Flow Behavior and Distribution of Embolus-Model Particles at the Terminal Bifurcation of the Human Internal Carotid Artery

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Abbreviations: ACA, anterior cerebral artery; ACoA, anterior communicating artery; ICA, internal carotid artery; MCA, middle cerebral artery; PCoA, posterior communicating artery

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Abstract

Background: To investigate the possible role of fluid mechanical factors in thrombo-embolism that occurs at a high rate in the human middle cerebral artery.

Methods: Isolated transparent cerebral arterial trees containing the terminal bifurcation of the internal carotid artery (ICA), where the ICA bifurcated into the middle cerebral artery (MCA) and the anterior cerebral artery (ACA), were prepared from humans postmortem. Then, flow behavior and distribution of embolus-model polystyrene particles in dilute suspensions at the bifurcation were studied in detail by means of flow visualization and high-speed cinemicrographic techniques.

Results: Large particles in suspensions flowing through the ICA migrated radially away from the vessel wall towards the axis of the ICA. It became more remarkable with increasing the flow rate in the ICA (Q_0), flow rate ratio Q_1/Q_0 (MCA/ICA), and particle diameter. As a result, redistribution of particles flowing in the ICA occurred at the bifurcation. The particles larger than 1 mm in diameter (≈1/4 of vessel diameter) selectively entered the MCA even when the flow rate ratio Q_1/Q_0 was decreased to as low as 0.34. By contrast, the particles whose diameters were smaller than 0.3 mm (≈1/10 of vessel diameter) and that did not show radial migration entered the MCA at the same rate as the flow rate ratio Q_1/Q_0.

Conclusions: Due to the flow-dependent migration of particles away from the vessel wall towards the axis of the ICA, large particles selectively enter the MCA to which the core flow of the ICA is generally directed. This might explain why the incidence of thrombo-embolism is higher in the MCA than in the ACA in humans.

Key words: Internal carotid artery; Anterior cerebral artery; Middle cerebral artery; Cerebral embolism; Embolization; Hemodynamics; Flow pattern; Particle flow behavior

1. Introduction

It has been shown that the incidence of cerebral arterial occlusion is variable among intracranial arteries.[1,2] Especially, there is a striking difference between the anterior cerebral artery (ACA) and the middle cerebral artery (MCA).[3] Several investigators considered that this occurs as a result of local thrombosis. However, it has been shown that the same phenomenon occur also in the case of embolism.[4,5] Embolism has been accepted as an important cause of intracranial occlusive vascular diseases, particularly of transient ischemic attacks.[2,6,7] Gacs et al.[1] recognized that there is a positive correlation between the paths of artificial emboli and the region of cerebral arterial occlusion, and indicated that there is certain regularity in the distribution of the region of occlusion even in the case of embolic occlusion, although the exact mechanism was not clear. It was also shown by Luessenhop et al.[8] that carried out artificial embolization using spherical beads for the case of cerebral arteriovenous malformation (AVM) that at the terminal bifurcation of the internal carotid artery (ICA), 90% of the beads entered the MCA territory even when the ACA and the MCA supplied the AVM equally. Similar results were reported by many other investigators.[9-11]

In most cerebral arteries, the terminal portion of the ICA is directed towards the MCA, and the diameter of the MCA is larger than that of the ACA. These geometrical characteristics are considered as the factors responsible for the biased distribution of large emboli (thrombi). Empirically, it is widely accepted that the geometry of the bifurcation, such as the branching angle, the diameter ratio of two daughter vessels, and so-called "pump effect" are important factors for the flow direction of large-sized emboli.[8-12] However, theoretical grounds for this conclusion are still lacking. The present work was carried out to elucidate the mechanism by which emboli are selectively carried to some certain arterial branches. To do so, we have prepared two isolated transparent cerebral arterial trees containing the terminal bifurcation of the ICA from humans postmortem by a method developed in our laboratory. Then by using

them, we studied the flow behavior and distribution of embolus-model particles flowing through the ICA at the bifurcation by means of flow visualization and cinemicrographic techniques.

2. Materials and Methods

2.1 Materials

Several cerebral arterial trees containing the terminal bifurcation of the internal carotid artery were obtained at autopsy in the Department of Pathology, Montreal General Hospital one full day after the death of the subjects that was unrelated to cerebrovascular disorders. Out of them, we chose only two arterial trees, obtained from a 64 year old male (Vessel I) and an 81 year old female (Vessel II), which we considered the best judging from their sizes and configurations, and used to prepare transparent arterial models. One of the reasons for that was due to the very laborious, time-consuming, and painstaking nature of the work involved in the preparation of the transparent arterial trees, film analysis, and preparation of drawings.

