable concentration of papaverine achieved in the buffer was approximately $1.6 \times 10^{-5}$ M, which is of the same order of magnitude as the maximum effective vasodilating concentration of papaverine in vitro. Drug release rates can be regulated as shown by modifying the composition and surface area of the sustained-release matrix. Ethylene vinyl acetate copolymer was selected as the sustained-release
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vehicle because, when properly purified, it is noninflammatory, is well tolerated even when implanted in the cornea, and has seen clinical use as a sustained-release vehicle.\(^5\)\(^1\)

The biological activity of our sustained-release preparation was demonstrated using a standard bioassay of basilar artery contraction.\(^5\) As our results indicate, there was a prompt and dramatic reversal of isometric tension in dog basilar artery rings regardless of whether the smooth-muscle tone was induced by a receptor ligand interaction (serotonin) or by the nonselective depolarization of smooth-muscle cells (20 mM KCl). Standard gas autoclaving of the sustained-release preparation using ethylene oxide did not alter the biological activity.

Intrathecal vasodilator therapy for the treatment of cerebral arterial spasm should minimize the risk of systemic hypotension and could complement the effect of calcium channel blockers. Therefore, continued research is needed to determine if the predicted efficacy of our implantable, sustained-release preparation of papaverine can be realized in an animal model of cerebral vasospasm.

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References


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