Chapter 4

Similar Ultrastructural Breakdown of Cerebrocortical Capillaries in Alzheimer’s Disease, Parkinson’s Disease, and Experimental Hypertension. What is the Functional Link?

Abstract

The brain, as an intensely active organ is highly dependent on a sufficient nutrient and oxygen availability in order to reach its optimal working capacity. It is well known that the vital supply of energy substrates is provided by the circulatory system, which splits up into a fine, terminal capillary network in target tissues. These capillaries are considered as important sites since the actual nutrient trafficking takes place through their walls. That is why an intact, preserved structure of the microvessels is crucial to fulfill their function. Since the brain is known to be particularly vulnerable to sub-optimal oxygen and glucose delivery, the intact morphology of capillaries is of paramount importance.

Several observations have indicated that the cerebral capillary ultrastructure is damaged in Alzheimer’s disease (AD). Curiously, the regional cerebral blood flow of AD patients is also significantly lower than in age-matched control individuals. Based on these data, it has been suggested that the decreased blood supply and the cerebrovascular alterations contribute to the development of dementia. However, we have observed similar capillary damage in Parkinson’s disease patients and chronically hypertensive rats in addition to AD cases, as presented here. These findings indicate that cerebral capillary damage is not exclusive for AD but occurs under other neurodegenerative disorders and hypertension, as well. We hypothesize that ultrastructural abnormalities of cerebral capillaries are causally related to decreased cerebral blood flow and create a condition, which favors neurodegenerative mechanisms including the development of dementia.
Introduction

Cerebrovascular structural abnormalities have frequently been reported in demented patients diagnosed to have Alzheimer’s disease (AD) (Perlmutter and Chui, 1990; Claudio, 1996; Kalaria, 1996; Buée, 1997). Capillary damage in AD brains consistently occurs in the form of capillary basement membrane thickening and collagen type IV accumulation, also known as fibrosis. These pathological alterations implicate several functional consequences: the thickening of the basement membrane can physically hinder nutrient transport to the neural tissue or the clearance of potentially toxic waste products from the brain, and may structurally affect the efficacy of important carrier systems of the blood-brain barrier. For example, a decreased density of glucose transporter sites supports the latest assumption and suggests reduced glucose availability in the affected brain regions (Harik, 1992; Horwood and Davies, 1994; Mooradian et al., 1997). Hence, the imposed metabolic crisis may be reflected by data indicating decreased cerebral glucose uptake and oxygen utilization in Alzheimer brains (Rapoport et al., 1991, Fukuyama et al., 1994; Blesa et al., 1996).

However, the observed cerebral capillary damage is not exclusive for AD. Similar ultrastructural alterations of the cerebral microvessel walls were described in aging and experimental cerebral hypoperfusion in rats (De Jong et al., 1990; De Jong et al., 1999). Cerebral hypoperfusion has drawn major attention in AD research, too. Regional cerebral blood flow measured by SPECT in AD patients demonstrated significant decrease in the hippocampal formation and temporal cortex, regions first and most severely affected in the disease (DeKosky et al., 1990; Eberling et al., 1992; O’Brien et al., 1992; Ohnishi et al., 1995). Interestingly, cerebral hypoperfusion was also found to coincide with hypertension due to possible, high blood pressure-related lack of autoregulation of cerebral blood flow or atherosclerosis (Nobili et al., 1993; Nakane et al., 1995). Supportive experimental evidence was obtained in spontaneously hypertensive rats where the observed reduced cerebral blood flow was reported to coincide with an additional tendency for poor learning skills (Fujishima et al., 1995; Katsuta, 1997).

In the present study, we overview the structural alterations of brain capillaries in neurodegenerative diseases and hypertensive rats and attempt to establish a comparison between them in the light of cerebral hypoperfusion.
Cerebrovascular Pathology in Neurodegenerative Diseases

The condition of cerebrocortical capillaries of AD and PD patients was investigated in post mortem samples from the cingulate cortex. The patients were selected based on neurological diagnosis and subsequent standard neuropathological characterization (Braak staging, CERAD scale, Lewy body score and substantia nigra pathology) (Braak and Braak, 1991; Mirra et al., 1991; de Vos et al., 1995; McKeith et al., 1996). The PD cases were further differentiated based on the presence of neuropathological alterations characteristic of AD. Thus, four separate groups were established: an AD group (n=5), a PD group without AD-like neuropathology (n=6), a PD group with AD-like neuropathology (PDd, n=4) and controls (n=5).