2.2. Preparation of Transparent Cerebral Arterial Trees

Transparent cerebral arterial trees containing the terminal bifurcation of the internal carotid artery (ICA) were prepared by modification of the method described by Karino and Motomiya.[13]

After rinsing the vessel with isotonic saline and clearing surrounding tissues, the inlet and all the exits of the arterial system were cannulated with short, thin-walled stainless steel pipes that fitted snugly to the vessels. All other branches, which included the anterior choroidal artery and small perforating arteries, were occluded by ligating them with suture thread or coagulating with a fulgurator. The cerebral arterial tree was then gently perfused with isotonic saline to wash out the blood, pressurized to a physiological mean arterial blood pressure of 100 mmHg by perfusing and immersing it in isotonic saline to stretch and set the geometry of the vessel as close as possible to its natural state in vivo, and then mounted on a three-dimensional supporting frame made of aluminum by ligating the cannula of the inflow

vessel and each branch onto the arms of the supporting frame. Subsequently, the arterial tree was histologically fixed by perfusing with and immersing it in a mixture of 2% glutaraldehyde and 4% formaldehyde in isotonic saline at a perfusion pressure of 100 mmHg. Then it was dehydrated by perfusing with and immersing in ethanol-saline mixtures of progressively increasing ethanol concentration under the same perfusion pressure, and finally suspended in pure ethanol. Finally, the arterial tree was filled with and immersed in oil of wintergreen (methyl salicylate) containing ethanol at 5% by volume under a perfusion pressure of 100 mmHg to render it transparent.

The arterial trees prepared by this method lost elasticity of natural living artery during the process of fixing, dehydrating, and rendering them transparent. However, the method ensured the preservation of the complex three-dimensional configuration of the natural cerebral arterial tree that we considered the most important factor for studying the flow behavior and distribution of embolus-model particles.

2.3. Experimental Procedures and Analysis

The inflow vessel (ICA) was connected via a flexible plastic tube to a plastic bottle used as a head tank. Each of the outflow vessels was connected via a flexible plastic tube to a conical flask used as a collecting reservoir. The arterial tree was then placed vertically with the inlet at the bottom in a glass chamber filled with the same liquid used to render the vessel transparent (methyl salicylate containing ethanol at 5% by volume), and the area of interest on the arterial tree was trans-illuminated with a condensed parallel light from a tungsten-filament lamp or a mercury arc lamp. The rest of experimental procedures and methods of analysis were almost the same as those described elsewhere.[14,15] Therefore, only a brief explanation will be given here.

A dilute suspension of a mixture of 30, 80, 100, 115, 300 μm- and 1.0 mm-diameter polystyrene microspheres (density $\rho_s = 1.06$ g/cm^3; Duke Scientific Corp., Palo Alto, CA) in methyl salicylate (oil of wintergreen) containing ethanol at 5% by volume (density $\rho = 1.16$

g/cm^3, viscosity μ= 2.6 mPa s, refractive index n_D = 1.53) was used as a substitute for blood (density ρ = 1.06 g/cm^3, viscosity μ= 4.0 mPa s).

The arterial tree and the entire flow system were filled with the suspension, and the fluid was then subjected to steady flow through the arterial tree. A series of experiments were carried out within a physiological range of inflow Reynolds numbers*, Re_o, evaluated in the ICA proximal to the flow divider of the ICA from 330 - 710 (in terms of blood flow rate; 200 - 490 ml/min) while varying the ratio of flow rate in the MCA to that in the ICA (Q_1/Q_0). The blood flow rate in the ICA used as a reference was 364 ml/min (289 ~ 494 ml/min, n = 11) reported by Hardesty et al. [16]

In doing the flow experiments, the flow into the posterior communicating artery (PCoA), which was the smallest in diameter among the three outflow vessels, was stopped during the flow experiments to avoid obstruction of the vessel by large particles and keep the flow conditions constant. Experiments were carried out under several different conditions in the flow rate ratio between the ACA and the MCA by varying the height of the two collecting reservoirs. The behavior of individual suspended particles flowing in the cerebral arterial tree was observed through a magnifying lens system attached to a cinecamera and photographed on a black & white 16-mm cinefilm with a 16 mm high-speed cinecamera at a film speed of 2,000 pictures per second. The flow experiment and filming were repeated 6 times under the same flow condition, and the particles that were photographed on these films were counted as the number of particles that entered each of the branches. Experiments were carried out also under the condition of pulsatile flow by superimposing a sinusoidal oscillatory flow (frequency 2 Hz) in parallel with steady flow to study the effect of sinusoidal oscillations on distribution of particles at the bifurcation.

