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Microthrombi formation after severe head trauma

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Summary. This study was undertaken to look for trauma-related fibrinous microthrombi in traumatized human brains. Fifty brains from patients with variable time intervals between trauma and death were fixed in 10% formaldehyde. Sections from the contusioned area and from the corresponding area of the contralateral hemisphere were embedded in paraffin and 50 non-traumatized brains were used as controls. After sectioning and embedding, 10 µm sections were stained with haemalum and eosin (HE) and phosphotungstic acid-hematoxylin (PTAH). Stained fibrinous microthrombi were counted in each hemisphere and in control sections. More microthrombi could be found in the contusioned areas of the brain than in the contralateral side or in control sections.

Key words: Microthrombi – Brain injury – Hypoperfusion – Brain edema

Zusammenfassung. Das Ziel dieser Studie war es, traumaabhängige Mikrothromben im menschlichen Gehirn nachzuweisen. Zu diesem Zweck wurden 50 Gehirne von Patienten nach Verkehrsunfall mit tödlichem Ausgang in 10% Formalin fixiert und ausgewählte Stücke davon später in Paraffin eingebettet. Das Zeitintervall zwischen Unfall und Tod war unterschiedlich lang. Als Kontrollen wurden 50 Gehirne von nicht traumatisierten verstorbenen Patienten verwendet. 10 µ Schnitte wurden schließlich mit Hämalaun und Eosin sowie mit PTAH gefärbt. Die sich dunkelblau färbenden Fibrinmikrothromben wurden in jeder der Hemisphären und in den Kontrollschnitten gezählt. Mikrothromben konnten in wesentlich höherer Anzahl in den traumatisierten Zonen des Gehirns gefunden werden, als auf der nicht traumatisierten Gegenseite und in den Kontrollschnitten.

Schlüsselwörter: Mikrothromben – Schädel-Hirn-Trauma – Hypoperfusion – Hirnödem

Introduction

Severe head injury in humans is often complicated by complex secondary or delayed pathophysiological events such as edema, ischemia and elevated intracranial pressure. Each event can lead to further damage to the traumatized tissue. One of the most common responses to tissue damage is the aggregation of platelets and formation of thrombi (Weller et al. 1983; Williams et al. 1977).

The studies of Obrenovitch and Hallenbeck (1985) have shown that microthrombosis in the brain decreases cerebral blood flow, impairs the function of the blood brain barrier and leads to structural damage of nerve and glial cells.

The development of postischemic microthrombi has been described in experimental and clinical studies by Cervós-Navarro et al. (1984), Sampaolo et al. (1987) and Heye et al. (1991). In 1987 Hekmatpanah demonstrated the occurrence of collapsed vessels and intravascular clots after brain trauma in rats.

Based on these findings our objective was to demonstrate the existence of post-traumatic fibrinous microthrombi in human brains. To the best of our knowledge the existence, effects and distribution of microthrombi in human brain after trauma has not yet been studied.

Methods

Fifty brains from deceased casualties aged between 4 and 87 years who suffered severe head trauma prior to death were taken and fixed in 10% unbuffered formaldehyde. The time between trauma and death ranged from a few hours to 5 months and 9 patients died immediately after trauma. The survival time after injury can be seen in detail in Table 1.

One patient died from massive pulmonary embolism 5 months after trauma and was therefore not included in the statistical analysis. In 20 cases head injuries were caused by traffic accidents, 18

Table 1. Survival time after trauma in detail. 0 h = death immediately after trauma ($n = 49$)

	n		*n*
0 h	9	2–5 d	6
0–12 h	8	5–9 d	2
12–24 h	6	9–14 d	4
24 h–2 d	6	> 14 d	8

h = hours, d = days

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patients suffered accidents at home, 6 suffered industrial accidents and 5 were victims of bodily harm. Polytrauma with combined skull fracture was seen in 8 cases, isolated head trauma with skull fracture was seen in 19 cases and isolated head trauma without skull fracture in 22 cases.

The dura mater was closed in all these cases. The cause of death was malign brain edema in 35 cases, cardiac arrest in 9 cases and bronchopulmonary infection in 5 cases. In no case was generalized disseminated intravasal coagulation observed which is also applicable to our control group. Fifty patients who had died from other causes were taken as our control group. These patients suffered exclusively from cancer of the lung (25 cases) and the breast (14 cases) as well as melanoma (10 cases) prior to death. Causes of death were cardiac arrest, bronchopulmonary infection and renal failure, but no cerebral metastasis. After fixation for 2 weeks the brains were dissected at various levels and cut into 2 cm thick coronal sections. Specimens exclusively from the contusioned area of the coup (not from the contrecoup area) and from the noncontusioned corresponding area of the contralateral hemisphere as well as from the control brains were embedded in paraffin. The contusioned areas were macroscopically classified as small (diameter 1–3 cm), medium (diameter 3–5 cm) and large (diameter > 5 cm).