The samples were processed for electron microscopic analysis: the tissue was imbedded in glycide ether and cut to ultrathin sections. The sections were mounted on copper grids and contrasted with 5 % aqueous uranyl acetate and Reynolds lead solution. The analysis was performed with a Philips 200 electron microscope.

We focused on deviations of the capillary walls and defined the following criteria: 1. when the basement membrane (BM) demonstrated local thickening exceeding double width compared to an intact BM segment of the same capillary, or showed duplications or branching, we considered the abnormality as basement membrane thickening (BMT) (Fig. 4.1.B). 2. The appearance and accumulation of collagen fibers in the BM, occasionally

invading the cytoplasm of an embraced pericyte, was taken as fibrosis (Fig. 4.1.C). 3. membranous inclusion bodies and swollen pericytic profiles indicated pericytic degeneration (Fig. 4.1.D). One hundred capillaries per case were screened and the percentage of capillaries with any of the above-described abnormalities was calculated.

The data show that the percentage of microvessels with BMT and fibrosis has considerably increased in AD as well as in PD and PDD (Fig. 4.2). The occurrence of BMT was almost three times higher in the neurological diseases than in controls pointing to an obvious and remarkable increase of BMT (Fig. 4.2.A). Similar tendency was observed in fibrosis, but the individual variance prevented statistical significance (Fig. 4.2.B). In contrast, the percentage of capillaries with degenerating pericytes was found comparable in all groups (Fig. 4.2.C) suggesting no specific involvement of pericytic degeneration in the pathology of AD or PD. Thus, the capillary wall, specifically the basement membrane appears to be the major site of cerebrovascular damage in the investigated neurodegenerative disorders.

**Cerebrovascular Pathology in Hypertension**

The effects of hypertension were investigated on the cerebral capillaries of the frontoparietal cortex of Wistar-Kyoto (WKY, n=6) and spontaneously hypertensive stroke prone rats (SHR-SP, n=6). Blood pressure measurements were taken on the tail on every fourth week for a period of 20 weeks, starting when the animals were 40 weeks old. On the 60th week, the rats were perfused with 2% paraformaldehyde, 0.05% glutaraldehyde and

![Figure 4.2](image)

**Figure 4.2.** Cerebral capillary damage in Alzheimer’s disease (AD) and Parkinson’s disease with or without Alzheimer-like neuropathology (PDD and PD, respectively). A: percentage of capillaries with basement membrane thickening (BMT). B: percentage of capillaries with fibrosis. C: percentage of capillaries with degenerative pericytes. ∗<0.05, ∗∗<0.02.
0.2% picric acid in 0.1 M phosphate buffer. The brains were cut on a vibratome at 50 µm and the slices were routinely embedded in glycid ether. Ultrathin sections were prepared of the frontoparietal cortex, mounted on 200 mesh copper grids and contrasted for EM analysis.

For the examination of microvessels, we followed the guidelines described above for the human samples, thus, the same three categories of capillary abnormalities were established: basement membrane thickening (BMT), fibrosis and degenerative pericytes. The percentage of capillaries with abnormal features was expressed as percentages of the total number of capillaries encountered.

The cortical capillaries of hypertensive animals suffered a considerable damage. The frequency of BMT increased with 25 % in the SHR-SP group (Fig. 4.3.A) while the individual capillaries were endowed with more advanced forms of BMT compared to the alterations observed in the WKY group. Usually, larger segments of a particular capillary demonstrated clearly more pronounced alterations in the SHR-SP animals. Fibrosis, which was nearly absent in normotensive controls increased remarkably in the hypertensive cases (Fig. 4.3.B). At the same time, the condition of pericytes proved to be unaffected by high blood pressure (Fig. 4.3.C). The occurrence of capillaries with BMT or fibrosis correlated with the blood pressure values taken before perfusion (Fig. 4.4.A and 4.4.B), whereas, pericytic degeneration showed no such relationship (Fig. 4.4.C).

