

Lectin (UEA-1) reaction of capillary endothelium with reference to permeability in autopsied cases of cerebral infarction

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Summary. The relationship between endothelial reactivity to *Ulex europaeus* agglutinin-1 (UEA-I) and the permeability of the vascular wall in human autopsied cases of cerebral infarction was studied. Sections from the cerebral cortex were reacted with horseradish peroxidase UEA-1 to demonstrate the surface membrane of endothelial cells. Albumin in the neuropil of sections was demonstrated for the estimation of increased vascular permeability. The results showed that endothelial reactivity to UEA-I was reduced in cases where death had occurred 3 to 5 days after onset of cerebral infarction. Reactivity was also diminished in cases where death had occurred after 13 and 25 days; these cases showed fresh ischemic lesions caused by re-attacks of infarction. Albumin extravasation into the neuropil was demonstrated in these intermediate cases. Chronic cases, dying after more than 52 days, showed no reduction of endothelial reactivity to UEA-I and no albumin extravasation was proved.

It was concluded that UEA-1 can be employed as a useful morphological marker for evaluation of endothelial function and vascular permeability.

Key words: Capillary permeability - Cerebral infarction - Endothelium - Lectins

Introduction

Lectins have been used as histological markers since the 1960s to evaluate renal capillaries (Yonezawa et al., 1983) or determine tumor origins (Tatematsu et al., 1982). *Ulex europaeus* agglutinin 1 (UEA-I), one of the lectins, has already been known to bind with human vascular endothelium (Mazzuca et al., 1982). Recently, the blood-brain barrier (BBB) has received much attention in cases of ischemic cerebrovascular disease in relation to brain edema. The vascular endothelium of the brain is said to play an important role in the BBB (Kerenyi et al., 1975). This study was carried out to determine whether or not UEA-I could be used as a marker to evaluate cerebral endothelial cell damage in autopsied specimens from cases of cerebral infarction.

Materials and methods

The subjects of this study were 14 patients who had died of ischemic cerebrovascular diseases. They consisted of 7 males and 7 females, ranging in age from 64 to 91 years (average, 76.4 years), who had died from 36 hours to 6 months after the onset of the cerebral infarct. They were divided into three groups, as follows: Group 1; 3 cases who died at an early stage (36 hours to 5 days after onset), Group 2; 6 cases who died at an intermediate stage (13 days to 27 days after onset), and Group 3; 5 cases who died at a chronic stage (52 days to 6 months after onset). In all these 14 cases, major arterial obstructions were recognized clinically by angiography. Angiographical recanalization was observed in 4 cases from Group 2 during their clinical courses. The autopsied brains were immersed in 10% buffered formalin for fixation. After 2 weeks or more, ischemic foci which showed the most severe damage and control tissues which showed no or very little ischemic

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Endothelial permeability and lectin

change were trimmed off. Only cortical tissues from the same location in all cases were chosen as the materials of our study to ensure constant vascularity.

The paraffin-embedded sections were deparaffinized, dehydrated and washed three times in PBS (pH 7.4.). They were then incubated overnight, at room temperature, with horseradish peroxidase-conjugated UEA-1 (EY Laboratory Inc.). The horseradish peroxidase was visualized histochemically with DAB (3-3' diaminobenzidine tetrachloride) and fresh hydrogen peroxide. Finally, the sections were washed in PBS. These specimens were observed by light microscopy and analyzed quantitatively using a color image processor (Magiscan 2, Nikon Co., Ltd., Tokyo) combined with the light microscope. Using the light microscope the treated specimens were observed under a magnification of 200 and the color image was obtained on the screen of the image processor simultaneously. The total area of UEA-1-positive sites, which were stained dark brown and restricted to the vascular endothelium, was obtained. The percentage of UEA-1-positive areas in each field was calculated automatically, and percentages were obtained for 10 fields. The mean values of these 10 fields were regarded as parameters of UEA-I reactivity.

In a control case, we used electron microscopical observation to confirm the reactive site of UEA-1 on the vascular endothelium. In this case, the extracted brain was perfused with Karnovsky fixative from catheters which were inserted into the bilateral carotid and vertebral arteries. Tissue sections were incubated overnight at 4°C with horseradish peroxidase-conjugated UEA-1. After incubation of these sections with 0.005% DAB Tris-HCl solution for 30 minutes, 0.005% DAB Tris-HCl solution (to which H₂O₂ was added at a concentration of 0.05%) was added and washed out immediately with PBS. The sections were embedded in EPON after fixation in 1% OsO₄, according to the routine method, and ultrathin sections were observed by electron microscopy.