Sections (10 µm thick) were stained with HE and PTAH (Phosphotungstic acid-hematoxylin). In PTAH stains, fibrin is visualised as a deep blue intraluminal material which is oganized in laminar tracks. PTAH was used for determination of fibrinous microthrombi. The slides were observed under a light microscope and examined independently by 2 investigators. Examination was performed by counting the number of fibrinous microthrombi in the grey matter in 10 microscope fields (Magnification × 100). Only intracapillary thrombemboli were evaluated where capillaries were defined as small lumen vessels, less than 15 µm in diameter. Thrombi in postcapillary venules and spastic arteries were therefore included.

Results were expressed as the mean ± standard deviation (SD) and matched for survival time after trauma, age of the patients, as well as for size and localization of the contusion. The difference in the number of microthrombi between the corresponding areas of both hemispheres and control brains and between the various age groups was evaluated using a t-test for unpaired and paired samples. The differences of the number of microthrombi between the various size and location groups were also analyzed by means of the t-test.

The correlation between the number of microthrombi and survival time after trauma was evaluated by calculating Pearson's correlation coefficient.

Table 2. Age of patients, size and localization of the contusioned area of the brain

Patient's age					
0–10	11–30	31–50	51–70	< 70	Total
6	9	10	12	12	49
Localization of contusion					
Frontal	Temporal	Parietal	Occipital		
11	21	8	9		
Diameter of contusion					
0–1 cm	2–3 cm	4–5 cm	> 6 cm		
15	16	12	6		

Results

Of the patients investigated, 9 died immediately after trauma, 14 died within the 1st day, between the 2nd and 9th day, 14, and after the 9th day, 12. The age distribution of the patients can be seen in Table 2. The size and localization distribution are also shown in Table 2. The highest number of microthrombi was found in the contusioned grey matter of the traumatized brains (mean 17.4 ± 14.1). The lowest number was found in the control group of non-traumatized brains (mean 1.7 ± 2.6), whereas a mean of 5.1 ± 5.4 was found in the sections of the corresponding areas (non-contusioned) of the contralateral hemispheres.

In order to detect statistically significant differences between the groups we performed t-tests for unpaired (control group vs. contralateral hemisphere) and paired (contusioned area vs. corresponding area of the contralateral hemisphere) samples. All groups differed significantly from each other.

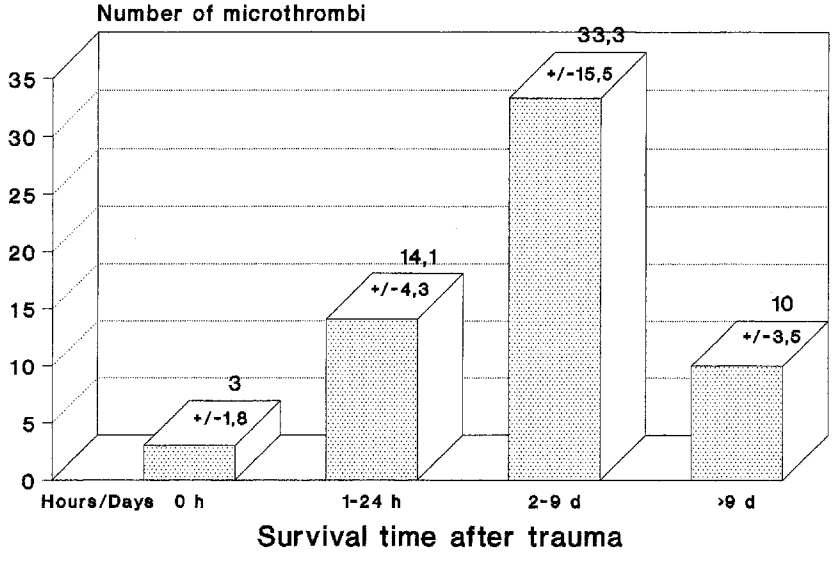

Fig. 1. Mean number and standard deviation of fibrinous microthrombi in the grey matter of brain post-trauma. 0 h = death immediately after trauma

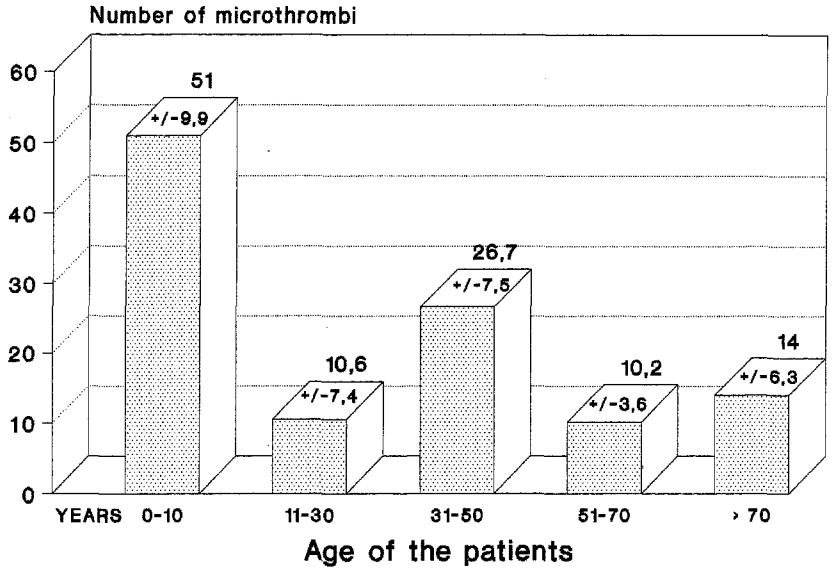

Fig. 2. Mean number and standard deviation of fibrinous microthrombi posttrauma matched for age of the patients