Figure 4.3. Cerebral capillary breakdown in spontaneously hypertensive rats at 60 weeks of age. A: percentage of capillaries with basement membrane thickening (BMT). B: percentage of capillaries with fibrosis. C: percentage of capillaries with degenerative pericytes. WKY: Wistar-Kyoto rats (control); SHR-SP: spontaneously hypertensive, stroke prone rats. ∗<0.05, ∗∗<0.01.
**Discussion**

Here we have shown that neurological conditions such as AD and PD are accompanied by a progressive degeneration of cerebrocortical capillary wall ultrastructure in a similar manner. Furthermore, comparable microvessel damage was observed in the frontoparietal cortex of chronically hypertensive rats. Figure 5 demonstrates typical examples of these degenerative features.

The microanatomical capillary damage observed here and previously by others (Perlmutter and Chui, 1990; Claudio, 1996; Kalaria, 1996; Buée, 1997) coincides with alteration of additional, functional vascular factors in AD and possibly in PD. For example, clinical measurements of regional cerebral blood flow by the non-invasive SPECT method have indicated that AD is accompanied by a decreased regional cerebral blood flow in the hippocampal formation and temporal cortex (DeKosky et al., 1990; Eberling et al., 1992; O’Brien et al., 1992; Ohnishi et al., 1995). This phenomenon was also described in the temporal cortex of PD patients (Kawabata et al., 1991; Varma et al., 1997) but the data coming from different research groups is not uniform on this issue (Bissessur et al., 1997). The theory that the vascular ultrastructural changes, like those described in the present study, and reduced cerebral blood flow are functionally related, was tested in an animal model. Experimental cerebral hypoperfusion was created by a bilateral carotid artery occlusion (2VO) (De Jong et al., 1999; de la Torre et al., 1992; Ni et al., 1994) which yields to 25-30% reduction of regional cerebral blood flow (Ni et al., 1994), corresponding well with...
the values recorded in AD. Subsequently, the condition of hippocampal capillaries was examined and a significant increase of BMT and fibrosis was found (De Jong et al., 1999). Therefore we propose that hypoperfusion is a causal factor in the development of capillary alterations observed in the 2VO model, and possibly in AD and PD.

Reduced cerebral blood flow was also recorded in hypertensive patients by PET and the 133Xe-inhalation method (Nobili et al., 1993; Nakane et al., 1995). In addition, parallel experiments revealed a comparable hypertension-induced cerebral hypoperfusion in spontaneously hypertensive rats (Fujishima et al., 1995; Katsuta, 1997). Our own findings in the hypertensive animals show that the cerebral capillary wall is damaged in a similar fashion to hypoperfused rats, as well as to AD/PD patients: the basement membrane is thickened and contains collagen deposits. Fibrosis gives a considerable contribution to aberrations in the hypertensive animals, which is, nonetheless, more pronounced and uniform among cases than either in experimental hypoperfusion or the here presented neurological diseases (AD or PD). At the same time, the increase in BMT appears higher in 2VO rats and AD/PD than in a hypertensive condition. We suggest that these differences emerge due to different stages and degree of cerebral hypoperfusion. BMT and fibrosis are thought to be in a functional relationship and their relative ratio can change in the course of time as pointed out by De Jong et al. in aging rats (De Jong et al., 1990).

Our hypothesis that the same mechanisms are involved in the generation of capillary damage in the 2VO model of cerebral hypoperfusion, spontaneous hypertension in SHR-SP rats and AD/PD finds support in the following behavioral data. Both 2VO rats and SHR-SP animals were independently tested in the Morris water maze and radial arm maze for their learning skills (de la Torre et al., 1992; Wyss et al., 1992; Ni et al., 1994; Pappas et al., 1996; Ohta et al., 1997; De Jong et al., 1999). The experiments provided corresponding results in the sense that both the 2VO animals and the SHR-SP rats performed worse than their controls in the learning paradigms. By putting the findings into a sequential order, we may speculate that cerebral hypoperfusion gives rise to cerebrocapillary damage which, in turn, is responsible for a mild decay of learning skills probably by preventing the optimal transport of sufficient nutrients to the neural tissue and subsequent neuronal dysfunction.
In conclusion, we propose that cerebral hypoperfusion is a mild but persistently prevailing condition shared by neurological disorders - AD or PD - and chronic hypertension, and that the constantly low flow rate triggers pathologic malformations of the cerebral capillary walls. This chain of degenerative events may be an underlying mechanism of mild memory deficits in chronic hypertension and PD, and probably contributes to a largely enhanced cognitive failure pre-established by numerous additional neuropathological factors in AD (Fig. 4.6).
Chapter 4

Figure 4.6. Causal events of cerebrovascular parameters leading to mild cognitive disorders.

References