In order to evaluate the microvasculature permeability in these cases, we employed albumin as a marker. The other sections from the same material were reacted with anti-human albumin rabbit serum by the avidin-biotin complex method. These specimens were incubated with normal goat serum (blocking serum) and treated with biotinylated antiserum for 30 minutes at room temperature, then reacted with avidin-biotinylated horseradish peroxidase complex for 60 minutes. The peroxidase was localized with DAB and fresh hydrogen peroxide. In addition, routine hematoxylin eosin staining was conducted.

By these methods, UEA-1 reactivity was compared between ischemic lesions and controls in the three groups. In addition, the extravasation of albumin and the histological findings obtained by hematoxylin eosin staining were also investigated.

Results

Figure 1 shows the reactivity of normal endothelium to UEA-1. The deposition of DAB, which appears dark brown, can be recognized on the surface of the

endothelium. This reactivity was not affected by digestion with endogenous peroxidase and was abolished by absorption of fucose.

The results obtained in this study were as follows.

Group 1: These were 3 patients who died from 36 hours to 5 days after the onset of ischemic attack. Case 1, where death occurred 36 hours after onset, showed considerable edematous changes in hematoxylin eosin staining, but the reactivity of the vascular endothelium for UEA-1 was preserved. Case 2, where death occurred after 3 days, and case 3, where death occurred 5 days after onset, showed significant edema and diapedetic changes, but only swelling of the endothelial nuclei was apparent in hematoxylin eosin preparations (Fig. 2A). The reactivities for UEA-I, however, were markedly reduced in these two cases (Fig. 2B). Extravasation of albumin into the neuropil was confirmed in these three cases (Fig. 2C).

Group 2: The six patients of this group died from 13 to 27 days after the onset of ischemic brain insult. In cases 4, 5, 6 and 8, recanalization of the occluded internal carotid artery was demonstrated clinically by angiography. In hematoxylin eosin staining, these 4 cases all showed relatively chronic ischemic changes, fat-laden macrophages and astrogliosis, and acute signs, such as severe edema and fresh haemorrhagic changes. The reactivity of endothelium to UEA-1 was diminished significantly where hematoxylin eosin staining disclosed acute ischemic alterations (Figs. 3A, 3B). However, the reactivity was preserved where the tissue showed only relatively chronic ischemic changes. Extravasation of albumin into the neuropil was observed where reactivity to UEA-1 was reduced (Fig. 3C). In cases 7 and 9, no recanalization was demonstrated in the clinical course. These cases showed good reactivity of the vascular endothelium to UEA-1 and extravasation of albumin was limited to the astroglial cytoplasm only.

Group 3: These were five patients, who died from 52 days to 6 months after the onset of ischemic cerebrovascular disorders. Cases 10, 11 and 12 where death occurred from 52 days to 2 months after a stroke, showed gliosis around the ischemic focus. The glial fibres had not become markedly proliferated. These organizing processes, therefore, seemed to be at a relatively early stage in comparison with cases 13 and 14. Endothelial reactivity to UEA-1 at the ischemic focus in these cases was almost the same as that of the control specimen. In cases 13 and 14, where death occurred after 4 and 6 months, respectively, the organizing glial reaction processes were at a considerably late stage (Fig. 4A). The endothelial reactivities for UEA-I at the foci were more remarkable than at the control sites in these cases (Fig. 4B). Extravasation of albumin was not demonstrated in these five chronic cases (Fig. 4C).

Table 1 shows the ratio of UEA-1-positive areas to each total visual field at ischemic foci and in control specimens. These values were an average for 10 visual fields. In group 1, the patient who died only 36 hours after onset showed no significant difference between the ischemic focus and the control site with regard to UEA-I reactivity. The other two cases, however, showed significant reduction of reactivity in the damaged cortex. In addition, the four patients from group 2 who showed clinical signs of recent

Endothelial permeability and lectin

re-attack, and had accompanying acute ischemic tissue damage histologically, showed a remarkable reduction of UEA-1 reactivity in their ischemic lesions. These changes were statistically significant. On the other hand, group 3, in which organizing processes were considerably advanced,

statistically showed more distinct reactivity for UEA-1 in ischemic lesions than in control specimens. The circles in Table 1 indicate that positive extravasation of albumin into the neuropil was present.

Table 1. Ratio of UEA-1 positive area to each visual field in the ischemic foci and the control specimens. These values are average of 10 visual fields. Circles mean positive extravasation of albumin into the neuropil and squares indicate presence of recanalization during clinical course.

