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Pharmacokinetics of controlled-release polymers in the subarachnoid space after subarachnoid hemorrhage in rabbits

GUSTAVO PRADILLA, M.D., PAUL P. WANG, M.D., FEDERICO G. LEGNANI, M.D., JAMES L. FRAZIER, M.D., AND RAFAEL J. TAMARGO, M.D.

Department of Neurosurgery, School of Medicine, The Johns Hopkins University, Baltimore, Maryland; and Ospedale San Gerardo, Monza, Italy

Object. Implantation of controlled-release polymers into the subarachnoid space to deliver drugs for treatment of vasospasm after subarachnoid hemorrhage (SAH) is currently of interest. Among the issues regarding local delivery of drugs in the subarachnoid space, however, are the extent of diffusion and the rate of release of the loaded agents. In this study Evans blue dye (EBD) was loaded into controlled-release polymers and its pharmacokinetic properties were determined in vitro and in vivo by using a rabbit model of SAH.

Methods. Ethylene–vinyl acetate copolymer (EVAc) was loaded 40% (w:w) with EBD and its pharmacokinetics were spectrophotometrically determined in vitro by examining three EBD–EVAc polymers. Additional polymers were implanted either into the frontal lobe or into the cisterna magna of 16 New Zealand White rabbits. Subarachnoid hemorrhage was induced in eight of the animals by an injection of 1.5 ml of arterial blood into the cisterna magna. The animals were killed 3 or 14 days postoperatively, their brains and spinal cords were harvested, and samples of each were placed in formamide for dye extraction and quantification. Specimens were examined macroscopically and the concentrations of EBD were determined with the aid of a spectrophotometer.

The EBD–EVAc polymers continuously released EBD over a 133-day period. The controlled release of the dye into the subarachnoid space in either location resulted in staining of the entire central nervous system (CNS) in rabbits when the polymers were placed either on the frontal lobe or in the cisterna magna. The EBD diffusion covered a distance of at least 40 cm. The presence of blood in the subarachnoid space did not interfere with the diffusion.

Conclusions. In this study the authors define the rate and extent of diffusion of EBD from controlled-release polymers placed in the subarachnoid space under conditions of SAH. Evans blue dye diffused through the entire rabbit CNS, covering a distance greater than that of the longest dimension of the hemicircumference of the subarachnoid space around the human brain. The pharmacokinetic properties of EBD–EVAc polymers are comparable to those of antivasospasm agents that are successfully used in animal models of SAH.

KEY WORDS • subarachnoid hemorrhage • vasospasm • controlled-release polymer • drug delivery • rabbit

CHRONIC vasospasm following aneurysmal SAH results from physiological changes that occur primarily in the subarachnoid space.[16,21,28] Vasospasm is the delayed luminal narrowing of intracranial arteries after those vessels are exposed to blood in the subarachnoid space.[15]

We and others have previously shown in animal models of vasospasm that the implantation of controlled-release polymers adjacent to arteries exposed to blood prevents vasospasm. Controlled-release polymers are devices into which drugs can be incorporated and released locally at the site of polymer implantation.[4,5,23,24,27] Local delivery of drugs directly into the subarachnoid space to treat CPHV has several theoretical advantages, such as bypassing the blood–brain barrier, decreasing systemic toxicity, and increasing the concentration of the drug at treatment sites.[14,22] In these models, however, the drug in the polymer has to diffuse a distance of only 5 to 10 mm to reach the blood-exposed arteries.[9,20,25,26]

One of the issues related to the local delivery of drugs in the subarachnoid space using controlled-release polymers is the potential extent and rate of diffusion of agents inserted in the human subarachnoid space. Diffusion of drugs in the subarachnoid space is determined primarily by molecular weight[18] and lipid/water solubility,[19] and can be affected by the presence of blood in the area. To assess whether polymeric drug delivery in the subarachnoid space of humans could effectively reach distant arteries in that area we tested the rate and extent of diffusion of a diazole dye delivered by controlled-release polymers placed at two locations within the subarachnoid space of the rabbit, in which the length of the CNS is approximately 40 cm. This length is greater than

Abbreviations used in this paper: CNS = central nervous system; CPHV = chronic posthemorrhage vasospasm; CSF = cerebrospinal fluid; DETA-NO = diethylenetriamine–nitric oxide; EBD = Evans blue dye; EVAc = ethylene–vinyl acetate copolymer; PBS = phosphate-buffered saline; SAH = subarachnoid hemorrhage.

