Experimental Study of the Pathogenesis of Moyamoya Disease: Histological Changes in the Arterial Wall Caused by Immunological Reactions in Monkeys

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Moyamoya disease is a progressive vascular disorder of unknown etiology. Theories of inflammatory and immunologic mechanisms have been proposed as the pathogeneses. We have designed a new method of administering N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) for experimental induction of moyamoya disease using an intravascular interventional technique combined with rod-shaped embolic materials made from lactic acid-glycolic acid copolymer. The embolic materials containing MDP were repeatedly injected into the right internal carotid artery of monkeys in the embolic group. Intravenous injections of MDP solution alone were performed in the intravenous group. Histological examination of the arteries demonstrated reduplication and lamination of the internal elastic laminae, which corresponded with findings of moyamoya disease in both groups. These histological changes occurred not only in the intracranial arteries on the embolization side, but also in the contralateral intracranial and even extracranial arteries. The changes were more prominent in the intravenous group than in the embolic group. We conclude that the systemic humoral factors induced by MDP in this study may be important in the pathogeneses of moyamoya disease. Our observations suggest that moyamoya disease is a systemic vascular disease and has an etiologic factor affecting both intracranial and extracranial arteries.

Key words: moyamoya disease, etiology, immunological reaction, intraarterial embolization, N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP)

Moyamoya disease is a progressive vascular disorder of unknown etiology [1]. The primary lesion of moyamoya disease is considered a stenotic change in the terminal internal carotid artery [2, 3]. The abnormal moyamoya vessels at the base of the brain are thought to be collateral circulation secondary to brain ischemia [1].

The stenotic lesions in this disease are caused by fibrous thickening of the intima. The thickened intima is generally devoid of acute inflammatory cell infiltration. The internal elastic lamina often shows reduplication and lamination consisting of newly formed fine elastic laminae in a layered fashion. The media becomes thinner, but the adventitia is not affected [4, 5]. The causal genesis of these pathological vascular changes and the manner of histological progression, however, remain unknown.

Theories of inflammatory and immunologic mechanisms have been proposed to explain the pathogenesis of moyamoya disease [6-8]. N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide: MDP) is thought to be a minimal component of the bacterial wall inducing experimental autoimmune diseases [9, 10]. MDP has been
utilized for experimental induction of moyamoya disease [11, 12]. However, these experimental studies have failed to induce characteristic intimal thickening followed by the formation of moyamoya vessels in animals [11, 12].

In the present study, we carried out experimental induction of moyamoya disease in monkeys to clarify the etiology of this disease with a new method of administering MDP into the terminal portion of the internal carotid artery using an intravascular interventional technique.

Materials and Methods

Six Japanese monkeys (Macaca fuscata) weighing from 7–11 kg were used in this study. All animal experiments were carried out in accordance with Policies and Guidelines for the Care and Use of Laboratory Animals at Okayama University Medical School. The animals were injected intramuscularly with ketamine hydrochloride (25 mg/kg) and atropine sulfate (0.05 mg/kg) immediately before the procedures to facilitate intubation and placement of an intravenous catheter. A lactate Ringer’s solution was administered intravenously at rates of 50 ml/h. Following endotracheal intubation, the animals were mechanically ventilated throughout the procedure. General anesthesia was subsequently induced with an intravenous administration of sodium pentobarbital (12.5 mg/kg). The animals were paralyzed with an intravenous administration of pancuronium bromide (0.1 mg/kg).

Angiographic study. After induction of general anesthesia, a femoral artery cutdown was performed in a sterile fashion, and a No. 4 French catheter was advanced into the internal carotid artery with the aid of a fluoroscope. Contrast medium, 2 ml of iopamidol (300 mgI/ml), was injected manually. A single film of the arterial phase of the lateral projection was obtained in each procedure.

Preliminary embolization study. Rod-shaped test embolic material (1 mm in diameter, 5 mm in length) was made of stainless steel wire and injected into the terminal portion of the right internal carotid artery at the base of the skull via the angiographic catheter in one monkey. Selective right internal carotid angiography was performed immediately after injection of the test embolic material.