After finishing the flow studies, the whole arterial tree was photographed together with a ruler on 35-mm color or black & white films. The developed 35-mm films were analyzed to obtain the inner diameter of each segment. The developed 16 mm cinefilms were subsequently projected onto a drafting table, and the movements of individual particle were analyzed frame by frame with the aid of a stop-motion 16 mm movie-analyzer to obtain detailed flow patterns and the number of particles that entered each of the ACA and the MCA.

To obtain detailed flow patterns at the bifurcation, observations were made from the dorsal side of the vessels at right angle with the common median plane of the terminal ICA and proximal portion of the two daughter vessels (ACA and MCA). Flow patterns were obtained by tracing the paths of small particles (30 - 115 μm) frame by frame. The paths of 0.3 and 1.0 mm-diameter particles were traced individually by dotting the center of the particles. The number of 0.3 mm-diameter particles was counted from the cinefilm taken under each flow condition. The number of 1.0 mm-diameter particles was obtained by counting the number of particles recovered from outflow fluid collected in each reservoir during the period of 180 sec.

Representative geometric and flow conditions such as the vessel diameter, D_0, mean volume flow rate, Q_0, mean fluid velocity, $\overline{U}$, and Reynolds number, Re_0 (= $D_0 \overline{U} \rho/\mu$, where ρ and μ are the density and the viscosity of the flowing fluid, respectively) were evaluated at the inlet of the arterial tree, that is, the ICA.

Statistical analysis of obtained data was carried out using a microcomputer and running Statview® 512. The results were expressed as a mean±SD of the mean of the group using the Student's *t*-test. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Anatomical Structure of the Cerebral Arterial Trees

Figure 1 shows photographs of two transparent cerebral arterial trees prepared and used in the present study. Measured inner diameters of the three vessels of the bifurcation are shown in the figure at the locations indicated by arrows. As shown in the figure, in both the arterial trees, the ICA was directed towards the MCA. In Vessel I shown in panel A, the branching angle between the axis of the ICA and that of the ACA and the MCA was 82º and 156º, respectively. In Vessel II shown in panel B, the corresponding value was approximately 60º and 170º, respectively.

Compared with the data reported by others,[17] the inner diameters of the ICA and the MCA of these two vessels and the inner diameter of the ACA of Vessel II were almost the same as the standard values, but the diameter of the ACA of Vessel I was larger than the reported value. The diameter ratio between the ACA and the MCA (diameter of

ACA/diameter of MCA, calculated by using the inner diameters at the bifurcation) was 0.97 in Vessel I and 0.77 in Vessel II, while the mean value reported was around 0.70.[17-19] The geometrical flow rate ratio, defined as the flow rate ratio calculated by assuming that the flow in the parent vessel was distributed to the two daughter vessels proportionally to their cross-sectional areas evaluated at the flow divider of the bifurcation, and expressed by the ratio of the flow rate in the MCA to that in the ICA (Q_1/Q_0), was 0.52 for Vessel I and 0.63 for Vessel II.

3.2. Distribution of Large Particles

It is believed that increased blood flow is the most important factor for emboli to be carried to a particular vessel. Under certain pathological states such as the case of intracranial arteriovenous malformation (AVM), abnormally large amount of blood flows to one particular vessel due to reduced peripheral resistance.[20-22] In subjects with an azygous ACA, the proximal ACA serves not only its own distal ACA (A2 segment) but also contralateral distal ACA (A2 segment) via the anterior communicating artery (ACoA). In such a case, large amount of blood, that is comparable or even greater than that to the MCA, flows through the proximal ACA (A1segment). Taking such abnormal situations into consideration, we have studied the effects of the inflow rate to the ICA and the flow rate ratio at the terminal bifurcation of the ICA on distribution of embolus-model particles at the bifurcation by varying the height of the head tank and peripheral resistance of two daughter vessels (ACA and MCA).