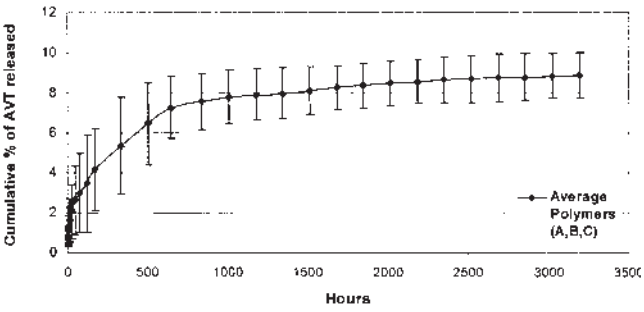

FIG. 1. Graph of the pharmacokinetic profile of EBD–EVAc polymers in vitro demonstrating ongoing sustained release of EBD over 3192 hours (133 days). AVT = azovannatrium (EBD).

the hemicircumferential distance of the human brain, which measures approximately 25 cm at its widest point.

In this study we evaluated the extent and rate of diffusion of a diazole dye with molecular and diffusion features similar to those of antivasospasm drugs that have been delivered by controlled-release polymers in animal models of SAH.

Materials and Methods

Polymer Preparation

Ethylene–vinyl acetate copolymer, 40% vinyl acetate by weight (Elvax 40W; DuPont, Wilmington, DE), and EBD (Sigma Chemical Co., St. Louis, MO) were dissolved into methylene chloride (Fisher Chemicals, Fairlawn, NJ) at a 6:4 ratio, as we have previously described.[23] Briefly, 2 ml of the solution was placed with the aid of a pipette in cylindrical glass molds (5 × 27 mm) at −70°C. The frozen mixture was extracted from the glass molds with a cold spatula. Methylene chloride was allowed to evaporate for 3 days at −20°C. The polymers were subsequently placed in a dessicator at room temperature for 5 days. From this procedure, dry 40% EBD–EVAc polymer rods were obtained, with a mean weight of 40 mg, a length of 12 mm, and a diameter of 1.5 mm.

Pharmacokinetic Studies

In Vitro Pharmacokinetics. The release rate of EBD from the 40% EBD–EVAc polymers was first determined in vitro. A standard absorbency curve at 620 nm was defined for EBD concentrations between 0 and 0.0025 mg/ml in Dulbecco PBS (Invitrogen Co., Grand Island, NY) at 37°C by using a Spectronic Genesis 5 spectrophotometer (Spectronics Instruments, Rochester, NY). Three 10-mg 40% EBD–EVAc polymers were suspended in separate glass vials, each containing 10 ml of PBS at 37°C. The polymers were sequentially transferred to fresh 10-ml aliquots of PBS at 30 minutes and at 1, 3, 6, 12, 24, 48, 72, 168, 336, 504, and 672 hours, after which the amount of EBD was determined spectrophotometrically by a comparison with the standard absorbency curve (Fig. 1).

In Vivo Pharmacokinetics. The brain and spinal cord of rabbits were harvested en bloc and samples were obtained from the frontal lobe, cerebellum, and brainstem, as well as the cervical, thoracic, and lumbar spinal cord. Tissue samples were weighed, immersed in 3 ml formamide (Aldrich Chemical Co., Milwaukee, WI), sonicated in a water bath, and incubated at 60°C for 24 hours to extract the EBD. Formamide suspensions with the extracted EBD were analyzed spectrophotometrically at 620 nm to establish the concentrations of the dye.

