Cerebrospinal fluid enzymes in acute brain injury

2 Relation of CSF enzyme activity to extent of brain injury

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SUMMARY The value of CSF enzyme estimations as indices of the extent of brain damage was studied in the experimental situation. Standard cold lesions of different severity were induced in cats. The activities of the enzymes CPK, HBDH, LDH, GOT, and ChE were studied at half hour intervals in the cerebrospinal fluid (CSF). The ventricular and cisternal fluid pressure, and the arterial blood pressure were monitored continuously. Significantly higher enzyme levels were found in the animals with more severe injuries of the brain.

In a previous paper Maas (1977) demonstrated a great increase in the activity of various enzymes in the CSF during the first seven hours after induction of a cold lesion in the cat brain. Although prognostic value has been attributed by various authors to the level of CSF enzyme activity in patients with severe head injuries (Smith *et al.*, 1960; del Villar *et al.*, 1973), few clinical or experimental studies have correlated the changes in CSF enzyme activity with the extent of brain damage in the acute phase after brain trauma (Smith *et al.*, 1960; Akashi, 1966; Florez *et al.*, 1975). To investigate this relationship standardised cold injuries of different severity were made in three series of cats. In one series a deep lesion was induced, in the second series a more shallow lesion was produced by freezing at a higher temperature, and in the third series a smaller area of cortex was frozen. CSF enzymes were studied at half hour intervals and differences analysed between the various series.

Materials and methods

Thirty-eight anaesthetised cats, weighing 2800-6400 g, otherwise unselected for age or sex, were studied. The experimental model as well as anaesthetic and surgical methods were as described by Maas (1977). Experimental brain injury was effected by induction of cold lesions to the brain. Before and after freezing, CSF samples of 0.35 ml were withdrawn from a cisternal catheter every half hour and analysed for the activity of the

following enzymes: creatine phosphokinase (CPK; EC 2.7.3.2), alpha hydroxybutyric acid dehydrogenase (HBDH), lactic dehydrogenase (LDH; EC 1.1.1.27) aspartate aminotransferase (GOT; EC 2.6.1.1.) and pseudocholinesterase (ChE; EC 3.1.1.8). Standard optimised Merck-1-test kits (E. Merck, Darmstadt) were used. Arterial blood-pressure, end expiratory CO₂, ECG, and ventricular and cisternal fluid pressures were monitored continuously and measured every half hour before withdrawal of CSF samples. Respiratory frequency and heart rate were also calculated. After the control period as described by Maas (1977) a cold lesion was induced in 31 of the 38 animals. The other seven animals, which served as a control series, were those used as controls in the earlier paper. Cold lesions were induced by the method of Beks *et al.* (1965).

The experiments were divided into four series:

Series 1: control series (seven animals). These animals received a complete sham operation, but no cold lesion was induced.

Series 2: in 14 animals an extensive cold lesion of the brain was induced by freezing 1.34 cm² of cortex for exactly six minutes at -40°C.

Series 3: in this series less extensive lesions of the brain were induced.

3a: in series 3a (nine animals) this was done by freezing the same area (1.34 cm²) for the same length of time, but at a higher temperature: -30°C.

3b: in series 3b (eight animals) a smaller lesion was induced by freezing a smaller area (0.97 cm²) for six minutes exactly at -40°C. After freezing, samples of CSF

(0.35 ml) continued to be withdrawn every half hour.

The experiments were usually terminated at an arbitrarily chosen time limit of 7.25 hours after induction of the cold lesion. If tentorial herniation developed, the experiments were terminated prematurely as CSF could no longer be obtained from the cisternal catheter in quantities sufficient for analysis. The criteria for brain stem herniation have already been described (Maas, 1977).

After the experiments, the animals were killed with an overdose of intravenous pentobarbitone. The brains were removed and fixed in a 4% formalin solution for two weeks and paraffin sections cut through the maximal diameter of the cold lesion. These sections were stained with haematoxylin and eosin (HE), periodic acid Schiff (PAS), trichrome and PAS, and according to the method of Kluwer-Barrera.

STATISTICAL ANALYSIS

The differences between the values measured at the various times (every half hour up to 7.25 hours after induction of the cold lesion), and the control values for each experiment were calculated. Statistical analysis of these calculated differences was performed at every time level using Wilcoxon's ranking test. The following series were compared: (A) control animals versus animals with cold lesions—control versus series 2, control versus series 3a, and control versus series 3b; (B) series with extensive cold lesions versus series with less extensive lesions—series 2 versus series 3a, and series 2 versus series 3b.

Difference in occurrence of tentorial herniation between the series with more and less severe injuries was analysed with Fisher's test.