Fibrinous microthrombi in the control group and in the contralateral hemisphere showed a significant mean difference for $P < 0.01$. The number of microthrombi in the contusioned area and in the contralateral side of traumatized brain tissue differed to an even higher degree ($P < 0.001$). Further statistical analysis revealed an increase in microthrombi in the posttraumatic brain as the survival time increased (Fig. 1). The number of microthrombi increased step by step towards the 9th day posttrauma and decreased afterwards. This result was confirmed by calculating Pearson's correlation coefficient. The correlation between the number of microthrombi and the survival time after trauma was significant for $P < 0.01$.

In addition we found a certain age-dependency of microthrombi (Fig. 2), which were most common in patients in the 1st, 3rd and 4th decades of life. The t-test confirmed significant differences between the various age groups ($p < 0.01$). A comparison of size and localization of the contusioned area did not show any significant differences.

Discussion

Due to the studies of Hekmatpanah (1987), Sampaolo et al. (1987) and Suzuki et al. (1990) we know that microthrombosis in the brain occurs as a general response to various noxious stimuli. It seems to be likely that fibrinous microthrombi also occur after brain trauma as a consequence of alterations in aggregatory activity. However, no information is available regarding the formation and development of microthrombi in posttraumatic human brain tissue. As mentioned by Hekmatpanah and Hekmatpanah (1985) and Hekmatpanah (1987), intravascular clots are demonstrable after brain trauma in rats. These studies, on the other hand, do not provide any data about possible alterations in aggregatory activity. Intravascular thrombi consisting purely of platelets are difficult to demonstrate in routinely stained preparations.

During complex interactions of thrombocyte aggregates, fibrinogen is polymerized to form fibrin, which provides mechanical stabilization of a primary platelet thrombus. A qualitative evaluation of the vascular thrombosis and fibrinous microthrombi can be achieved by staining the fibrin with PTAH.

Electron microscope investigations have revealed that the ratio of platelet thrombi and fibrinous thrombi is 5:1 (Heye et al. 1991). The analysis of fibrinous microthrombi, which is possible using PTAH staining, confirms the hypothesis of aggregatory alterations after traumatic brain injury. Histological methods of quantification showed that the maximum number of microthrombi could be counted in the grey matter of the contusioned area of the brain, while the noncontusioned corresponding area of the contralateral hemisphere expressed microthrombi to a significantly lesser degree. It could therefore be suggested that local elevation of fibrin degradation products after brain trauma is more marked and more prolonged in brain areas with severe contusion than in those with no or mild contusion.

Several authors (Drayer and Poser 1975; Goodnight et al. 1974; Keimowitz and Annis 1973) have documented disseminated intravascular coagulation after severe head injury. Our findings, however, support the hypothesis of "local" disseminated intravascular coagulation as expressed by Ueda et al. (1985). The significant correlation between the number of microthrombi and survival time after trauma also adds support to these findings. The increase of microthrombi up to the 9th day posttrauma is not inconsistent with the elevation of fibrin degradation products 7 days after trauma, found by Ueda et al. (1985). Correlation between the number of microthrombi and the age of the patients, on the other hand, seems to depend on mechanical factors. The high degree of posttraumatic brain edema in infants for example can be explained by the high vulnerability of the blood-brain barrier and maximum elevation of microthrombi in the 1st decade of life. It could be that microthrombi formation and brain edema are dependent on each other and

represent the result of an increased reactivity of brain tissue in the 1st decade of life.

The mechanism of formation of cerebral edema after trauma is not yet well defined, but may be caused by microthrombosis. One of the causes for the formation of microthrombi could be the so-called platelet-activating factor (PAF), a biologically active phospholipid, which is reputed to be a mediator in central nervous system injury (Feuerstein et al. 1990). Not only the formation of microthrombi, but also cerebral hypoperfusion, reduction of blood flow (Arvigo et al. 1985; Yamakami and McIntosh 1991; Dickman et al. 1991; Susi and Walls 1990) and aggravation of posttraumatic brain edema (Ishige et al. 1987) are thought to be partly triggered by PAF (Feuerstein et al. 1990; Snyder 1989). It can therefore be seen, that the formation of microthrombi in the brain is triggered not only by trauma, but also by other noxious stimuli. Furthermore, microthrombi seem to be one of the central secondary morphological events after brain trauma.

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