Experimental Design. The animals were randomized to two major experimental groups, each of which had a cohort with and another without SAH. Animals in Group 1 (eight animals) underwent SAH (four animals) or no hemorrhage (four animals) and were implanted with 40% EBD–EVAc polymers in the cisterna magna; the animals were killed at either 3 or 14 days postoperatively. Animals in Group 2 (eight animals) underwent SAH (four animals) or no hemorrhage (four animals) and were implanted with 40% EBD–EVAc polymers on the surface of the right frontal lobe; these animals were also killed at 3 or 14 days postoperatively.

Animal Preparation

Sixteen New Zealand White rabbits (*Oryctolagus cuniculus*; Robinson Services, Inc., Winston-Salem, NC), each weighing between 1.5 and 2.5 kg, were used in this experiment. The rabbits were kept in standard animal facilities, one animal per cage, and given free access to Baltimore city water and rodent chow; the Animal Care and Use Committee of The Johns Hopkins University School of Medicine approved all experimental protocols.

Anesthesia. The animals were anesthetized with a mixture of ketamine (50 mg/kg [100 mg/ml ketamine HCl; Abbott Laboratories, Chicago, IL]) and xylazine (10 mg/kg [100 mg/ml XYLA-JECT; Phoenix Pharmaceutical, St. Joseph, MO]), which was injected intramuscularly.

Surgical Technique. After induction of anesthesia and prior to surgery, the cervical or cranial region was shaved with clippers and prepared with alcohol and povidone–iodine solution. Ceftriaxone sodium (50 mg/kg) was prophylactically administered intramuscularly.

Polymer Implantation

Cisterna Magna Implantation. A midline incision was made in the suboccipital region from the inion to C-1. The underlying connective tissue was dissected from the occipital bone and lamina of C-1 and the dura mater was exposed and opened parasagittally in a linear fashion. The 40% EBD–EVAc polymer was placed in the cisterna magna, after which the dura was closed with cyanoacrylate and a piece of Gelfoam was fitted into the insertion site. The fascia was closed using a running 3-0 Vicryl suture and the skin was closed with staples.

Frontal Lobe Implantation. A midline incision was made extending from the midpupillary line to the lambdoid suture. The galea was dissected off the skull, a burr hole was made using a high-speed drill, and a 1 × 1–cm craniectomy was made. The dura mater was opened with a 16-gauge needle and an EBD-containing polymer was inserted. The dura was closed with cyanoacrylate and a collagen hemostat was fitted into the insertion site. The skin was closed with a running 2-0 nylon suture.

Induction of SAH

Using a 26-gauge needle, we aspirated CSF from the cisterna magna through the exposed atlantooccipital membrane. The central ear artery of the rabbit was identified and between 1.5 and 2 ml of blood was aspirated from the vessel by using a 25-gauge butterfly needle. The blood was slowly reinjected into the cisterna magna through the exposed dura mater. The rabbits were placed prone and inclined 30° with the head down for 30 minutes to ensure blood clot formation around the basilar artery.

The rabbits were monitored postoperatively until they were mobile and then they were transferred to their cages and observed. Animals displaying signs of pain or discomfort were given buprenorphine (0.01–0.05 mg/kg subcutaneously every 6–12 hours) and acetaminophen (10–15 mg/kg per rectum every 4–6 hours). To kill the rabbits, anesthetic agents were given as previously described and sodium pentobarbital was administered by an intracardiac injection.

Timing of Polymer Placement

The EBD–EVAc polymers were put in place 30 minutes after SAH in cohorts with SAH and immediately after exposure of the dura mater in the non-SAH cohorts.

Statistical Analysis

The concentrations of EBD in the CNS of rabbits are presented as micrograms of EBD extracted per gram of tissue and are expressed as mean values ± standard errors of the means. These values were