Table 1 shows the results of the distribution of 0.3 and 1.0 mm-diameter particles at the terminal bifurcation of the ICA of Vessel I obtained in steady flow under three different conditions. The flow conditions were expressed in terms of the flow rate in the ICA (Q_0) and the ratio of the flow rate in the MCA to that in the ICA (Q_1/Q_0). Distribution rate of particles was expressed as the rate of particles that entered the MCA within all the particles that passed through the ICA. While doing flow experiments, it was observed that some of the 1.0

mm-diameter particles aggregated and formed a large particle. Therefore these particles were separated and listed as a subgroup of 1.0 mm-diameter particles that had a diameter of greater than 1 mm.

As shown in the table, the distribution rate of 0.3 mm-diameter particles was very close to the flow rate ratio. In contrast to this, the probability of large particles having diameters greater than 1.0 mm to enter the MCA was higher than that of 0.3 mm-diameter particles at the flow rate ratio (MCA/ICA) of 0.61 and over. At the flow rate ratio of 0.61, 64.2 ± 1.4 (Mean ± SD) % of 0.3 mm-diameter particles entered the MCA. In the case of large sized particles, 71.1% of 1 mm-diameter particles and 91.8% of greater than 1 mm-diameter particles passed through the MCA. The differences between these values (between 0.3 mm- and 1.0 mm-diameter, and 1.0 mm- and greater than 1 mm-diameter particles) were all statistically significant ($p < 0.05$). Moreover, at the flow rate ratio of 0.77, all of the particles having diameters greater than 1.0 mm entered the MCA.

The results of particle distribution in Vessel II are shown in Table 2. As it was observed in Vessel I, it was also observed in Vessel II that 0.3 mm-diameter particles entered the MCA and the ACA at almost the same rates as their flow rate ratios. In contrast to this, 1.0 mm-diameter particles showed a very different distribution. At a flow rate ratio of 0.62 and over, 1.0 mm-diameter particles selectively entered the MCA, and the differences between the distribution rate of 1.0 mm-diameter particles and that of 0.3 mm-diameter particles were statistically significant ($p < 0.05$) under each flow condition. The tendency was same even at the flow rate ratio of 0.34 where the volume flow rate in the ACA became almost double of that in the MCA, and more than a half of the 1.0 mm-diameter particles entered the MCA. However, under the flow rate ratio of below 0.20, they changed their directions and entered the ACA preferentially, and especially at the flow rate ratio of 0.12, all the particles that passed through the ICA entered the ACA. The results of the distribution of 1.0 mm-diameter particles under the condition of pulsatile flow are shown in the brackets in Table 2. They

showed the same tendency as those observed under the condition of steady flow but the values were a little higher than those obtained under the condition of steady flow.

Table 3 shows the effects of pulsation and the flow rate in the ICA (Q_0) on distribution of 1 mm-diameter particles at a fixed flow rate ratio ($Q_1/Q_0 = 0.61$) in Vessel II. As shown in this table, particle distribution was greatly affected by the flow rate (flow velocity) in the ICA, and as the flow rate increased, the fraction of particles that entered the MCA also increased. Comparison of the data obtained in steady flow with those obtained in pulsatile flow showed that the probability of particles to enter the MCA was a little higher in pulsatile flow than steady flow at each flow rate.

3.3. Flow Patterns

Figure 2 shows the general flow pattern (**A**) and the velocity distribution (**B**) in the common median plane of the bifurcation at the terminal bifurcation of the ICA in Vessel I obtained at the flow rate ratio (Q_1/Q_0) of 0.61. Streamlines were represented by the paths of small particles (30 - 115 μm, less than 1/10 of vessel diameter). As shown in **A**, the particles in the mainstream of the ICA moved obliquely to the axis of the vessel from the medial side (ACA side) to the lateral (MCA) and upper (dorsal) direction due to the lateral and anterior curvature of the supraclinoidal segment of the ICA. The particles located relatively close to the axis of the ICA entered the MCA directly. The particles located close to the medial (ACA side) wall of the ICA entered the ACA directly. The particles in the secondary flow indicated by the dashed particle paths that were deflected at the lateral (MCA side) wall of the ICA traveled along the ventral wall passing under the mainstream of the ICA and entered the ACA. Judging from these particle paths, the stagnation point where the flow actually split into two branches was located at or very close to the carina of the bifurcation. The velocity profile at the termination of the ICA was flattened as shown in **B**. This was the same as the result obtained by Takeuchi and Karino using human cerebral arterial systems containing the carotid siphon and the terminal bifurcation of the ICA.[23]