Results

DEVELOPMENT OF TENTORIAL HERNIATION

Eight of the 14 animals in the series with a severe cold injury (series 2) developed tentorial herniation within 7.25 hours of induction of the cold lesion. Five of the animals developing herniation did so within 2.5 hours of induction of the lesion. These five animals were excluded from further

enzyme analysis as the survival time was too short for an increase in CSF enzyme activity to occur. One experiment in this series was terminated prematurely because mechanical obstruction of the cisternal catheter had made it impossible to obtain further samples of CSF. The number of animals developing tentorial herniation within the 7.25 hours standard study period in series 2 and in series 3a and 3b are summarised in Table 1.

Statistical analysis using Fisher's test showed that the probability of tentorial herniation occurring less often in series 3a or 3b (less severely injured) than in series 2 (more severely injured) was $p=0.80$ and $p=0.90$ respectively. These probabilities are not statistically significant, but they do show a tendency for tentorial herniation to develop less often in the series with the less severe injuries (series 3a and 3b) than in the series with more extensive lesions (series 2).

DIFFERENCES IN CSF ENZYME ACTIVITY AND IN THE VENTRICULAR FLUID PRESSURE (VFP) BETWEEN THE CONTROL SERIES AND THE SERIES WITH COLD LESIONS

All three series of animals with cold lesions (2, 3a, and 3b) developed a statistically significant increase in the activities of the enzymes CPK, HBDH, LDH, and GOT in the CSF, when compared with the control series. Cholinesterase was significantly increased in series 2 and 3b but not in series 3a (-30°C). The ventricular fluid pressure (VFP) was raised in all animals with a cold lesion.

DIFFERENCES IN CSF ENZYME ACTIVITY AND IN VFP BETWEEN THE SERIES WITH MORE SEVERE AND WITH LESS SEVERE INJURIES

A statistically significant (Wilcoxon: $p<0.05$) difference of activity of all the enzymes studied in the CSF developed between the series with severe injuries (series 2) and the series with less severe injuries (3a and 3b) from approximately 2.75 hours after induction of the cold lesion. These differences are summarised in Figs 1–4 which show the median values of CSF enzyme activity and the median VFP in the three series.

In 23 of the 26 experiments, either a maximal

Table 1 Number of animals developing tentorial herniation after severe and less severe cold injuries

Series	No herniation	Brain stem herniation	Mechanical obstruction of cisternal catheter	Total number of experiments
Control	7	0	0	7
Series 2	5	8	1	14
Series 3a	5	3	1	9
Series 3b	6	2	0	8

Fig. 1 Median values of CPK activity in the cerebrospinal fluid after induction of cold lesions of different severity in cats. The animals in series 2 were severely injured, those in series 3a and 3b less severely.

value of the activities of the enzymes in the CSF, or a plateau level (a more or less constant raised enzyme activity) was reached. This 'maximal or plateau value' was defined as the level of enzyme activity which did not subsequently increase by more than 10%. Figure 5 shows that this 'maximal value' was highest in the series with the more severe injuries.

One might expect this 'maximal value' to be reached later in the animals in series 3a or 3b than in those in series 2 (severely injured). This, however, was not the case (Fig. 6).

From these findings it may be concluded that higher enzyme activities are encountered in the CSF of the more severely injured animals, but that the time span in which the 'maximal activity' is reached does not differ.

Fig. 2 Median values of α -HBDH activity in the CSF after induction of cold lesions of different severity in cats.

VALUE OF CSF ENZYME ACTIVITY AND VENTRICULAR FLUID PRESSURE RELATED TO SEVERITY OF BRAIN INJURY

The VFP was higher in series 2 than in 3a or 3b. The difference was statistically significant (Wilcoxon: $P < 0.05$). However, in series 3a (-30°C) this statistical significance was only transitory, and was not present later than four hours after induction of the lesion.

The question arose whether the CSF enzyme activity, although obviously offering different information than the VFP, was not also more valuable in discriminating between the groups with injuries of different severity. In Figs 7 and 8 the observed values of the CPK activity in the CSF and the VFP are shown at hourly intervals for series 2 and 3a.

