Cisternal Talc Injection in Dog Can Induce Delayed and Prolonged Arterial Constriction Resembling Cerebral Vasospasm Morphologically and Pharmacologically


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BACKGROUND
The possible role of inflammation in the pathogenesis of cerebral vasospasm has been noted in recent studies. In order to examine the role of inflammation, we examined the vasocontractile activity of talc, which is known to cause severe inflammation, using a canine cisternal talc injection model.

METHODS
Under general anesthesia, a sterile talc powder suspended in saline was injected into the cisterna magna of the dog. Serial vertebral angiography and postmortem histologic changes of the harvested basilar artery were examined. The morphologic and pharmacologic features of talc-induced vessel spasm were compared with the usual autologous blood-induced artery spasm.

RESULTS
Cisternal injection of sterile talc powder caused no early spasm, but induced definite basilar arterial constriction 2 days after injection. This vascular constriction was observed to continue up to 7 days after injection. Ultrastructural study of the constricted vessel revealed several morphologic changes, such as corrugation of the elastic lamina, subintimal proliferation, migration of smooth muscle cells, detachment of endothelial cells, etc.; findings that are compatible with the changes observed in vasospasm. Pharmacologic study showed a moderate decrease in the maximal constriction to KCl and UTP. Endothelium-dependent relaxation was markedly disturbed, while endothelium-independent relaxation was preserved. These pharmacologic properties were also similar to those reported in vasospasm.

CONCLUSIONS
Our present study indicates that the several changes of vascular properties, which had been considered to be specific to cerebral vasospasm, can be regarded as a nonspecific biomechanic defense reaction against the foreign body. The analysis of the common pathway from talc and autologous blood to vasospasm may lead to the pathogenesis of cerebral vasospasm.

KEY WORDS
Cerebral vasospasm, cisternal talc injection, experimental model, inflammation, subarachnoid hemorrhage.

Cerebral vasospasm with secondary cerebral ischemia following aneurysmal subarachnoid hemorrhage is still the leading cause of death or disability after aneurysmal rupture. In spite of many investigations, the pathogenesis of cerebral vasospasm remains unknown. The histologic findings of large numbers of inflammatory cells in and around the spastic vessel [9,34] have suggested the possible role of inflammation in the pathogenesis of vasospasm. On the other hand, blood components are still considered to be essential for the pathogenesis.
of cerebral vasospasm because of the vasoactivity of erythrocyte lysate *in vitro* [4,10,25,27,29].

In order to study the effects of blood components and the inflammatory reaction separately, we examined the possible vasocontractile activity of talc using the canine cisternal talc injection model. Talc is the powder of crystallized hydrous magnesium silicate, and has long been used as a powder for surgical gloves. This material is widely recognized to cause severe inflammation with granuloma formation [1,15,33,35,39]. This study attempted to show the possible vasoactivity of talc powder, and to demonstrate the similarity of the morphologic and pharmacologic features of talc-induced vessel spasm to usual autologous blood-induced artery spasm.

**Materials and Methods**

Twenty-two adult mongrel dogs, each weighing 8.5–13.0 kg, were used for these studies. All experiments were performed according to the National Institutes of Health “Guide for the Care and Use of Laboratory Animals,” revised 1985. Twelve dogs were used for the morphologic study. Animals were anesthetized with an intravenous administration of 30 mg/kg of sodium pentobarbital, orally intubated, and fixed with a stereotaxic frame. As previously described [24], the effect of arterial $p$CO$_2$ on basilar arterial diameter was negligible when $p$CO$_2$ was within 30–45 mm Hg. Based on this result, all of the dogs breathed spontaneously, and their $p$CO$_2$ ranged from 34.2–44.7 mm Hg. Using an aseptic technique, the right vertebral artery was exposed in the lower neck and cannulated with a polyethylene catheter (0.86 mm), through which vertebral angiography was performed using 8 ml of meglumine diatrizoate. One gram of talc powder, sterilized with ethylene-oxide gas was suspended in 7.5 ml of normotonic saline, and percutaneously injected into the cisterna magna over 1 minute, followed by the removal of the same volume of cerebrospinal fluid. Animals were placed in the nose-down position for 15 minutes. Another angiogram was taken 1 hour later to evaluate the presence of early spasm. Further angiograms were taken on days 2 and 7 for the evaluation of delayed spasm. The luminal diameter of the basilar artery was measured at five prefixed locations on the angiograms, and the mean value was defined as the calculated basilar arterial diameter. All films were randomly measured three times by a single investigator. The range of error of measurement on angiogram was evaluated, and the coefficient of variation was 3.7%, indicating the reproducibility of measurements.