Apart from the general flow pattern, the movements of 0.3, and 1.0 mm- and larger-diameter particles were also traced separately under the same flow condition as that of the general flow pattern obtained at $Q_1/Q_0 = 0.61$, by plotting the center of each particle. The results are shown in Fig. 3, **A** and **B**. As shown in **A**, 0.3 mm-diameter particles (≈1/10 of vessel diameter) were found to travel very closely to the vessel wall of the ICA in the same manner as small particles (30 - 115 μm), and also the flow pattern obtained by tracing 0.3 mm-diameter particles was almost the same as that obtained with small particles. However, as shown in **B,** 1.0 mm- and larger-diameter particles (greater than 1/4 of vessel diameter) traveled away from the vessel wall as shown by many particle paths. Furthermore, on entering the daughter vessels, a striking tendency of convergence of particle paths was recognized with 1 mm- and larger-diameter particles. These particles were located in the core flow shown in the general flow pattern, and were carried to the MCA.

Figure 4**A** shows the general flow pattern in Vessel I represented by the paths of small particles (30 -115 μm) at the flow rate ratio (Q_1/Q_0) of 0.34. As shown in the figure, a vortex was formed at the entrance of the MCA adjacent to the outer wall (hip) of the bifurcation due to large deviation of the flow rate from the geometrical flow rate ratio ($Q_1/Q_0 = 0.52$). Compared with the general flow pattern obtained at the flow rate ratio of 0.61, the number of streamlines (particle paths) that entered the MCA decreased, and the stagnation point indicated by the letter "P" shifted towards the MCA. However, the particles located close to the axis of the ICA and carried by the core flow still entered the MCA. Also the 1 mm-diameter particles that were located near the axis of the ICA were carried by the core flow, and entered the MCA as shown in Fig. 4**B**.

4. Discussion

4.1. Effects of Particle Size and Radial Migration of Particles

In the present study, it was observed that, in the ICA, particles greater than 1.0

mm-diameter traveled apart from the vessel wall. Furthermore, on entering the daughter vessels, they showed a strong tendency to gather in the core region of each vessel. It was considered that this occurred as a result of the radial migration of particles away from the vessel wall.

It is well known that rigid spheres, liquid drops, and even human red blood cells flowing in a tube migrate away from the tube wall, and the larger the diameter of the particle, and the faster the fluid velocity, the faster they migrate.[24-26] In our present study, as shown in Figs. 2A and 3**A**, the particles smaller than 0.3 mm-diameter (≈1/10 of vessel diameter) were found to travel very closely to the vessel wall of the ICA, and the rate of their distribution to the MCA and ACA was almost the same as the flow rate ratio. This implied that in the case of the particles whose diameters were 0.3 mm and smaller, there was no effect of particle migration on their distribution. In contrast to this, particles greater than 1.0 mm-diameter (≈1/4 of vessel diameter) traveled apart from the vessel wall in the ICA as shown in Fig. 3**B**. This resulted in redistribution of particles at the bifurcation, and particles greater than 1 mm in diameter selectively entered the MCA, suggesting the involvement of particle migration in the ICA in biased distribution of emboli in the MCA and ACA in clinical cases.

4.2. Effect of the Direction of Core Flow

In the present study, the particles smaller than 115 μm in diameter were found at any radial position, even at the very vicinity of the vessel wall, at the termination of the ICA as it can be seen in the flow pattern shown in Fig. 2**A**. This implied that no radial migration occurred with these particles, and hence they were distributed evenly in the flowing fluid. This was supported by the results shown in Table 1 that the distribution rate of these small particles in the ACA and MCA was almost the same as the flow rate ratio in these vessels. By contrast, 1 mm-diameter particles, and especially the particles larger than 1 mm-diameter, had already converged to the core region of the ICA. Due to that the distribution of large particles was greatly affected by the direction of the core flow (represented by the particle paths

located in and close to the center of the vessel). Furthermore, the size of the particle itself was an important factor. The mean internal diameter of the normal ICA was reported to be 3.6 mm.[17] Therefore, when a 2 mm-diameter particle flows through this vessel, a part of the particle is already within the core flow. Usually, the core flow has a velocity higher than that of the peripheral flow. Therefore it could carry the particle with a velocity higher than that of the peripheral flow. After all, the larger the size of particles, the more easily they obey the flow direction of the core flow. In both the Vessel I and Vessel II used in our present study, the core flow in the ICA was directed to the MCA as shown in Fig. 2**A**. Therefore, the large-sized particles that had already converged to the core region of the ICA were easily carried to the MCA, resulting in large differences between the distribution rate of 0.3 mm-diameter particles and that of 1 mm- and larger diameter particles at the bifurcation as shown in Table 1.

