

The Mechanism of Embolic Watershed Infarction: Experimental Studies

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ABSTRACT: The mechanism for the preferential distribution of emboli to cerebral arterial borderzone regions, known to cause some watershed infarcts was studied. We hypothesized that emboli of a specific size range are selectively directed to the arterial borderzones due to the tendency of emboli to bypass the small arterial branches which emerge proximal to major borderzones. To test this hypothesis we perfused the brains of cadavers with suspensions of 90-210 μm glass microspheres and chemically extracted the particles from various arterial territories and a watershed zone. Particles in the 150-210 μm size range were found to be preferentially distributed to the watershed zone whereas particles less than 150 μm in size were randomly dispersed in leptomeningeal arteries of all vascular regions. To assess the role of branch size on the concentration of emboli at bifurcations, we perfused artificial analogs of evenly and unevenly branching bifurcations with suspensions of 90-150 μm and 150-210 μm particles. Branching cylinders with symmetrical branches contained the same concentration of particles, independent of particle size. In contrast, when one branch was one-quarter the size of the other, the concentration of 150-210 μm particles in the asymmetric branch was approximately 65% of the main trunk. Particles 90-150 μm in size were evenly distributed despite variation in branch size. These results indicate that emboli, of a limited range of size, may be selectively propagated to the distal ramifications of subarachnoid arteries located in the watershed zone rather than diverging into small calibre branches which arise along the way.

RÉSUMÉ: Études expérimentales du mécanisme de l'infarctus embolique des zones de partage du lit vasculaire Le mécanisme de la distribution préférentielle des embolies aux régions limites du réseau artériel cérébral, connues comme causant des infarctissements des zones de partage du lit vasculaire, a été étudié. Nous avons émis l'hypothèse que les embolies de certaines tailles spécifiques sont dirigées sélectivement aux zones artérielles limites à cause de la tendance des embolies à éviter les petites branches artérielles qui émergent à proximité des zones frontières importantes. Afin de vérifier cette hypothèse, nous avons perfusé le cerveau de cadavres avec une suspension de microsphères de verre de 90-210 μm et nous avons extrait chimiquement les particules de différents territoires artériels et d'une zone de partage du lit vasculaire. Les particules de la taille 150-210 μm ont été retrouvées préférentiellement distribuées à la zone de partage du lit vasculaire alors que les particules plus petites que 150 μm étaient dispersées de façon aléatoire dans les artères leptoméningées de toutes les régions vasculaires. Pour évaluer le rôle de la taille des branches artérielles sur la concentration des embolies aux bifurcations, nous avons perfusé des analogues artificiels à bifurcations symétriques et asymétriques avec des suspensions de particules de 90-150 μm et de 150-210 μm . Les cylindres à branches symétriques contenaient la même concentration de particules, indépendamment de la taille des particules. Par contre, quand une branche était le quart de la taille de l'autre, la concentration de particules de 150-210 μm dans la branche asymétrique était approximativement 65% de celle du tronc principal. Les particules de 90-150 μm étaient distribuées également en dépit de variations dans la taille des branches. Ces résultats indiquent que les embolies, d'une certaine gamme de tailles, peuvent sélectivement gagner les ramifications distales des artères sous-arachnoïdiennes situées dans la zone de partage du lit vasculaire plutôt que d'être déviées vers les branches de petit calibre qui prennent naissance sur leur trajet.

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Cerebral borderzone or watershed infarcts (WI) are ischemic lesions which occur along the boundary zone between major cerebral arterial territories. The majority of WI are caused by diminished perfusion of distal arteries^{1,2} usually due to systemic hypotension³ or stenosing carotid atherosclerosis.⁴ However, there are some cases of WI that are not explained by cerebral

hypoperfusion but are seemingly the result of vascular occlusion by thromboemboli,^{5,6} cholesterol emboli,^{5,7,8,9} or tumour emboli.⁵

The underlying mechanism for embolic WI is not known. However, two distinct mechanisms to account for the selective distribution of emboli have been proposed emphasizing arterial

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branch angle⁵ and arterial branch diameter.⁶ The latter mechanism suggests that emboli, of a limited range of size (approximately 200 μm), travel to the distal branches of cerebral arteries located in the watershed zones, rather than diverging into arterial branches of smaller diameter, due to the tendency of the emboli to remain in the centre of laminar flow.^{10,11} Accordingly, cerebral microemboli are almost exclusively concentrated in borderzone-bound arteries and when vessel diameters diminish in the borderzone region, emboli become arrested causing infarction. For the first time we report experimental validation of this explanation by showing (1) preferential size specific distribution of particles to watershed zones in a cadaver, and (2) size dependent distribution of suspended particles in unevenly branching cylinders as models of arterial bifurcations.