Nine animals were sacrificed by exanguination after the final angiogram. Three were sacrificed on day 2 to observe the early morphologic changes. Following the infusion of 300 ml of heparinized normotonic saline into the vertebral artery, intravital perfusion-fixation was performed with 200 ml of 4% phosphate-buffered paraformaldehyde under 150 cm H$_2$O of perfusion pressure. Since this fixation technique has been well established [14] and widely been used for the morphologic studies of the canine basilar arteries after subarachnoid hemorrhage [8,19,31,32], it might be indisputable that this method is not responsible for the changes observed. The basilar artery was dissected under an operating microscope and immediately cut into rings (about 4 mm long). These were fixed in 2.5% glutaraldehyde with postfixation in 1% osmium tetroxide. After dehydration through a graded series of ethanol solutions, they were transferred to propylene oxide, and embedded in Epon 812. Ultra-thin sections were made with an LKB V ultramicrotome. Samples were doubly stained with uranyl acetate and lead citrate, and observed with a HITACHI H-7000 transmission electron microscope.

Ten dogs were used for the pharmacologic study. Three were not treated as normal controls, and seven were treated in the same fashion previously described. Among the seven treated dogs, three were sacrificed on day 7, and four sacrificed on day 2. Animals were sacrificed by exanguination and the basilar arteries were harvested. Rings of about 4 mm long were cut from the basilar artery. Each specimen was suspended between L-shaped stainless steel rods in a 37°C organ chamber filled with modified Krebs-Ringer bicarbonate solution (composition (mM): NaCl 120; KCl 4.5; MgSO$_4$ 1.0; NaHCO$_3$ 27.0; KH$_2$PO$_4$ 1.0; CaCl$_2$ 2.5; and dextrose 10.0) bubbled with a 95% O$_2$ and 5% CO$_2$ gas mixture. The preparations were allowed to equilibrate at 37°C for 90 minutes before use. The resting tension was adjusted to 2.5 g. Contractile force was recorded isometrically with a force-displacement transducer (model WT-611T; Nihon Kohden, Tokyo, Japan) and displayed on a chart recorder (model CDR-12A; Toa Electronics Ltd., Tokyo, Japan). The individual 50% effective dose (ED$_{50}$) for uridine triphosphatase (UTP) was determined in each ring by obtaining a full concentration-response curve ($10^{-5}$–$10^{-4}$ M) for the agonist. Maximal contraction to KCl and UTP was examined using the vessels obtained on days 2 and 7. As described later, the vessels obtained on day 7 were rigid enough to contract to UTP. Therefore, the following experi-
Graph showing the change of basilar arterial diameter after talc injection (n = 9). Significance: *** = p < 0.001, compared with the diameter before the talc injection.

Relaxation in arterial rings affected by UTP was expressed as the percent change of maximal relaxation in response to papaverine (3 × 10^{-4} M).

The data were expressed as mean values ± standard error of the mean. Differences among the groups were assessed by analysis of variance. The minimum level of significance was set at p = 0.05.

**RESULTS**

The change in basilar arterial diameter was expressed as a percentage of the original diameter before talc injection. One hour after talc injection, the average arterial diameter was 98.5 ± 3.0% (mean ± S.E.M.), and early spasm was not observed in the talc injection model. However, marked constriction of the basilar artery was observed on day 2. This severe arterial constriction persisted to day 7. The average arterial diameters on days 2 and 7 were 61.5 ± 2.2% and 62.6 ± 3.5%, respectively (Figure 1).

Postmortem morphologic examination revealed several histologic changes in the constricted artery (Figure 2). On day 2, endothelial cells were desquamated and partly detached from elastic lamina.
Vasospasm Induced by Cisternal Talc Injection

Their cytoplasmic organelles, such as mitochondria, lysosomes, and rough endoplasmic reticulum, were increased. Elastic laminae were folded, corrugated, and partly torn off. Smooth muscle cells had irregular cell membranes and their nuclei were deeply cleft. Smooth muscle cells on the luminal side had migrated into the subintima. Subintimal proliferation still was not observed. Endothelial cells and smooth muscle cells contained intracytoplasmic vacuoles of various sizes.

On day 7, these ultrastructural changes were more prominent. Endothelial cells had more intracytoplasmic vacuoles and severely degenerated organelles. Tight junctions between endothelial cells were often lost, and the desquamated endothelium apart from the elastic lamina formed extensive gaps that included membrane materials. As numerous smooth muscle cells migrated through the fissure of the internal elastic lamina, the subintima was greatly proliferated and elastic laminae were often torn off. The smooth muscle cells were irregularly shaped (amoebae-like formation) and had rich organelles and few myofilaments. Collagen fibrils were increased in the widened interstitial smooth muscle cells. These histologic changes were compatible with those observed in vasospasm [6,31,32,36], although the degree of change was more prominent in the talc model.

