Reactions of Granular Pericytes in a Rabbit Cerebrovascular Ischemia Model

BRIAN JEYNES, PH.D.

SUMMARY This study was undertaken to examine some of the quantitative and qualitative changes which might occur in the cerebral granular pericyte population when comparing control and embolically stressed animals. Eight animals, male NZW 2-2.5 kg rabbits, were given intracarotid sublethal injections of human atheroma concentrated at 125 mg/ml. Eight others received 1 cc of sterile saline. Two hours after injection the brains were fixed with either neutral buffered formalin or 3% buffered glutaraldehyde by cardiac perfusion at 110 mmHg pressure. The brains were removed and sliced, anteroposteriorly, in 3 mm slices. Each slice from five brains for each condition was then processed for paraffin sectioning and staining with Hematoxylin and PAS. Two animals for each condition underwent the same treatments and were processed for frozen sectioning and staining with Oil Red O. One animal for each condition had each brain slice sectioned by vibratome (40 μm) and was processed appropriately and examined by either fluorescence microscopy, standard electron microscopy or acid phosphatase electron microscopy. In the groups stained with Hematoxylin and PAS, granular pericytes were counted for each of the first five levels of each brain for both the control and experimental conditions. The results of this study reconfirmed that these cells were granular pericytes; that is, they were autofluorescent and PAS positive; were surrounded by a basement membrane, were acid phosphatase positive and contained a heterogenous granular cytoplasmic inclusion. Furthermore, after ischemic insult, the number of granular pericytes increased significantly at two hours. It was also shown that these cells appeared capable of accumulating lipid components of the injected atheroma from the vessel lumen.

PERICYTES are cells enclosed within a common basement membrane with the endothelial cells of small vessels, particularly at the capillary and post capillary venule level in most tissues of the body.1,2 A subpopulation of these pericytes, the so-called “granular” pericytes, are believed to function primarily as phagocytes.3,4 Although the origin of these cells is unknown, most current hypotheses support either a perivascular mesenchymal or hematogenic origin. The study of these cells in the brain is of particular interest to us, not only because little is understood about their biology and functions under non-pathologic conditions, but also because they have been particularly observed in some disease processes.5-7 This paper focuses on the reaction of cerebral granular pericytes under conditions of cerebral ischemia, provoked by occlusive thromboembolism and induced in an animal model which is being used to study stroke and TIA (Transient Ischemic Attack). In particular, we were interested to quantitatively determine the extent to which granular pericytes were present under “control” circumstances and whether their numbers were influenced by ischemic stress. Further, we wanted to see if injected intra-luminal materials were phagocytosed by these cells under the stress condition.

Materials and Methods

Male NZW rabbits 2–3 kg, received 1 cc saline (control) or 50 mg of human atheromatous material (HAM) concentrated at 125 mg/ml. The atheroma was removed from severely atherosclerosed aortas, taken at autopsy, under surgically clean conditions and prepared as described previously.8 These materials were injected into the surgically exposed left common carotid artery of anaesthetized animals. Anaesthesia was induced and maintained by I.V. injection of Somnitol concentrated at 32.5 mg/ml. Each condition consisted of a population of eight animals. Two hours after injection, the brains were either fixed by cardiac perfusion, for either light or transmission electron microscopy, or removed only partially fixed for frozen sectioning. Brains to be used for light microscopic examination with either paraffin or frozen sections were fixed with neutral buffered formalin. Those to be examined by electron microscopy were fixed with 3% buffered glutaraldehyde. The brains were sliced mid-sagittally and the ipsilateral injection half was cut anteroposteriorly in 3 mm slices. Granular pericytes were examined in sections taken from slices for each of the conditions in the following ways: (1) the brains of two animals taken from each condition were processed for frozen sectioning and stained with Oil Red 0, to determine whether lipid components of the injected material might be absorbed by these cells; (2) the brains of five animals taken from each condition were processed for light microscopy with paraffin sections which were stained with Hematoxylin and PAS, in order to count the number of granular pericytes per section (granular pericytes are PAS positive). Counts were made on sections from the entire rostral surface of left brain slices one through five; (3) each of the slices for one brain fixed in 3% buffered glutaraldehyde for each condition was cut on a vibratome to produce sections which were 40 μm thick. One section from each level was used to examine the autofluorescence of the pericytes and another section was processed for TEM acid phosphatase.