Group	Case No.	Aye	Sex	Duration from onset to death	Control (%)	Ischemic lesion (%)
II	1 ●	73	male	36 hours	1.026+0.168	1.097+0.297
	2 ■			3 days	1.590+0.321	0.954+0.821*
	3 ●			5 days	1.264+0.267	0.611+0.231**
	4 ●■	64	female	13 days	2.262+0.841	0.186+0.176**
	5	79	male	19 days	1.911+0.463	0.397+0.447**
	6 ●■	68	male	20 days	0.378+0.125	0.056+0.075**
	7	71	male	24 days	1.620+0.342	2.063+0.404
	8 ●■	69	male	25 days	2.505+0.681	1.143+0.852**
	9	75	female	27 days	0.311+0.096	0.237+0.088
III	10	75	female	52 days	2.516+0.447	2.649+1.437
	11	90	female	55 days	0.298+0.074	0.240+0.102
	12	88	female	69 days	1.568+0.287	1.148+0.404
	13	73	male	4 months	1.467+0.555	2.143+0.448*
	14	81	female	6 months	1.530+0.298	2.016+0.430**

●; Albumin extravasation into the neuropile (+)
 ■; Acute changes due to recanalization (+)
 *; p<0.05] — t test
 **; p<0.01] — t test

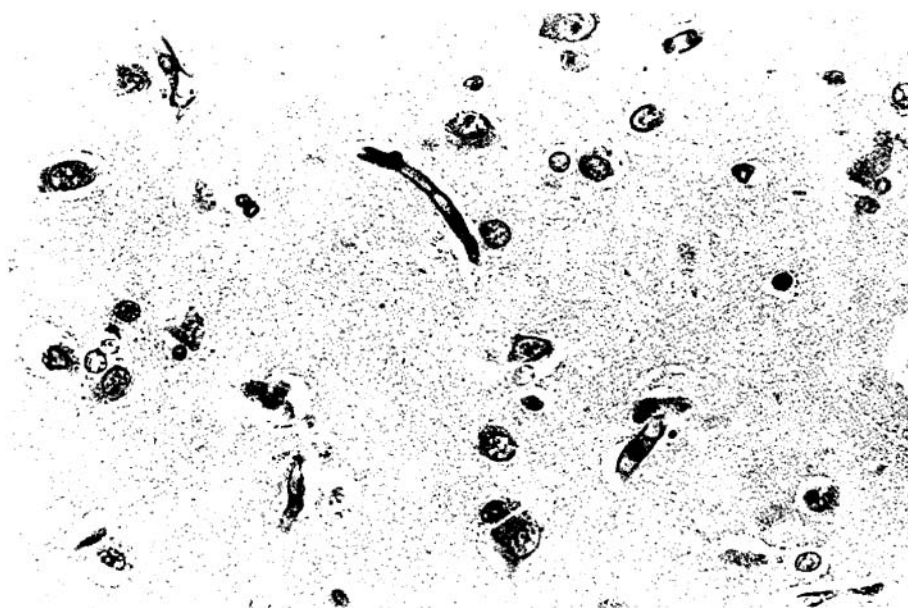


Fig. 1. Reactivity of normal endothelium of cerebral vasculature for UEA-1. The deposit of DAB, which is seen dark brown, is recognized on the surface of the endothelium (treated by UEA-1 peroxidase, x 2001