Pharmacologic study showed that maximal contraction to KCl and UTP was moderately diminished even on day 2, and was severely decreased on day 7. Since the control contraction by UTP was minimal on day 7, the following studies for relaxation were performed using arterial rings on day 2. Normal control arterial rings contracted with UTP relaxed in response to arginine vasopressin, which causes endothelium-dependent relaxation in a concentration-dependent manner (Figure 3A). However, even 10^-6 M vasopressin failed to relax the arterial ring in the talc model (Figure 3A). Endothelium-independent relaxation in the talc group was preserved in response to papaverine and sodium nitroprusside (Figure 3B). These pharmacologic properties were basically similar to those reported in the SAH model [18].

**DISCUSSION**

Our present study demonstrated that cisternal talc injection induced a delayed and prolonged arterial constriction, which morphologically [6,31,32,36] and pharmacologically [18] mimicked cerebral vasospasm. A few authors have reported that foreign bodies other than blood components in the sub-arachnoid space can induce severe arterial constriction [28,37]. Peterson et al [28] found in the canine model that the cisternal injection of dextran or latex beads led to moderate to severe chronic vasoconstriction. Yanamoto et al [37] observed dose-dependent arterial narrowing after a cisternal injection of polystyrene latex beads in the rabbit. Neither of them, however, mentioned a similarity between the resulting vascular constriction and conventional cerebral vasospasm. We revealed that the properties of talc-induced vessel constriction were highly similar to those of vessels in vasospasm, both morphologically and pharmacologically. These characteristic changes in properties have been considered to be specific to vasospasm induced by autologous blood. Our study indicates that these changes in properties are not always specific to cerebral vasospasm but can be regarded as a nonspecific biologic defense reaction against the foreign materials.

The mechanism underlying talc-induced inflam-
formation has been well examined because talc, which has been used as a lubricating agent for surgical gloves, has been recognized to cause foreign body reactions in a variety of tissues [1,15,33,35,39]. Tanaka reported in his rat-pleuritis model [35] that talc-induced inflammation was observed in as short a time as 15 minutes. According to his report, pleural liquid rapidly increased in volume within the first 30 minutes, then disappeared at 48 hours. Sheikh et al [33] examined the sequential histologic changes after talc-implantation in the abdominal muscle in rats. They observed an acute inflammatory response on day 1, followed by a chronic and persistent inflammatory response and granuloma formation by day 3. This granulomatous reaction with talc crystals in giant cells is a specific finding in chronic talc-induced inflammation [15,33,35,39]. Therefore, this inflammation implies a biologic defense reaction to eliminate the foreign body—talc particles. Since talc is a simple mineral, it does not behave as an antigen. It is recognized as "nonself" by the non-immunologic mechanism [35], and is scavenged by macrophages and/or polymorphocytes. Therefore, talc-induced inflammation can be considered a consequence of the biologic reaction to eliminate foreign bodies by non-immunologic recognition.

The same is true of the elimination of extravascular homologous blood cells. In the case of subarachnoid hemorrhage, the erythrocytes in the subarachnoid space must be eliminated. Most of the investigators have paid attention to hemolysis for the elimination of erythrocytes [29], and have examined the vasoactivities of the erythrocyte lysate or breakdown products [5,7,12,21,26,30]. The histologic findings, however, of erythrocytophagocytosis by macrophages [2,11,13,22,23] definitely show the elimination of erythrocytes by scavenging. The recognition of extravascular homologous erythrocytes by macrophages [38] is explained by the loss of sialic acid from sialoglycoprotein in the membrane of erythrocytes [3,16,17]. Since this recognition does not require any antibody or complements [20], it is defined as nonimmunologic recognition. Therefore, the recognition mechanisms for talc and homologous erythrocytes are considered to be essentially the same.

It follows from what has been said that the common pathway from talc and extravascular erythrocytes to cerebral vasospasm is recognition and elimination by the non-immunologic recognition mechanism. Of course, the results shown above do not always directly implicate that the underlying mechanism in the talc-induced vascular constriction is same as that in the conventional cerebral vasospasm. Our study simply suggests that the several changes in contractile vessel can be regarded as a non-specific biologic defense reaction. Nevertheless, the similarities in property between talc-induced contractile vessel and artery in vasospasm still remain worth noting. Therefore, the further analysis of talc-induced vascular constriction may lead to the better understanding of the pathogenesis of cerebral vasospasm.

REFERENCES

16. Khansari N, Fudenberg HH. Phagocytosis of senes-


COMMENTARY

The authors observed delayed arterial narrowing (vasospasm) in a canine model utilizing cisternal injection of talc. They make the important conclusion that "the analysis of the common pathway from talc and autologous blood to vasospasm may lead to [an understanding of] the pathogenesis of cerebral vasospasm." In my opinion, inflammation is important in the pathogenesis of cerebral vasospasm. I speculate that future studies will demonstrate that cellular adhesion molecules are an important part of the story.

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