MATERIALS AND METHODS

Cadaveric Model

In three cadavers (12 hours post mortem) with minimal atherosclerosis and no neurologic history, the internal carotid arteries in the neck were isolated and the right common carotid artery ligated. The left internal carotid artery was cannulated, the cannula attached to a perfusion apparatus and the cerebral circulation flushed with physiologic saline under near systolic constant pressure (300 mL) until effluent from the jugular veins was clear. The perfusion apparatus was then filled with perfusate containing anticoagulated whole blood with a suspension of 90-120 μm glass microspheres (Glass Microcarrier Beads, Sigma, St. Louis, MO) in a total volume of 300 mL. The left cerebral hemisphere was then perfused with 1.3×10^6 microspheres under constant pressure and a flow rate (10 mL/s). The skull was then opened, the brain removed and one gram of leptomeningeal arteries were stripped from each of the left anterior and middle cerebral arterial territories and the anterior-middle cerebral arterial watershed zone. Collected arteries were solubilized in 35% nitric acid (37° C, 12 hours) and microspheres extracted by centrifugation (200 g, 2 minutes). Microspheres were enumerated with a light microscope, and size was determined using a calibrated ocular graticule. Values are expressed as per cent particles recovered per vascular region from one representative cadaveric perfusion.

In Vitro Model

Artificial metal bifurcations were made as analogues to cerebral arterial bifurcations, similar to previous work.¹¹ Isolated segments were constructed with a constant trunk diameter of 4 mm and emergent branches either 1 mm to 4 mm in cross sectional diameter. The branch angle was constant at 60 degrees. Individual segments were perfused with known concentrations of particles (800-1000 particles/mL in 13% Glycerine to match viscosity of blood) in the 90-150 μm size range and in other experiments using particles 150-210 μm in size. Segments were perfused at a constant flow rate of 10 mL/s, using a peristaltic pump to generate pulsatile flow, and the effluent from the various size branches and the trunk were collected. The concentration of particles from the even and uneven branches were determined by microscopic enumeration of 150 μL aliquots. Results are expressed as number of particles/mL in the respective branches. The reported values are means and standard deviations of three separate perfusion experiments.

RESULTS

Cadaveric Model

Microspheres in the 90-150 μm size range were found to be randomly dispersed in leptomeningeal arteries of the various territories (Figure 1a). However, microspheres in the 150-210 μm size range were preferentially distributed to the watershed zone (Figure 1b).

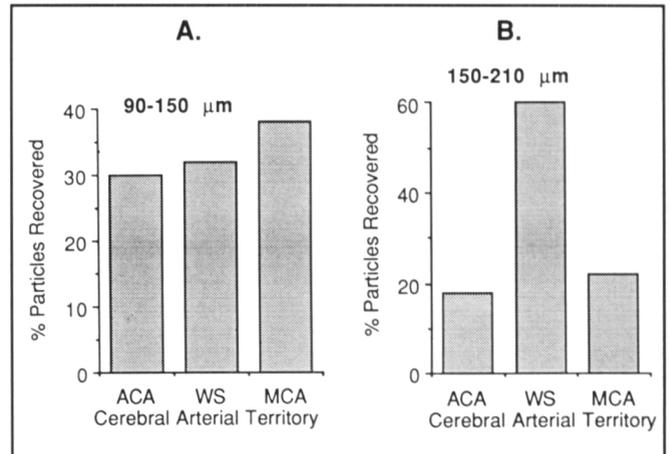


Figure 1 — (A) Relative percentages of 90-150 μm particles recovered from leptomeningeal arteries from the anterior cerebral (ACA), middle cerebral (MCA) and watershed (WS) territories. Particles in the 90-150 μm are evenly distributed to all vascular regions. (B) — Particles in the 150-210 μm are preferentially distributed to the watershed (WS) zone.

In Vitro Model

Particles in the 90-150 μm size range were evenly distributed to both branches at the bifurcations independent of the size of the branches (Figure 2). When particles in the 150-210 μm range were perfused through evenly branching bifurcations, the concentration of particles was similar in both branches (Figure 3a). However, when particles in the 150-210 μm range were perfused into the unevenly branching bifurcations, the asymmet-

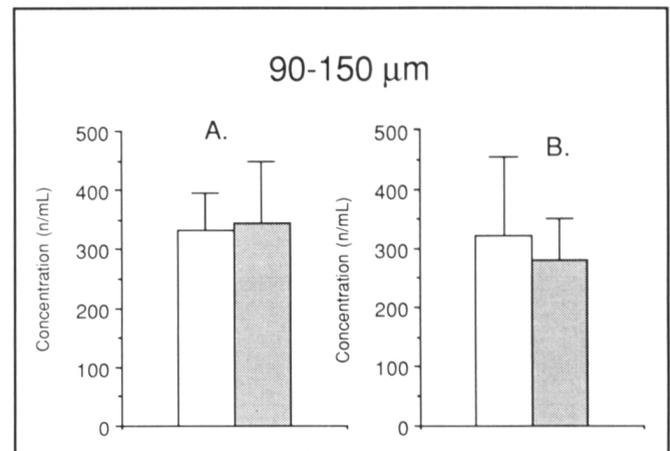


Figure 2 — (A) Bifurcations with two branches of the same size (shaded and clear bars) perfused with 90-150 μm particles showing even distribution of particles in both branches. (B) Bifurcations with one branch one-quarter the size (shaded) of the other (clear) show even distribution of 90-150 μm particles.