It is known that the migration velocity of a rigid sphere increases with increasing flow velocity and also by the imposition of oscillatory flow.[27] The results of our present study showed the effects of these factors. As shown in Table 3, under the condition of steady flow, the fraction of particles that entered the MCA increased with increasing the flow rate in the ICA (Q_0). The effect of pulsation was not so evident.

4.3. Effect of Branching Angle

It is considered that branching angle plays very important role in distribution of emboli. Karino and Goldsmith studied flow patterns in models of branching vessels using glass-made T-junctions with various branching angles and diameter ratios.[28] They showed that when the flow rate of two daughter vessels are the same, the core flow always enters the geometrically favorable branch, that is, the branch that has a larger branching angle between the parent vessel and the vessel, or that has a larger diameter.

In our present study, in both Vessel I and Vessel II, the terminal portion of the ICA was

directed towards the MCA whose branching angle between the ICA and the MCA was 156º in Vessel I and 170º in Vessel II, and diameter was a little larger than that of the ACA. Under these geometrical conditions, in Vessel I, the core flow (represented by the particle paths located in and close to the center of the vessel) entered the MCA even when the flow rate ratio (Q_1/Q_0) decreased to as low as 0.34. In Vessel II that had a wider branching angle between ICA and MCA (170º) and a larger diameter difference between the two daughter vessels (ACA/MCA = 0.77) than those of Vessel I, the core flow entered the MCA more easily than the case of Vessel I. This resulted in differences in the distribution of 1 mm-diameter particles between the two vessels. At the flow rate ratio (Q_1/Q_0) of around 0.6, 95.5% of the particles entered the MCA in Vessel II, while in Vessel I, 71.1% of the particle entered the MCA. Therefore, it could be said that the geometrical factor, especially the branching angle between the parent and its daughter vessels, is an important factor for determining the direction of the core flow of the parent vessel.

4.4. Effect of Flow Rate Ratio

In studying the flow patterns shown in Figs. 2 to 4 carefully, it was noticed that there was certain regularity in the flow that entered the ACA and MCA. The flow that entered the ACA came from the peripheral area of the ICA, while the flow that entered the MCA came from the center and dorsal portion. If we cut the ICA along the line **a-a'** drawn at the inlet of the bifurcation as shown in Fig. 5A, and look at the cross-section, the two areas will be seen completely separated by a border called a separation surface[29-31] as shown schematically in Fig. 5B. Here, the size of the area increases or decreases proportionally to the flow rate in the ACA and MCA as shown in the figure. Therefore, it is important in which area the core flow is included.

In our present study, in both the Vessel I and Vessel II, the ICA was directed towards the MCA. Therefore, the core flow was included in the MCA area as shown in the figure. Due to

that, large-sized particles were carried selectively to the MCA with increasing the flow rate ratio (Q_1/Q_0) as shown in Table 2. However, as the flow rates of two daughter vessels become close, the areas occupied by the flow to the ACA and MCA become also close. In such a case, geometrical factors such as the branching angle and the diameter ratio show a big effect on distribution of large particles since they determine the direction of the core flow. However, when the flow rate of the MCA is decreased further, large particles change their direction and enter the ACA preferentially as shown by the results presented in Table 2. This means that large deviation in flow rate from normal physiological flow rate overcomes geometrical inferiority. Such a condition may occur in subjects with an arterio-venous malformation (AVM) in the ACA territory and also with an azygous ACA that serve both ACA distributions. In such cases, it is likely that the embolization rate of the ACA becomes very close to or even higher than that of the MCA.

With regard to the results obtained from clinical studies, Luessenhop et al.[8] carried out artificial embolization of cerebral AVM using spherical balls. They showed that 90% of emboli were carried to the MCA even in the cases of AVM that was supplied equally by the ACA and MCA. However, difficulty of embolization was also reported by many investigators[9,11] in the cases of AVM that was supplied mainly by the ACA or equally by the ACA and MCA, although the AVM that was supplied mainly by the MCA became a good indication of artificial embolization.[8,11,12] Yet, Kricheff et al.[32] reported a successful case in which the feeding artery was enlarged but had an acute branching angle, and insisted that large flow rate was more important than the branching angle. All these results agree well with our experimental results.