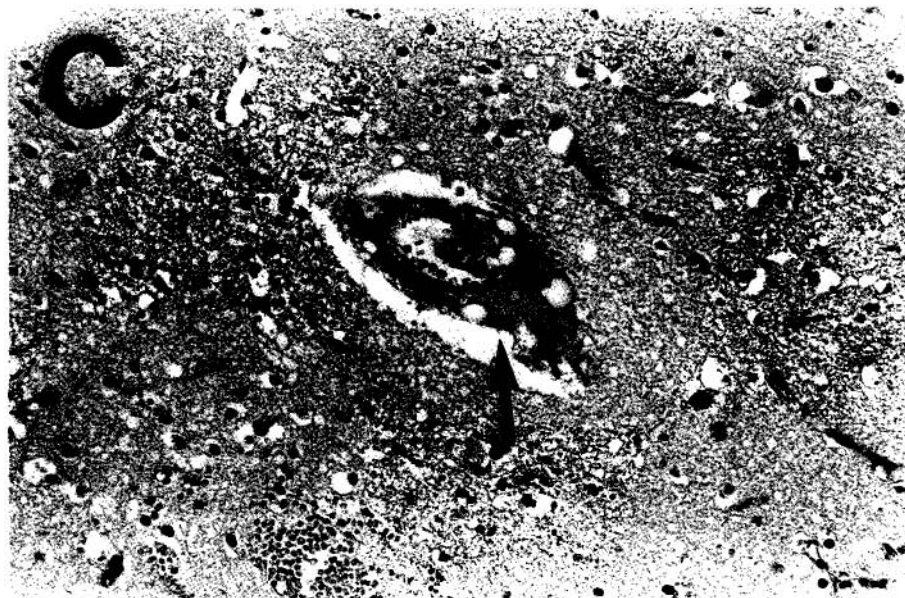
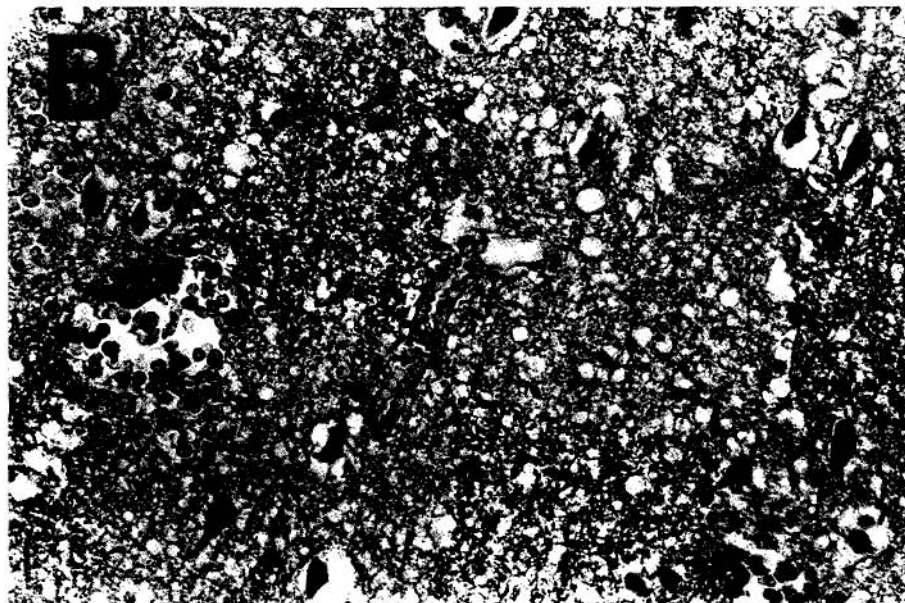
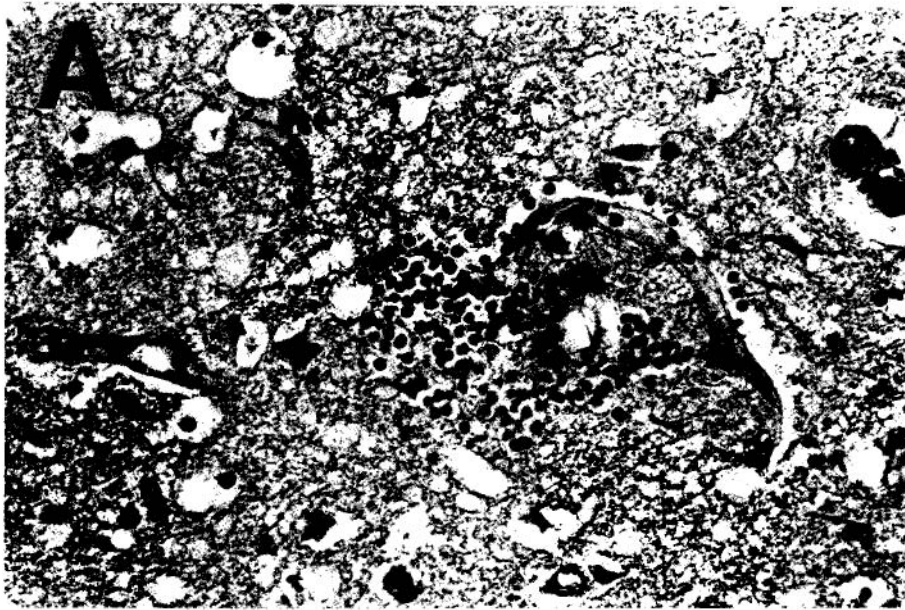


Fig. 2. Ischemic foci in case 3 (acute case, died after 5 days). A: significant edema, diapedesis and swelling of the endothelial nuclei (H.E. x 400). B: reduced reactivities of endothelium for UEA-1 (treated by UEA-1 peroxidase, x 400). C: extravasation of albumin into the neuropil around the vessel (arrow). (treated by antihuman albumin rabbit serum, x 200)

Fig. 3. Ischemic foci of case 8 (intermediate case, died after 25 days and experienced recanalization during process). **A:** numerous fat-laden macrophages and acute haemorrhagic changes (H. E. x 200). **B:** markedly reduced UEA-1 reactivity in the same lesion (treated by UEA-1 peroxydase, x 200). **C:** extravasation of albumin into the neuropil around the vessel (arrow). (treated by antihuman albumin rabbit serum, x 200)