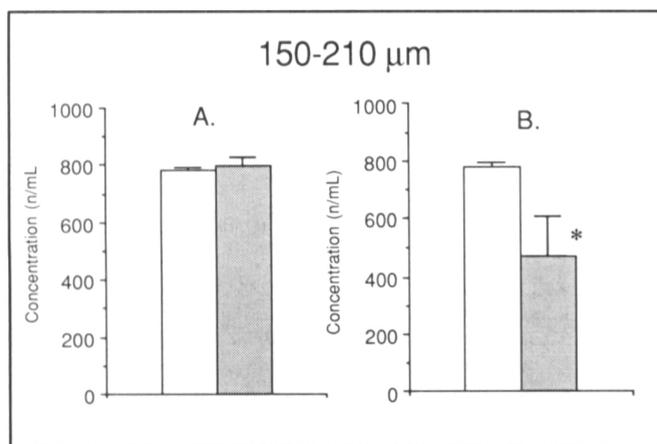


Figure 3 — (A) Bifurcations with two branches of the same size (shaded and clear), perfused with 150-210 μm particles show even distribution in both branches. (B) Bifurcations with one branch one-quarter the size (shaded) of the other (clear) contain fewer 150-210 μm particles/mL. (* indicates $p \leq 0.05$ by ANOVA)

ric branch (one-quarter of the parent trunk) was found to have 65% of the concentration of particles in the main trunk. Decreasing the branch diameter produced a relative concentration drop in particles contained in the asymmetric branch.

DISCUSSION

Infarcts along the boundary regions of major cerebral arterial distributions, known as watershed infarcted (WI) are usually correlated with either cerebral hypoperfusion or embolic occlusion of distal arterial segments in the brain.⁴ Studies using the hypotensive-primate model reproduce the topography of infarction found in human cases of WI with presumed cerebral hypoperfusion.^{1,3} However, no experimental model has reproduced selective embolization to watershed zones.^{1,2}

In the present experiments, microspheres 150-210 μm in diameter were selectively distributed to watershed zones in cadavers, when suspended in whole blood, rather than being randomly dispersed. In addition, as previously reported in an animal model, artificial emboli in the 90-150 μm range were non-selective in arterial distribution throughout the brain^{1,2} indicating that preferential sorting of emboli occurs for particles of a limited size range. This is consistent with previous observations which indicate that emboli overlying WI are seldom less than 200 μm in size, and very rarely less than 100 μm in size.⁹

Two mechanisms have been proposed to explain the preferential distribution of emboli in the cerebral circulation. The first, proposed by Torvik and Skullerud,⁵ emphasized the sharp angulation of branching vessels, suggesting that emboli are unlikely to enter such branches. However, in vitro flow studies indicate that branch angle is likely less important to the fluid dynamics in arterial branches compared to the asymmetric diameter of the branch vessel as compared to the parent vessel.¹³ On this basis, we emphasized an alternative property of the cerebral circulation to account for selective watershed embolism,⁶ suggesting that particulate emboli in arterial flow are more likely to remain in a centralized stream and not diverge into asymmetric arterial branches characteristic of cerebral arteries. In support of the latter mechanism, in vitro flow models indicate that 1) particles

flowing in cylindrical tubes tend to aggregate in a central stream,¹⁴ 2) fluid which enters asymmetric cylindrical branches is derived from the peripheral stream of the parent vessel rather than the central stream^{10,15} and 3) experimental studies of suspended particles in laminar flow are subjected to "phase separation" where particles tend not to diverge into branches emerging from main trunks.¹¹

Our experimental system using isolated artificial bifurcations reproduces the phase separation effect indicating that arterial branches with small diameters relative to parent arteries may contain fewer emboli than evenly branching arteries in vivo. Therefore, in the case of embolic WI, if emboli remain in the central stream of blood in major subarachnoid arteries rather than entering asymmetric branches, the majority of emboli become watershed bound and arrest only when the arterial lumen tapers to the size of the emboli, in the most distal arterial segments.

Experimental studies show that phase separation occurs only with specific ratios of emboli diameter to artery diameter.¹¹ One possibility which can be inferred from the in vitro flow studies is that particles of greater size are more centrally located in arterial flow when suspended in a medium with relatively smaller sized particles. In this way, under natural conditions and in the cadaveric and in vitro systems reported here, 90-150 μm emboli would diverge into asymmetric arterial branches and not be specifically localized to watershed zones.

In summary, the selective distribution of emboli in the cerebral circulation previously reported to cause WI has been reproduced in a cadaveric model. Using a perfusion system of isolated arteries, uneven arterial branches tend to contain less emboli than even branches suggesting that arterial branch diameter is important to stream sorting of emboli in watershed bound arteries.

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