Preparation of immuno-embolic material. In a sterile manner, lactic acid-glycolic acid 50:50 copolymer, molecular weight 10,000 (LGA-50), was mixed with MDP and formed into a rod-shaped material (1 mm in diameter, 5 mm in length) containing 1 mg of MDP in each piece. This dosage of MDP corresponded to an approximately 20% of the intravenous injection described below. We selected a smaller quantity of MDP to avoid an acute general reaction, e.g. shock, because no prior data for intraarterial injection of MDP were available.

Experimental study with immuno-embolic material. The other 5 monkeys were divided into 3 groups. In the embolic group (3 monkeys, No. 1–3), the sensitization was performed by intravenous injection of MDP (500 µg/kg) via a superficial vein of the forearm. Because no prior dosage data were available for MDP in primates, we selected a dose similar to that reported previously for use in rats [11] and cats [12]. Rod-shaped immuno-embolic material was injected 3 or 4 times into the terminal portion of the right internal carotid artery at the base of the skull via an angiographic catheter advanced into the right internal carotid artery after sensitization. Angiograms were obtained before and after the embolization procedure. In the intravenous group (1 monkey, No. 4), sensitization was performed in a similar fashion as described above, and intravenous injection of MDP (500 µg/kg) via a superficial vein of the forearm was repeated 4 times after the sensitization. We could not prepare the same number of monkeys in the intravenous group as the embolic group because of the limited number of animals. In the control group (1 monkey, No. 5), angiography was performed 3 times without sensitization or injection of MDP (Fig. 1).

Histological examination. In the embolic group, the animals were sacrificed on days 49, 78, and 163 postsensitization, respectively. Different durations for the experimental studies were established to examine whether the histological changes become more prominent with passing time because of a chronic progressive characteristic of moyamoya disease. In the intravenous group, the animal was sacrificed on day 162 postsensitization. In the control group, the animal was sacrificed on day 85 after the initial angiography. Thoracotomy was performed on the left side at the level of the fifth intercostal space in each animal. The left ventricle of the heart was cannulated, and the right atrium was opened widely. Ten-percent formalin was infused via the cannula for 5 min at a pressure of 100 mmHg for intraarterial perfusion. Subsequently, the brain, extracranial carotid arteries, heart, and kidneys were removed and placed in 10% formalin at
room temperature for a week. Under an operating microscope, the terminal portions of the internal carotid arteries, the proximal portions of the anterior, middle, and posterior cerebral arteries, and whole segments of the posterior communicating and basilar arteries were carefully dissected and removed from the fixed brain. The common, internal, and external carotid arteries were dissected. The coronary and renal arteries were also dissected and removed. The segments of the arteries removed from the fixed specimens were cut into small pieces (Fig. 2). The samples were dehydrated through a graded ethyl alcohol series and cleared in xylene. Following paraffin embedding, 8-μm sections were cut with a steel knife using a rotary microtome, floated on an albuminized slide, and allowed to dry overnight at room temperature. The samples were examined using a microscope after hematoxylin-eosin and elastica van Gieson staining.

Results

Angiographic study. Initial selective internal carotid angiography of 6 monkeys demonstrated that the terminal portion of the internal carotid artery and its branches were morphologically the same as the human carotid system. In the preliminary embolization study, the rod-shaped stainless steel test embolic material was seen in the terminal portion of the internal carotid artery at the base of the skull; the internal carotid artery remained patent; and its intracranial branches were well demonstrated after embolization (Fig. 3). It was therefore concluded that the single rod-shaped piece of embolic material (1 mm in diameter, 5 mm in length) used in this experiment did not obstruct the terminal portion of the internal carotid artery and dissolved in the patent arterial flow. In the embolic and intravenous groups, the stenotic changes of the terminal internal carotid artery and its branches were not observed even on the final angiograms, and there was no appearance of moyamoya vessels. Although these vascular changes are thought to be essential to the presence of moyamoya disease, we failed to produce these changes in this experimental study. In the control group, no angiographic changes were seen.