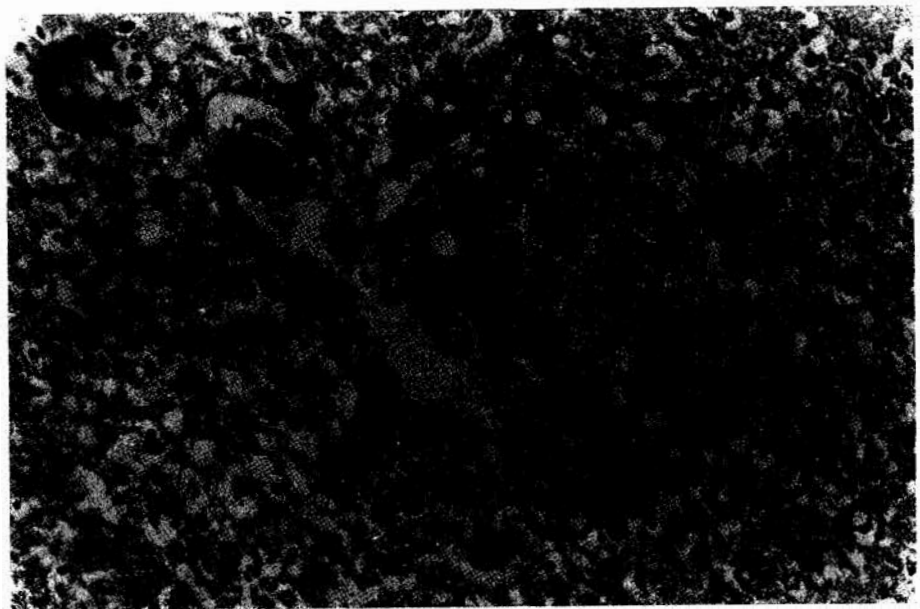
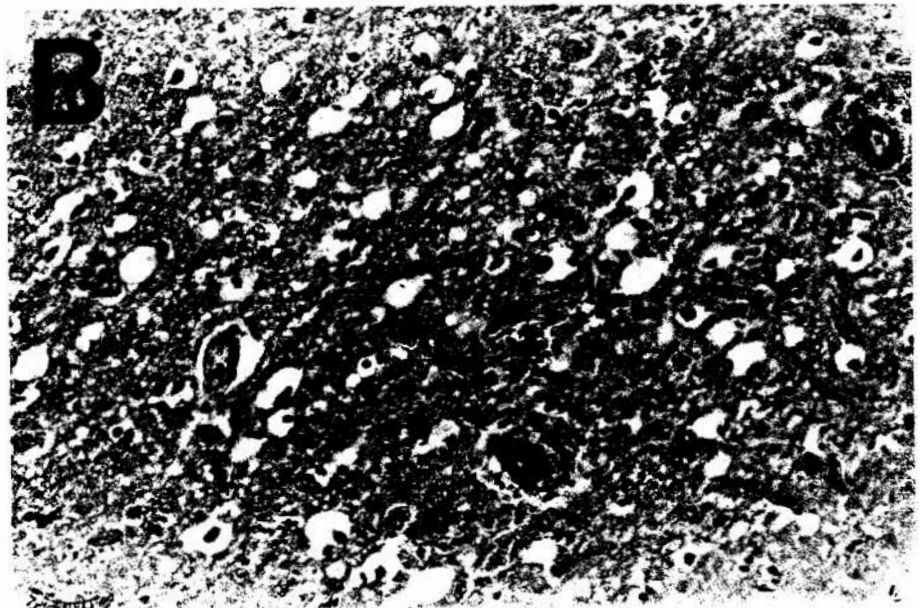
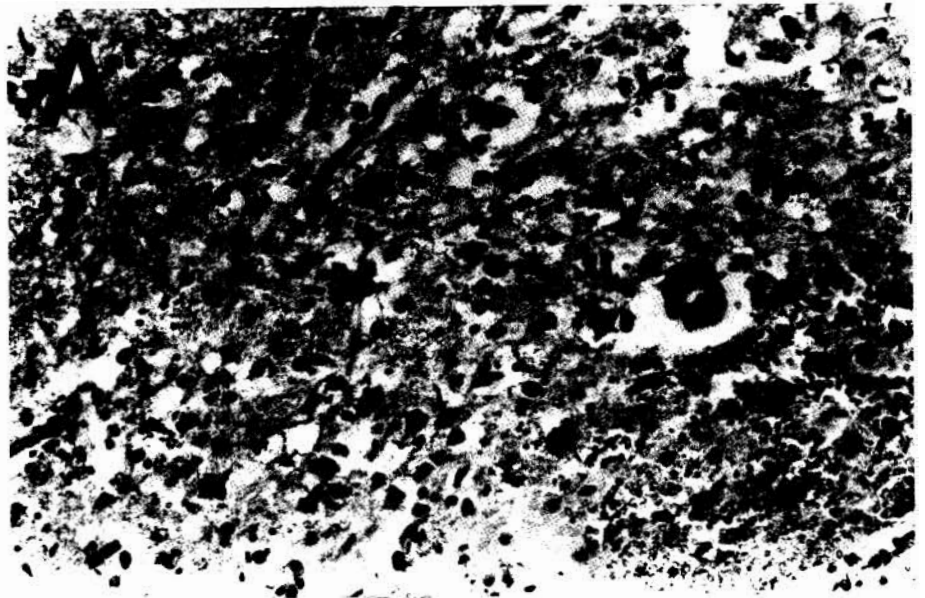