In the present work, we have investigated the cause of the difference in the incidence of cerebral embolic occlusion between the ACA and MCA territories. As a result of our model flow experiments using embolus-model particles, we arrived at a conclusion that it occurs due to imbalanced distribution of large emboli at the terminal bifurcation of the ICA that was

caused by the radial migration of particles away from the vessel wall. In most normal cases, the terminal portion of the ICA is directed to the MCA, and the diameter of the MCA is larger than that of the ACA. Therefore, the flow rate in the MCA is expected to be larger than that in the ACA. Here, with respect to the actual flow rate in cerebral arteries, Hassler[33] calculated mean flow rates in main branches of the circle of Willis in normal cases based on the flow velocity measured by Doppler sonography and the radiological intravascular mean diameters. He obtained 142.6 ml/min for the MCA and 91.1 ml/min for the ACA. Therefore, the average flow rate ratio for the MCA (Q_1/Q_0) under normal physiological condition is estimated to be about 0.61. At this flow rate ratio, large emboli enter the MCA preferentially due to the hemodynamic effect described above. In our present study, under similar conditions (Q_1/Q_0 = 0.61, Q_0: 260~270 ml/min), 71.1% of 1 mm-diameter particles and 91.8% of 2 mm- or larger-diameter particles entered the MCA in Vessel I, and 95.5% of 1 mm-diameter particles entered the MCA in Vessel II. These values are very close to the clinical data of Gacs et al.[14] that reported the incidence of occlusion of the MCA and ACA as 93.2% and 6.8%, respectively.

It is widely accepted that the important factors that govern the course of emboli (thrombi) at the terminal bifurcation of the ICA are pump effect, branching angle, and diameter ratio of two daughter vessels.[8,9,12] Our results provided a theoretical ground for this empirical conclusion, although it is limited to the terminal bifurcation of the ICA. Furthermore, we added one more factor, that is, the particle migration away from the vessel wall as the most important factor that determine the ultimate course of emboli (thrombi).

4.5 Generalizability of experimental data

The conclusions described in our manuscript were made using the data obtained from a small sample. With respect to this, it was desirable to perform the experiments on several other vessels. However, even if we had carried out the experiments with some other arterial models whose diameters and branching angles were different from those of the arterial models

used in our present study, we would have obtained very similar results since the phenomenon of the radial migration of large particles certainly occurs in the ICA in any model. We consider that the conclusions we obtained are generalizable.

4.6 Limitation of the model

As explained in the Method section, the arterial trees prepared by our method lost elasticity of the vessel wall and biological and biochemical functions of the endothelial cells of natural living artery. However, all the components constituting the arterial wall and supporting the integrity of the arterial tree (such as the endothelial cells, medial- and adventitial tissues) were not lost. Therefore, we do not consider that the loss of these histological and biological factors put limitations to our model.

With respect to the effect of elasticity (distensibility) of the vessel wall on the flow and radial migration of particles, there will be some differences between a rigid and a distensible model under the condition of pulsatile flow. In a distensible model, the fluid velocity takes a little lower value in the systolic period, but higher value in the diastolic period than those corresponding velocities in a rigid model. However, the differences are very small. If we consider the whole cardiac cycle, the effect of the distensibility on the radial migration of particles will be negligible. We do not consider that the lack of distensibility in our arterial system puts a limitation to our model.

We consider that our model may not apply to only the following two cases.

(1) Emboli which cannot be regarded as spheres such as disk-shaped and elongated-fibrous emboli, since we do not know whether non-spherical emboli undergo the radial migration away from the vessel wall.

(2) Flow of hypercythemic blood, since the movements of emboli might be interfered and suppressed by the presence of crowded blood cells.

Conclusions

Due to the radial migration of particles away from the vessel wall towards the axis of the ICA, redistribution of particles occurs at the terminal bifurcation of the ICA, and large particles selectively enter the MCA to which the core flow of the ICA is generally directed. The phenomenon becomes more remarkable with increasing particle diameter, the flow rate in the ICA, and the flow rate ratio Q_1/Q_0 (MCA/ICA). This might explain why the incidence of thrombo-embolism is higher in the MCA than in the ACA in humans.