From the Faculty of Medicine, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada.

Address correspondence to: Brian Jeynes, Ph.D., Faculty of Medicine, Health Sciences Center, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada A1B 3V6.

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staining. Sections examined for autofluorescence were excited by ultraviolet light with a UG5 excitation filter, and were observed and photographed on a Zeiss microscope. Vibratome sections to be processed for TEM acid phosphatase were processed in the following way: after the 40 μm sections were cut, they were washed in the incubation buffer (Sucrose-Tris/Maleate) and incubated in a 0.1 M sodium β-glycerophosphate, 0.02 M lead nitrate buffered medium (pH 5.2) at 37°C for 60 min (after Barka and Anderson). The sections were then washed in buffer, post fixed in buffered OsO₄, embedded in Epon 812, and sectioned for thick and subsequently thin sections. The samples were examined on a Philips 300 EM operated at 80 Kv.

Results

The pericytes were easily identifiable in thick Epon sections stained with Toluidine Blue. They were characterized by the presence of large, spherical and globular inclusions (granules) within the cytoplasm. These granular pericytes were readily found in both control and embolic conditions (fig. 1). The cells were found distributed throughout the brain slices examined and were associated with arteriolar-sized or smaller vessels. Within the limits of this study we observed no significant qualitative differences in the granular pericytes either between the embolic or control animals or between cells associated with the specific vessel types. In the embolic condition, the granular pericytes did not appear to be more especially associated with occluded or damaged vessels. (The specific morphology of atheroembolic occlusions are described in previous works.⁸ ¹⁰)

The granular contents of these pericytes have been previously¹¹ and were found here to be autofluorescent, acid phosphatase positive; and the cell PAS positive. This latter characteristic was advantageous in counting the cells in whole sections at the five levels. To be considered a positive count, the cells had to be PAS positive and in close association with an arteriolar sized or smaller vessel (fig. 2).

There was a significant increase in the number of granular pericytes when comparing embolically occluded versus control cerebral vasculature. This was true for each of the five brain levels examined (fig. 3). As progressively more caudal sections were examined, the number of pericytes per slice increased. This increase presumably reflects the presence of an increasing surface area. However, control numbers of granular pericytes for all slice levels ranged between 52 and
CEREBROVASCULAR GRANULAR PERICYTES IN ISCHEMIA

FIGURE 4. Vessel in frozen section. Oil Red 0 positive materials are shown (arrowheads) in what are believed to be granular pericytes. HAM injected animal × 1000.

68 percent of the number of cells in comparable experimental sections.

Many granular pericytes in the atheroembolic condition contained Oil Red 0 positive granules (fig. 4). None were found in control sections. With this frozen section technique, however, it was frequently difficult to ascertain whether these Oil Red 0 positive pericytes were in close proximity to either an occluding embolus or to a damaged vessel wall segment.

TEM examination of these cells showed their prominent basement membrane, a distinguishing characteristic for these cells (fig. 5). The TEM morphology of the granules showed them to be a heterogeneous population. Many were membrane bound while others seemed to consist of an aggregation of small spherical globules. The granules were of varying composition and electron density and varied from 0.5–1.5 μm in diameter (fig. 5). Although there was some small evidence of both luminal and abluminal plasmalemmal pinocytotic activity, no cells were observed which appeared to be involved in extensive absorption of observable extracellular material. However, many of these cells demonstrated an extensive smooth endoplasmic reticulum.