TABLE 1

*Extraction values of EBD in rabbits with SAH**

Location	EBD (μg/g tissue)			
	No SAH		SAH	
	Day 3	Day 14	Day 3	Day 14
Group 1—cisterna magna				
frontal lobe	0.86 ± 0.50	1.26 ± 0.18	1.15 ± 0.52	2.05 ± 0.00
cerebellum/brainstem	2.60 ± 1.31	1.93 ± 0.13	1.84 ± 0.09	1.63 ± 0.25
cervical region	3.63 ± 0.06	6.76 ± 1.66	6.01 ± 3.61	7.28 ± 6.54
thoracic region	3.62 ± 1.05	3.48 ± 0.07	2.45 ± 0.87	1.03 ± 0.08
lumbar region	1.93 ± 0.74	3.50 ± 1.13	6.46 ± 3.33	2.15 ± 0.36
Group 2—frontal lobe				
frontal lobe	0.50 ± 0.32	3.27 ± 0.35	2.64 ± 0.45	1.30 ± 0.00
cerebellum/brainstem	1.20 ± 0.02	2.47 ± 0.81	1.41 ± 0.00	1.29 ± 0.16
cervical region	0.68 ± 0.14	3.12 ± 0.89	3.20 ± 1.30	1.41 ± 0.48
thoracic region	1.44 ± 1.11	4.43 ± 0.43	2.97 ± 1.47	1.48 ± 0.75
lumbar region	1.01 ± 0.48	2.81 ± 0.46	0.94 ± 0.37	3.35 ± 0.79

* The EBD–EVAc polymers were implanted into the cisterna magna in Group 1 animals and on the frontal lobe in Group 2 animals. Values are expressed as means ± standard errors of the means.

obtained by averaging the micrograms of EBD per gram of tissue obtained in every subgroup and were calculated by comparing the spectrophotometric absorbance of the EBD that was extracted from a specimen section by using the formamide with the absorbance measured in a previously constructed standard curve of EBD in formamide. A comparison of mean micrograms of EBD per gram of tissue was performed using the Student t-test. Probability values lower than 0.05 were considered significant.

Results

Pharmacokinetic measurements of 40% EBD–EVAc polymers in vitro showed a sustained release of EBD, which continued up to 3192 hours (133 days) (Fig. 1). The concentrations of EBD that were released were consistent among the three polymers analyzed.

Placement of 40% EBD–EVAc polymers in the subarachnoid space of rabbits resulted in the diffusion of EBD through the entire length of the CNS regardless of the site of polymer implantation or the presence of blood (Table 1). Animals in Group 1 were implanted with EBD–EVAc polymers into the cisterna magna. In Group 1 animals that were not subjected to SAH, extracted EBD was present at all sites measured, with a peak concentration of 3.63 ± 0.06 μg EBD/g tissue in the cervical region at 3 days and one of 6.76 ± 1.66 μg/g in the cervical region at 14 days post-SAH (Fig. 2 *upper left*). Animals implanted with EBD–EVAc polymers into the cisterna magna after induction of SAH also were found to have extracted EBD present at all sites measured, with a peak concentration of 6.01 ± 3.61 μg EBD/g tissue in the cervical region at 3 days and one of 7.28 ± 6.54 μg/g tissue in the cervical region at 14 days post-SAH (Fig. 2 *lower left*).

Similarly, animals in Group 2 were implanted with EBD–EVAc polymer on the right frontal lobe. In the Group 2 animals that were not subjected to SAH, extracted EBD was found at all sites analyzed, with a peak concentration of 1.44 ± 1.11 μg EBD/g tissue in the thoracic region at 3 days and one of 4.43 ± 0.43 μg/g tissue in the thoracic region at 14 days (Fig. 2 *upper right*). Induction of SAH did not interfere with EBD diffusion after placement of polymers on the frontal lobe. Evans blue dye was detected in all sites measured with a peak concentration of 0.94 ± 0.37 μg EBD/g tissue in the lumbar region at 3 days and one of 3.35 ± 0.79 μg/g tissue at 14 days (Fig. 2 *lower right*).

Discussion

In this article we describe the release kinetics of a diazole dye delivered by controlled-release polymers in the subarachnoid space of rabbits with and without SAH. We found that EBD was released from EBD–EVAc polymers in a sustained fashion in vitro. We also found EBD–EVAc polymers placed at two different locations in the subarachnoid space of rabbits released EBD, which diffused throughout the subarachnoid space covering the entire CNS of the rabbit (distance of ~40 cm), regardless of the presence of blood in the subarachnoid space. Diffusion of EBD was also noted in an intraparenchymal distribution as seen in Fig. 3. The amount of intraparenchymal EBD, however, was not specifically measured. Although the pharmacokinetics described here correspond to the dye, those of controlled-release polymers that deliver drugs used to treat experimental vasospasm in animal models, such as ibuprofen or DETA-NO, exhibit faster release curves. If necessary, these curves could be optimized in several ways, according to the specific molecule released. Alternatives include addition of hyperosmolar agents such as sucrose or mannitol, lyophilization with bovine serum albumin, or the use of a polymer with a lower molecular weight, among others.