Histological examination. Histological findings of the obtained arteries are summarized in the Table 1. In the embolic and intravenous groups, observed histological changes consisted of reduplication and lamination of the internal elastic lamina. The original internal elastic lamina showed a layered structure expressed as reduplication by the addition of newly formed
elastic fibers (Fig. 4). In the portion exhibiting marked change, numerous fine elastic fibers showed signs of lamination (Fig. 5). Interruption of the internal elastic lamina was not seen. In the embolic group, changes were seen not only in the intracranial arteries on the right side of the animal, but on the left side and in the extracranial arteries. However, the degree of histological change did not differ among the 3 monkeys in the embolic group, despite the different experimental durations. In the intravenous group, the changes were more prominent than those of the embolic group and were seen in the intracranial arteries on both sides and in the extracranial arteries, especially in the renal arteries. However, no morphological difference was observed between the both groups, e.g. a difference in the shape of each internal elastic lamina. There was no finding of intimal thickening in either group. In the control group, histological changes were not observed.

Discussion

The primary lesion of moyamoya disease is considered

Table 1  Summary of histological findings

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Five monkeys were divided into 3 groups (No. 1–3, embolic group; No. 4, intravenous group; No. 5, control group). Histological findings were assessed as (+) when reduplication of the internal elastic lamina was seen, as (+++) when reduplication and lamination of the internal elastic lamina were seen, and as (−) when there were no histological changes seen. (NA) indicates that histological change was not assessed due to an insufficient specimen.

A1, proximal portion of the anterior cerebral artery; BA, basilar artery; CCA, common carotid artery; CoA, coronary artery; C1, the terminal portion of the internal carotid artery; ECA, cervical external carotid artery; ICA, cervical internal carotid artery; L, left; M1, proximal portion of the middle cerebral artery; PcoA, posterior communicating artery; P1, proximal portion of the posterior cerebral artery; R, right; RA, renal artery.
to be a stenotic change of the terminal internal carotid artery [2, 3]. In addition, fibrous intimal thickening associated with reduplication of the internal elastic lamina is a prominent histological feature [4, 5, 13, 14]. However, the causal genesis of these pathological vascular changes and the manner of histological progression remain unknown. Abnormal moyamoya vessels at the base of the brain in this disease are thought to be collateral circulation secondary to brain ischemia [1].

As observed in clinical studies of moyamoya disease, many patients demonstrate chronic inflammation above the neck before the onset [1], and pathological changes of the stenotic artery resemble chronic organized arteritis due to an immunological reaction [15]. Therefore, an inflammatory and immunological theory has been proposed [6-8], and experimental studies have been conducted with the intent of inducing this disease in animals [11, 15]. Kasai et al. have reported pathological arterial changes in mongrel dogs resulting from experimentally induced immunological arteritis. In their experiment, changes such as thickening of the intima and reduplication of the internal elastic lamina corresponding with the findings of moyamoya disease were observed primarily at the terminal portion of the internal carotid artery; these changes were insufficient to cause stenosis of the artery in their experiment [15].

MDP is thought to be a minimal component of the bacterial wall inducing various biological effects in a living body [16], e.g., modulation of immune responses [17-19] and induction of experimental autoimmune diseases [9, 10]. MDP was first utilized for experimental induction of moyamoya disease by Suzuki et al. [11], who carried out repetitive intravenous or intrathecal administration of MDP solution in Wistar rats. They observed histological changes consisting of disruption of the internal elastic lamina, degeneration of the media, and minimal intimal thickening primarily in the terminal portion of the
internal carotid artery. These changes, however, were insufficient to cause stenosis of the artery in their experiment [11].

The establishment of an experimental method of inducing moyamoya disease is essential to elucidating the causative factors in this disease. The arterial changes reported from previous experimental studies have been quite similar to those of moyamoya disease, although they have been minimal and insufficient to cause stenosis of the artery followed by the formation of moyamoya vessels [11, 15].

It remains unclear why the intimal thickening of moyamoya disease is found primarily at the terminal portion of the internal carotid arteries [11, 15]. There have been few reports concerned with the vulnerability of certain parts of the artery in this disease. Kasai et al., based on their experimental results, have hypothesized a participation of the autonomic nerve system originating from the superior cervical ganglion with regard to the vulnerability of the terminal portion of the internal carotid arteries [15]. Further studies must be carried out to test this hypothesis.