Discussion

The results of this study indicate that granular pericytes are present in many areas of the adult rabbit brain under “control” conditions, which supports the results obtained by Fleischhauer in cats.3 (Their presence under normal conditions has also been observed in this laboratory in samples of human cerebral tissue taken at autopsy, unpublished data). The granular pericytes observed in this study conform to morphological criteria accepted in the literature. Unlike contractile pericytes, these cells were not observed to contain microfilamentous structures and the cytoplasmic matrix of these cells was light. They are contained within a common basement membrane with the endothelium; they are autofluorescent and PAS positive3.12,13 and are acid phosphatase positive.14 The envelopment within a common basement membrane with the endothelium is important here to distinguish these cells from other phagocytic cells such as microglia.

The structure of the granules are variously described in the literature. Pericytal cytoplasmic inclusion structures described have included vacuoles.15,16 which were not observed in this laboratory, and in some instances substantive and discrete granules.16,15,17 As observed in this study, the granules are spherical, but their contents and electron density are highly variable. This suggests either different contents, as for example in selective sequestration sites for catabolites, or a different processing and/or manufacturing of the content materials. Some of the granules appear to be lipids and others variously staged lysosomal structures. The presence of pericytal lysosomal enzymes has been demonstrated in maturing cerebral vessels previously and would be consistent with their phagocytic role.15,16,18-20 Although some of the granules, especially in older animals, might be lipofuscin12 others found little such evidence in old rats.17 Granule containing leptomeningeal cells have also been referred to in literature.21-22 These cells, which were also observed in this study, contain granules identical in morphology to those in the pericytes. Their role in the subarachnoid spaces is also not well understood, but appears to be one of removal of waste materials.

The results of this study suggest that granular pericytes respond to an insult by an increase in their number. This does not necessarily imply an increase in the number of pericytes, only an increase in their granular form. One investigation reported the impression of increased numbers of granular perivascular cells in degenerating rabbit thalamic nuclei.23 Others reported no apparent (unquantified) increase in the number of pericytes after intraventricular and subsequent parenchymal spreading of HPR in rat brains.24 Considering those observations and the results of this present experiment, it would appear that under pathological con-

FIGURE 5. Micrograph of a typical cerebral granular pericyte. It demonstrates the morphological heterogeneity of the pericyte granules. Note that some granules are membrane bound and that others appear to be coalescing from smaller globules. The homogeneity of the granules is also variable. Note the prominent basement membrane (arrows). HAM injected animal × 9300.
ditions of ischemia and/or infarct, pericytes might be called upon to deal with increased accumulations of catabolites or debris. Related to this role is the question of whether or not these pericytes are mobile. Some researchers believe that the pericytes are not mobile at least in mature animals. Other studies seem to suggest that pericytes, at least in immature tissue, may be able to migrate away from the vessel wall.

With respect to the function of these cells, there is no doubt of their phagocytic role. The work of Cancilla’s group demonstrated that these cells will accumulate HRP injected into intraparenchymal sites, thus showing that they are capable of absorbing materials from surrounding tissue. This current study has also shown that they may be capable of absorbing luminal contents such as lipids under stress conditions. However, whether or not damage to the vessel wall is necessary for this to occur, or whether permeability is increased at the site of a vascular occlusion, is unresolved. One investigation did show, however, that pericytes could pick up exogenous protein from the blood in immature mice. The source of the phagocytized material in pericytes which become granular is still somewhat unclear. Under conditions of ischemic stress resulting in perivascular parenchymal infarction, the source might seem obvious. Also, under conditions which damage the vessel walls, such as might have occurred in this study when injected lipid material could have damaged the vessel wall at sites of occlusion, luminal material could have been phagocytized by the pericyte. However, in many instances in the experimental condition and at all sites of the “control” animals, there was not morphological evidence of vessel wall damage of parenchymal infarct in the presence of granular pericytes. This strongly supports the view of Mato and his colleagues that pericytes might be involved in the removal of catabolites and/or debris from the brain on a routine basis. If this is true, then one of two events might subsequently occur: (1) the pericyte is involved in the possible degradation and subsequent transport of the by-products into the vascular lumen through the blood-brain barrier, and therefore a component of the barrier; or (2) the pericyte accumulates these materials throughout the life of the organism or the life span of the cell. To date, no studies have attempted to clarify either of these possibilities. It has been observed, however, that the phagocytic capability of these cells seem to decrease with age in rats.