The CNS of the New Zealand White rabbit has a length of approximately 40 cm from the olfactory bulbs to the filum terminale. Evans blue dye released by EBD–EVAc polymers stained the full length of the rabbit's CNS when placed in the subarachnoid space at the frontal lobe or the cisterna magna. The dimensions of the CNS in the New Zealand White rabbit are comparable to the hemicircumferential distance of the human brain, which is the distance that a drug would have to diffuse from a polymer to reach the first- and second-order vasculature around the human brain. Such a comparison takes into consideration that compared

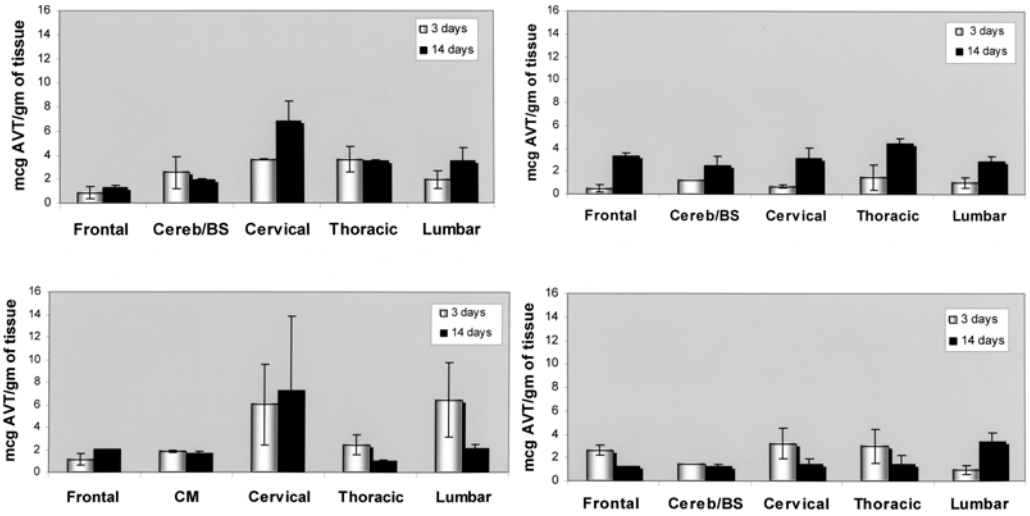

FIG. 2. Bar graphs demonstrating diffusion of EBD. *Upper Left:* Implantation of 40% EBT–EVAc polymers in the cisterna magna of rabbits without SAH resulted in diffusion of EBD with a peak concentration of 3.63 ± 0.06 μg EBD (AVT)/g tissue in the cervical region at 3 days and one of 6.76 ± 1.66 μg/g tissue in the same region at 14 days. *Lower Left:* Induction of SAH in rabbits did not interfere with the release of EBD from 40% EBD–EVAc polymers placed in the cisterna magna (CM), with a peak concentration of 6.01 ± 3.61 μg EBD/g tissue in the cervical region at 3 days and one of 7.28 ± 6.54 μg/g tissue in the same region at 14 days. *Upper Right:* Placement of the 40% EBD–EVAc polymers in the frontal lobe of the rabbits did not have an effect on EBD release. The EBD diffused through the CNS of the animals with a peak concentration of 1.44 ± 1.11 μg/g tissue in the thoracic region at 3 days and one of 4.43 ± 0.43 μg/g at 14 days in the same region. *Lower Right:* Presence of blood in the subarachnoid space did not interfere with the diffusion of 40% EBD–EVAc polymers placed in the frontal lobe of the animals. The concentration of EBD was 0.94 ± 0.37 μg/g tissue in the lumbar region at 3 days and 3.35 ± 0.79 μg/g tissue at 14 days. Cereb/BS = cerebellum/brainstem.

with the rabbit, the subarachnoid space in humans is more capacious and contains more CSF, which is osmotically and electrolytically comparable to its rabbit counterpart,[11,13] and thus is theoretically more favorable to drug diffusion from polymers.