The experimental induction of substantially thickened intima in the terminal carotid arteries may be key to the causative mechanism of this disease. Therefore, we designed a new method of administering MDP to the terminal internal carotid artery using an intravascular interventional technique. LGA-50 was chosen as a matrix of soluble embolic material. The rod-shaped material (1 mm in diameter, 5 mm in length) made from LGA-50 was dissolved in 37°C phosphate buffer for 7–10 days [12]. In this study, rod-shaped embolic material did not obstruct the terminal portion of the internal carotid artery, leading us to conclude that the material dissolved in the patent arterial flow. MDP contained within the material appeared to be released continuously at the terminal portion of the internal carotid artery after the embolization procedure. We therefore expected a direct effect of MDP on the vascular wall in the terminal internal carotid artery.

The histological changes observed in this study, however, were restricted to the internal elastic lamina, and intimal thickening, a prominent feature of moyamoya disease, was not observed. The changes occurred not only in the intracranial arteries on the right side into which the immuno-embolic material was injected, but also in the contralateral arteries; in addition, changes were observed not only in the intracranial but also the extracranial arteries. Although we expected an effective application of MDP to the arterial wall from the vascular lumen in this study, the inner layer of the wall, i.e., the intima, showed no histological changes, and changes were observed only in the internal elastic lamina on both sides of the intracranial arteries and in the extracranial arteries. It was suspected that this systemic application of MDP was effective for the induction of vascular changes, even with an intraarterial application. Further studies of whether the sensitization alone causes the histological changes are necessary to elucidate the role of intraarterial MDP. It was also suspected that the dose of intraarterial MDP was too small to produce the required changes. Therefore, further studies utilizing a higher dose of intraarterial MDP are required.

In the present study, the degree of histological change did not differ among the 3 monkeys in the embolic group, despite different experimental durations and different total dosages of MDP. It was considered, however, that the limited histological changes and the relatively close experimental durations might have obscured any actual differences.

The changes in the internal elastic lamina were more prominent in the intravenous group than in the embolic group in this study. The larger total amount of MDP administered in the intravenous group than in the embolic group might have caused this difference. Further studies will be necessary to elucidate MDP’s mechanism of action in different methods of application, i.e., embolic and intravenous, with the same total dosage of MDP.

It is possible that the total dosage of MDP in this study was not sufficient to cause the substantial histological changes. We did not observe a vulnerability of the terminal portion of the internal carotid arteries, which is a characteristic finding of moyamoya disease. Therefore, studies utilizing a higher dosage of MDP will be necessary to investigate the dose dependency. It will also be desirable for a larger number of animals to be used in further studies.

Although the histological findings obtained in the present study are minimal, our observations do suggest that moyamoya disease is a systemic vascular disease involving an etiologic factor affecting both intracranial and extracranial arteries [4, 5, 20–24]. In this study, histological changes were found in the renal artery, which is characteristic of findings in human autopsy cases of moyamoya disease [4, 20–23].

Recent studies have shown that the thickened intima in moyamoya disease is composed primarily of a synthetic
phenotype of smooth muscle cells (SMC) with abundant extracellular matrices [25, 26]. The possibility that some growth factors play an important role in inducing the migration and proliferation of SMC in this disease has been raised [27–33]. Aoyagi et al. have shown that platelet-derived growth factor (PDGF), a major mitogen of SMC [34, 35], alters the response of cultured SMC derived from superficial temporal arteries (STA) of patients with moyamoya disease [27, 28]. They have also observed diminished proliferation responses to PDGF and down-regulation of the PDGF receptor in SMC from moyamoya patients.

Basic fibroblast growth factor (FGF) is an angiogenic factor as well as a potent mitogen for vascular endothelial cells and SMC [36, 37]. It has been reported that the amounts of basic FGF and its receptor are increased in the STA of moyamoya patients [29, 30] and that the levels of basic FGF are high in cerebrospinal fluid taken from patients with moyamoya disease [31, 32].

Hoho et al. have reported that the expression of transforming growth factor-β1, a multifunctional protein regulating cell growth and differentiation [38, 39], is significantly elevated in cultured SMC derived from the STA of moyamoya patients [33]. Thus, certain growth factors may contribute to the causative mechanism of moyamoya disease [27–33]. The mechanism of action of these factors, however, remains unclear. Further studies which combine experimental induction of moyamoya disease and analysis of the expression of growth factors must be performed to elucidate the mechanism promoting vascular changes in this disease.

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References