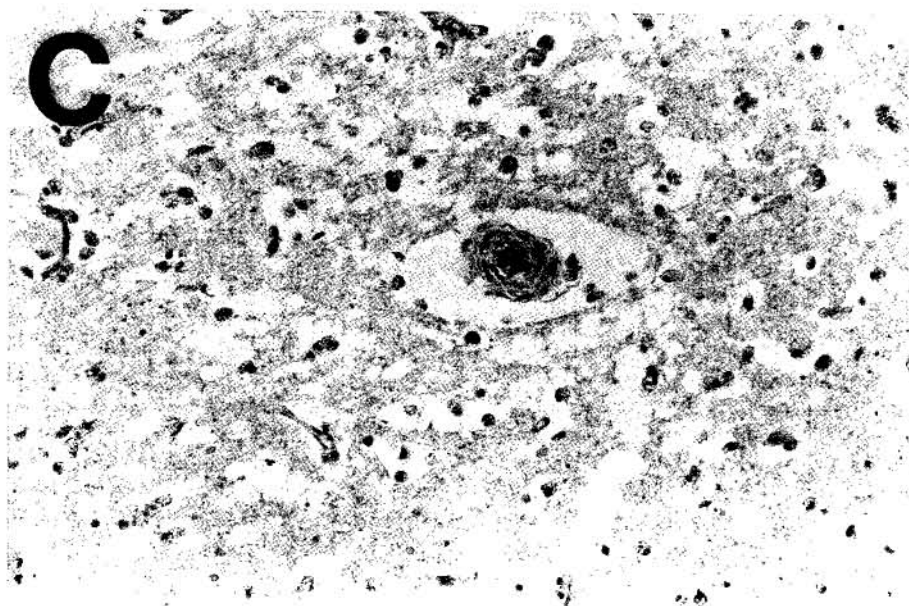
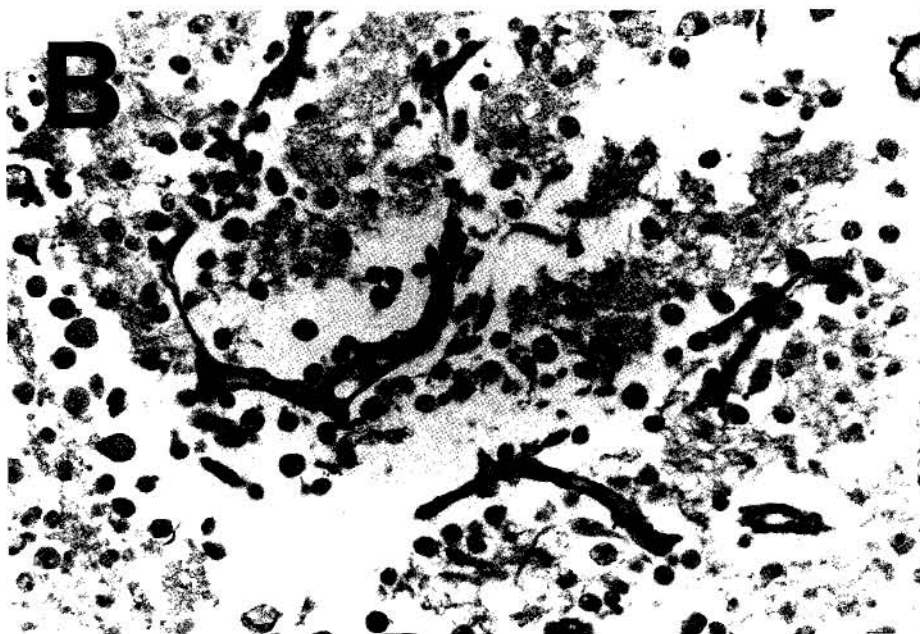
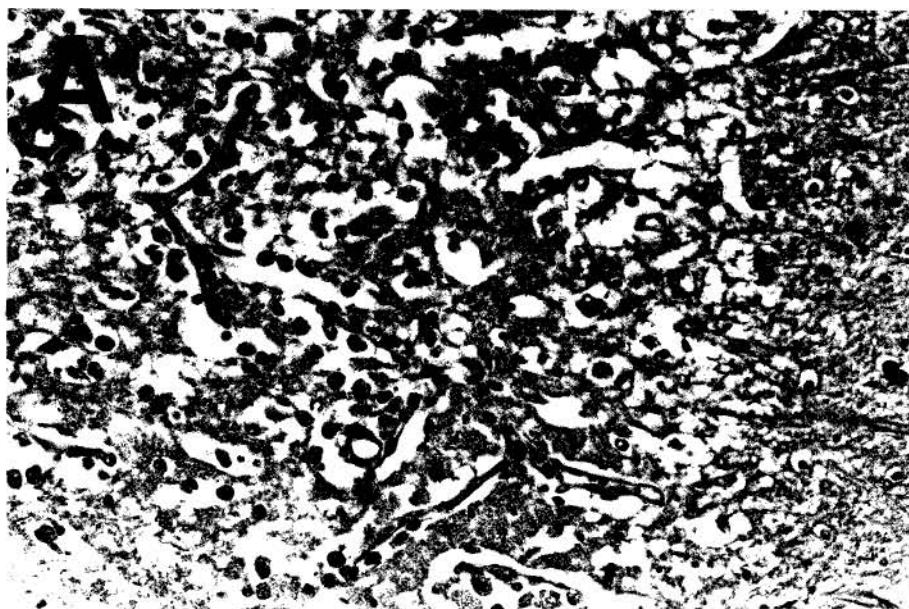