The lipid solubility and molecular weight of drugs are the most important physicochemical properties that determine drug diffusion.[22] The preferred way of expressing the lipid solubility of a substance is based on the partitioning distribution of the substance between octanol and water. This is expressed as the logarithmic value of the fraction (log P). The ideal log octanol/water partition coefficient (log P value) for penetration of the blood–brain barrier is in the range of 1.5 to 2.5.[10] Evans blue dye has a molecular weight of 960.8 and a log P value of 0.71. Ibuprofen and DETA-NO are drugs that have successfully prevented vasospasm in animal models.[6–9,25,26] Ibuprofen has a molecular weight of 206.3 and a log P value of 3.79; DETA-NO has a molecular weight of 163.2 and a log P value of −0.26. Therefore, both drugs have lower molecular weights and higher partition coefficients than EBD. This indicates that both ibuprofen and DETA-NO could have better diffusion rates than EBD in the subarachnoid space.

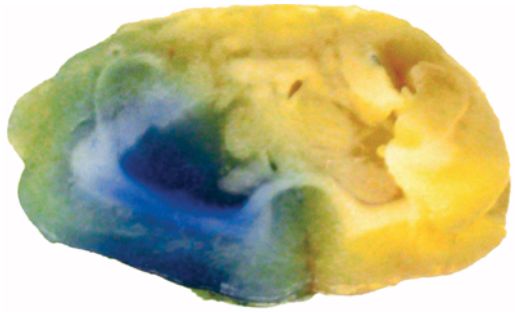

FIG. 3. Coronal section of the rabbit brain demonstrating intraparenchymal penetration of EBD 3 days after placement of a 40% EBD–EVAc polymer in the frontal lobe.

Treatment of CPHV with ibuprofen and DETA-NO delivered by controlled-release polymers has been successful in animal models of SAH.[25,26] Ibuprofen is an antiinflammatory agent that inhibits expression of intercellular adhesion molecule–1[3] and vascular cell adhesion molecule–1,[12] blocking leukocyte–endothelial cell binding;[17] prevention of experimental CPHV supports the strong role of inflammation in this entity. Activated leukocytes present in the subarachnoid space after SAH releases endothelins and NO antagonists,[1,2] altering vascular smooth muscle tone. Diethylenetriamine–nitric oxide is a zwitterionic triamine–NO donor that restores vascular smooth muscle tone, preventing experimental CPHV.

Previous studies in which controlled-release polymers have been used to treat experimental CPHV were performed by placing the polymers adjacent to the target vessel. In the rat femoral artery model the polymer is placed next to the artery to achieve periadventitial delivery of DETA-NO.[26] In the rabbit model of SAH the polymer is placed in the cisterna magna, after which DETA-NO travels through the subarachnoid space covering a distance of up to 10 mm to reach the basilar artery. In the primate model of SAH the polymer is placed in the chiasmatic cistern and the agent that is delivered similarly travels up to 10 mm to reach its target.

To prevent CPHV in patients, drugs delivered by controlled-release polymers placed in the subarachnoid space

during surgery would have to travel through the subarachnoid space a mean distance of 17 cm. This is the maximum length of the hemicircumferential distance from the basal cisterns (the site of anticipated polymer placement) to the diametrically opposed side in both an anteroposterior and a bicoronal plane. This experiment demonstrates that drugs delivered by EVAc polymers in the rabbit subarachnoid space diffuse widely in the CSF under SAH conditions, covering a distance greater than that of the mean hemicircumference of the human brain.

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Address reprint requests to: Rafael J. Tamargo, M.D., Department of Neurosurgery, School of Medicine, The Johns Hopkins University, Meyer Building 8–181, 600 North Wolfe Street, Baltimore, Maryland 21287. email: rtamarg@jhmi.edu.