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***Foot note**

The Reynolds number, Re, is a dimensionless parameter defined as $Re = DU\rho/\mu$, where D is the tube diameter; U, the mean fluid velocity; ρ and μ, the respective fluid density and viscosity. In physical terms, it is the ratio of a dynamic pressure, ρU^2, to a typical shearing stress, $\mu U/D$, or the ratio of inertial force to frictional force. It was discovered by Reynolds in his dye-injection experiments that in circular tubes with different diameters, the transition from steady, orderly laminar flow to turbulent flow, which was characterized by rapid and

continuous mixing of the fluid in a chaotic manner throughout the tube, occurs at approximately the same Reynolds number, ~2,000. The significance of his findings is that, for incompressible viscous fluids such as water and blood, if the Reynolds numbers are the same in geometrically similar vessels, one can assume a dynamic similarity of flow patterns even if the vessel diameters and the fluid velocities and viscosities are completely different. Thus, it is more convenient to express the flow conditions in terms of the Reynolds number, Re, than in terms of the flow rate. The numerical value of the Reynolds number expresses the degree of stability of laminar flow (at Re < 2,000) or intensity of turbulence (at Re > 2,000) of the flow. In the present study, we used methyl salicylate (oil of wintergreen) as a test fluid whose viscosity and density are different from those of blood. Therefore, we expressed the flow conditions in terms of the Re instead of blood flow rate.

Figure Captions

Figure 1. Photographs of isolated transparent human cerebral arterial trees containing the terminal bifurcation of the internal carotid artery (ICA), showing the geometrical structure of the bifurcation as observed postero-anteriorly normal to the common median plane of the ICA and its two daughter vessels. Measured inner diameters of the three vessels of the bifurcation are shown in the figure in mm at the locations indicated by arrows. **A** (Vessel I) is the right ICA bifurcation from a 64 year-old male. **B** (Vessel II) is the left ICA bifurcation from an 81 year-old female.

Figure 2. Detailed flow pattern (**A**) and distributions of axial fluid velocity (**B**) obtained in steady flow in a transparent human ICA terminal bifurcation (Vessel I) shown in **Fig. 1A** as observed normal to the common median plane of the bifurcation. The drawing was constructed by tracing the paths of small (30-115 μm in diameter) tracer microspheres. Solid lines represent the paths of particles located in or close to the common median plane; short dashed lines represent paths far out of the common median plane; long dashed lines or dash-dotted lines represent paths located between the first two types of paths. The arrow at "P" indicates the stagnation point where the flow actually split into two branches. The numbers at the outside and along the particle paths indicate, respectively, the inner diameter of the vessel in mm and particle translational velocities in mm/sec at positions shown. Numbers along the velocity profiles indicate the maximum velocity at the cross-section indicated. The numbers given under Q_1, and Q_2 indicate the fraction of the flow that entered the particular branch out of the total inflow Q_0. The following parameters were assessed at the inlet of the ICA. Re_0: Reynolds number, D_0: vessel diameter, Q_0: volume flow rate, $\overline{U}$: mean fluid velocity.

Figure 3. Tracing of the paths of 1 mm (**A**) and larger than 2 mm-diameter particles (**B**), showing a striking tendency of convergence of particle paths to the axis of the vessel.

Figure 4. A: Detailed flow pattern, as in Fig. 2, obtained by tracing the paths of small (30-115 μm in diameter) tracer microspheres flowing in steady flow in a transparent human

ICA terminal bifurcation (Vessel I) at $Q_1/Q_0 = 0.34$. The figure shows the complicated flow behavior of particles that entered the ACA and the formation of a vortex at the outer wall of the MCA that occupied more than a half of the cross-section at the entrance of the MCA. Note that the core flow of the ICA still entered the MCA directly. The arrows at "P" and "S" denote the respective locations of the stagnation and separation points. **B:** Tracing of the paths of 1 mm-diameter particles in steady flow at $Q_1/Q_0 = 0.34$, showing the convergence of particles to the center of the ACA on entering the branch. Note that the particles in the core flow of the ICA still entered the MCA.

Figure 5. A: Detailed flow pattern the same as that shown in Fig. 4 with a dashed line **a-a'** at the inlet of the bifurcation, showing the location to be looked at the cross-section of the ICA. **B**: Cross-section of the ICA at the location indicated by the dashed line **a-a'** in **A**, showing schematically the areas occupied by the flow that entered the ACA and MCA. Note that the two areas are completely separated by a border that is called a separation surface. The size of the area varies with the increase or decrease in flow rate in the ACA and MCA as shown in the figure.