Fig. 4. Chronic foci of case 13 (chronic case, died after 4 months). A: astrogliosis and a lot of fat-laden macrophages without edematous changes. (H.E. x 200). B: marked reactivities for UEA-1 (treated by LIEA-1 peroxydase, x 400). C: no extravasation of albumin (treated by antihuman albumin rabbit serum, x 200)



Endothelial permeability and lectin

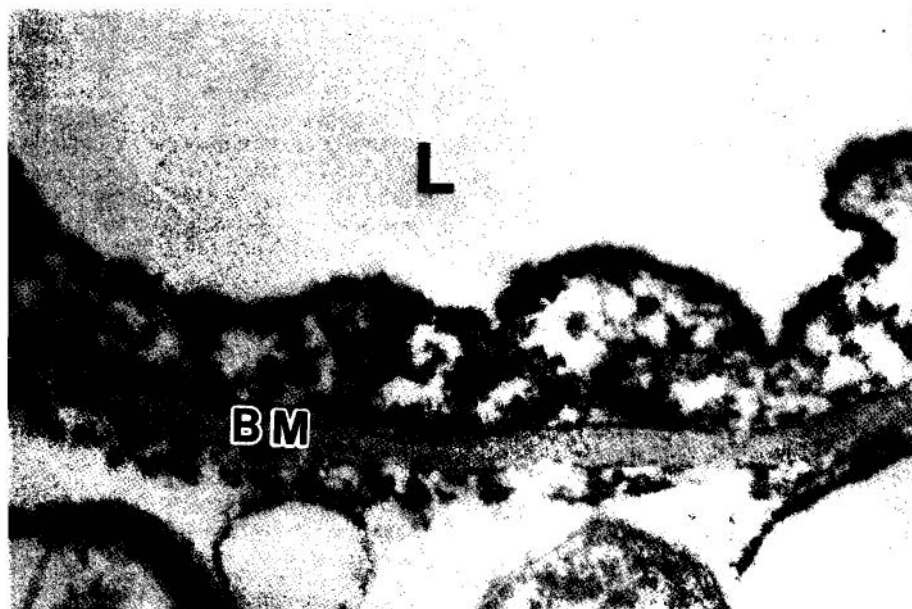


Fig. 5. Electron microscopical picture of normal brain capillary showing visualized UEA-1 on the surface of capillary endothelial cell membrane of the cerebrum as a continuous dark line. (L: vascular lumen, BM: basement membrane). x 17,000

Discussion

Lectins are kinds of protein or glycoprotein which combine with specific residues in mono- or oligosaccharide chains (Pena et al., 1981). The binding sites are nonreducing chain termini or subterminal positions (Simionescu et al., 1981). Among these lectins, *Ulex europaeus* agglutinin I (UEA-I), wheat germ agglutinin (WGA), and concanavalin A (Con A) have properties of reaction with human vascular endothelium (Yonezawa et al., 1983). Capillary endothelium reacts with UEA-I particularly strongly (Mazzuca et al., 1982). In the present study, we confirmed by electron microscopy that UEA-1 binds to the surface of the capillary endothelial cell membrane of the normal cerebrum. Endothelial cytoplasm also shows a positive reaction, although this may be rather artifactual (Fig. 5). With regard to ischemic changes of the endothelium, many morphological findings have been reported, such as disappearance of the endothelium, crater formation, flattening of convolutions and fibrin or platelets covering the endothelial surfaces (Fonkalsrud et al., 1976). In addition, increased numbers of endothelial vacuoles have been reported under ischemic conditions (Westergaard, 1980; Nag et al., 1981). These ultrastructural manifestations are closely related to insufficiency of the blood-brain barrier. However, perfusion fixation which is necessary for ultrastructural study can be conducted only occasionally in human autopsy cases. For this reason, there have been no histological methods for accurately evaluating the functional state of the capillary endothelium in autopsied human brains. We anticipated that UEA-1 reacting with saccharide chains on the surface of the cerebral capillary endothelial cell membrane might be a useful marker of endothelial cell damage and of capillary permeability in light and electron microscopy studies.