The granular pericyte population would appear to react differently under varying pathologic conditions. In this study their numbers are increased under ischemia. However, pericytes (not specifically the granular type) undergo degeneration in diabetic retinopathy; and more specifically in the retinal vessels only, not in the adjacent optic nerve or brain vessels. Further, granular pericytes have been observed in tissue samples in Krabbe’s disease,4 irradiation injuries,3 Tay-Sachs disease4 and CNS neoplasms.7

There are a considerable number of unresolved questions associated with granular pericytes. Among them are: What is their origin? What is the source and nature of the phagocytized material? What is the fate of that material and/or the granular pericytes themselves? Do these cells have a normal ongoing role in the breakdown and removal of catabolites and debris from the perivascular zone? What stimuli accelerate this role? Are they part of the blood-brain barrier as, for example, suggested by Van Deurs10 and Cancilla. What other roles might they perform? Can they migrate into the cerebral intercellular spaces? The further investigation of these cells could aid greatly in our understanding of the ability of the CNS to regulate the presence of waste materials under both normal and pathologic conditions, particularly such as might exist after TIA, stroke or amaurosis fugax episodes.

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Spontaneous Dissecting Aneurysms Of The Internal Carotid And Vertebral Arteries — Two Case Reports

JEAN-LOUIS MAS, M.D.* CATHERINE GOEAU, M.D.,* MARIE-GERMAINE BOUSSER, M.D.,*
JACQUES CHIRAS, M.D.,† JEAN-MICHEL VERRET, M.D.,† PIERRE-JEAN TOUBOUL, M.D.*

SUMMARY Two patients had acute spontaneous dissection of both internal carotid arteries and of one or both vertebral arteries. One had angiographic signs suggestive of fibro-muscular dysplasia and both were on oral contraceptives. They were treated with high dose heparin and made a good clinical recovery. A digital intravenous angiography performed two to three months later showed a complete recanalization of arteries involved.

These patients are similar to those reported as “idiopathic regressing arteriopathy” and “reversible angiopathy” which probably correspond to the same entity.

Report of Cases

Patient 1

A 40 year old woman was admitted to La Salpêtrière on February 5, 1982, two weeks after the acute onset of headache, neck pain, vomiting and of a bruit in the left ear. She was seen by her local physician who found trauma to the head or neck and her past medical and family history were not contributory. She had been on contraceptive pills for the last six years and she was advised to stop them. Over the next days, the bruit and headache increased in severity and developed over the whole of the head. Ten days after the onset of symptoms, she had a brief loss of consciousness and four days later she had a sudden episode of bilateral blurring of vision, which lasted 15 minutes. Clinical examination was normal except for the presence of a left Horner’s syndrome and a loud bruit over the left side of the head and neck. Blood pressure was 135/95 mmHg.

On admission the next day, clinical findings were similar. Routine laboratory investigations and detailed coagulation studies were normal. A CT scan with and without contrast injection was normal. A percutaneous transfemoral selective bilateral carotid and subclavian angiography showed abnormalities on all 4 vessels. On the right ICA (fig. 1-A), there was a severe extensive narrowing beginning just past the origin and extending up to the siphon which was filled retrogradely by the external carotid artery via the ophthalmic artery. On the left ICA (fig. 1-B), there were slight irregularities at the origin and distal to it, a severe narrowing extending up to the entrance into the carotid canal, with an aneurysmal dilatation at the C3 level. On the left VA (fig. 1-C), there was, distal to its origin, a moderate irregular narrowing extending up to the C5 level with two aneurysmal dilatations, one at the C6-C7 level and the other on its extracranial curve. The right VA (fig. 1-D) was hypoplastic and, in addition, showed a severe regular eccentric stenosis at the C3 level. The left VA filled both middle cerebral arteries. The intracranial circulation was otherwise normal. The abdominal aorta and renal arteries were normal. High dose Heparin calcium was started on Feb. 11. Over a period of two weeks, there was a steady improvement with a gradual
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B Jeynes

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