Two cases of group 1, where death occurred in an early stage, showed both a decrease of UEA-1 reactivity of the capillary endothelium and extravasation of albumin into the neuropil in ischemic foci. In addition, 4 intermediate cases of group 2, which had experienced recurrence of ischemic attacks and had acute lesions histologically, showed the same findings as those in the acute cases. On the other hand, two cases of group 2, which had no recurrence of ischemic insult, showed good reactivity of UEA-1 and albumin-positive staining that was restricted to the glial cytoplasm. This intracytoplasmic albumin staining may have been due to the appearance of resolution edema (Klatzo et al., 1980). Accordingly, we speculated that the BBB had already been repaired in these 2 intermediate cases, and we considered that there might be some relation between endothelial UEA-1 reactivity and increased capillary permeability. These capillaries showed no morphological alterations other than swelling of the endothelial nuclei in routine hematoxylin eosin staining. This supports the usefulness of UEA-1 in the evaluation of ischemic vascular damage. Endothelial reactivity for UEA-1 recovered about 3 weeks after the onset of cerebral infarction. This period agreed with that for endothelial regeneration (Fonkalsrud et al., 1976) and the recovery of BBB function after ischemia (Olsson et al., 1971). On the other hand, case 1, where death occurred only 36 hours after onset, demonstrated no reduction of UEA-1 reactivity. This case, however, showed considerable brain edema and albumin extravasation. Therefore, it can be speculated that UEA-1 is not suitable for the detection of very early stages of BBB insufficiency. We have, however, also studied the endothelial reactivity to lectin (WGA) in experimental animals by electron microscopy. The UEA-1 reactivity was visualized as continuous lines upon the endothelial surface in control Mongolian gerbils. Partial absence of UEA-1

reactivity was observed after 6 - hour ischemia with a re-perfusion period of 1 hour (Nishida et al., 1986). These ultrastructural alterations of the reactivity, however, could not be recognized by light microscopy. We can therefore consider that there may have been some ultrastructural changes in UEA-1 reactivity in our case 1, but that these were too fine to be demonstrated by light microscopy. We thought that this would warrant subsequent study.

On the other hand, UEA-1 reactivity in old ischemic lesions was more marked than that in controls. It has been reported that regenerated vessels in necrotic brain lesions have no basement membranes and are lacking in BBB function (Kawase et al., 1982). These morphological and functional changes might have some relationship with increased UEA-1 reactivity, but hematoxylin eosin staining showed neither necrotic changes nor enough scar formation to bring about vascular regeneration in our subjects. Therefore, it seems more likely that alteration of UEA-1 reactivity is due to tissue atrophy rather than vascular regeneration.

The UEA-1 reactivity was heterogeneous with each capillary even in one visual field of the damaged cerebral cortex. This agrees with previous observations that endothelial damage due to ischemia is not homogeneous but heterogeneous (Kerenyi et al., 1975).

Some authors have reported that BBB dysfunction is predominant in the arterioles (Westergaard, 1980). Our results, however, show that there are no differences of UEA-1 reactivity between arterioles and capillaries in ischemic cortical lesions.

UEA-1 reacts and binds with l-alpha-fucose which is present on the surface of the endothelium. Reduction of UEA-1 reactivity is therefore probably due to the loss of l-alpha-fucose. The endothelial surface has so-called "microdomains" which are generated by the preferential distribution of chemically different anionic sites and play an important role in transportation through the vessel wall (Simionescu et al., 1981). We can therefore speculate that a reduction of l-alpha-fucose may result in some changes of these "microdomains", thus causing functional changes in vascular permeability. Further biochemical investigations should be performed on this transportation mechanism.

UEA-1 has already been used as a morphological marker of blood vessels in various organs. Our present report shows that this lectin can be used to evaluate an endothelial functional state, permeability in the central nervous system. Our method seems to be a very reliable one for morphological studies of endothelial function after ischemic damage in autopsied human brains and in experimental animals.

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