It becomes evident that the CSF CPK levels differentiate better between the two series than does the VFP. This is also illustrated in Table 2, which shows the development of changes in CPK

Table 2 CPK activity in CSF and ventricular fluid pressure in two animals with a cold lesion, one severely injured, and one less severely injured

	Hours after induction of cold lesion	Control value	0.25	1.25	2.25	3.25	4.25	5.25	6.25	7.25
Experiment 35 (series 2)	VFP (mmHg)	3.2	5.0	16.3	21.3	28.0	30.0	30.0	26.5	33.5
	CPK (u/l)	5	4	5	1040	1100	830	610	600	430
Experiment 30 (series 3a)	VFP (mmHg)	2.1	2.5	10.6	16.8	24.5	34.0	100	†	
	CPK (u/l)	2	2	5	230	340	340	290

Fig. 3 Median values of GOT activity in the CSF after induction of cold lesions of different severity in cats.

activity in the CSF and in the VFP in a series 2 and a series 3a experiment. The VFP reached higher levels in the animal that was less severely injured, while the CPK activity was higher in the animal with the larger lesion. Apparently the development of raised VFP is not linearly related to the extent of brain damage. This phenomenon is also known in the clinical situation: patients (especially children) with relatively minor brain damage may develop explosive cerebral oedema.

HISTOLOGICAL STUDIES

All animals with a cold lesion exhibited cortical necrosis and cerebral oedema. Macroscopically, a slightly swollen, darkly discoloured area of cortical necrosis was present with evident hyperaemia.

Fig. 4 Median values of the ventricular fluid pressure after induction of cold lesions of different severity in cats.

The hemisphere in which the lesion had been induced was swollen, with displacement across the midline, and compression of the ventricle on the side of the lesion. Microscopically the lesions were essentially the same as described by other authors (Klatzo *et al.*, 1958; Clasen *et al.*, 1962; Go *et al.*, 1967). The borders of the necrotic lesion were sharply defined in the cortex, but the extent of the necrosis into the white matter was more difficult to delineate. Oedema was evident by less intense staining of the white matter in the hemisphere

Fig. 5 'Maximal values' of CPK activity observed in the CSF after induction of cold lesions to the brain in cats. The highest levels are seen in the more severely injured animals (series 2).

Fig. 6 Time span before maximal CPK activity is reached in the CSF after induction of cold lesions of different severity. No difference exists between animals with severe (series 2), and animals with less severe lesions (series 3a and 3b).

with the cold lesion, and by dispersion of the myelin fibres. Occasionally a complete status spongiosus resulted. The oedema was confined to the ipsilateral hemisphere, sparing the corpus callosum. In the necrotic zone all signs of cellular destruction were present. The cell bodies of the neurones were shrunk, exhibiting pyknosis (their nuclei being hyperchromatic with a triangular deformation). Karhyorrhesis and occasionally total cell lysis were present. The

Fig. 7 Levels of CPK activity observed in the CSF of cats with severe injuries (series 2) and less severe injuries (series 3a).

Fig. 8 Ventricular fluid pressure measured in cats after induction of cold lesions of different severity.

cytoplasm was eosinophilic and homogenisation of the Nissl substance was seen. The destruction of glial elements was less complete, but the white matter often showed dissolution. The necrosis proceeded to a greater depth in the length of the nerve fibres. In the necrotic zone hyperaemia of veins and capillaries was present, with erythro- and leucodiapedesis, and often haemorrhages, in a few experiments so extensive as to produce a haemorrhagic necrosis. It was difficult to make a quantitative estimation of the extent of brain damage, but it was possible to trace the extent of the necrotic area under the microscope at constant magnification. The average extent of the lesions in the three series is shown in Fig. 9.

As might be expected the largest area of destruction was observed in the series frozen at -40°C (series 2). In the series frozen at -30°C (series 3a), the average lesion was obviously more shallow, but the depth of the lesion in series 3b was similar to that of series 2. The average diameter of the lesion in this series, however, was obviously smaller than in series 2.

Fig. 9 Average extent of cold lesion as seen on a frontal section of the brain through the maximal diameter of the cold lesion. The area of necrosis and bleeding is obviously more shallow in series 3a and smaller in diameter in series 3b than in series 2 (more severely injured animals). — = Series 2, - - = Series 3a, . . . = Series 3b.

Discussion

Establishing a reliable prognosis in patients with severe head injuries can be very difficult. It will depend on many factors of which site and degree of brain damage are very important (Jennett, 1972). An easily obtainable and objective index of the extent of brain damage is desirable for giving a prognosis. The amount of enzymes released from damaged tissue into extracellular fluid (Go *et al.*, 1976) could be expected to be proportional to the extent of tissue damage. Enzymes will be transported from the extracellular fluid towards serum and CSF. Serum enzyme estimations would be both objective and easily obtainable but have not been found to be of prognostic value. Dubo *et al.* (1967) reported raised values of CPK in the serum of all patients studied with head injuries, as well as in 17 of 28 patients suffering from stroke or meningitis. However, no definite clinical correlations or prognostic inferences could be drawn from the serum CPK levels and isoenzyme analysis demonstrated that the CPK was mainly derived from muscle.

Kaltiala *et al.* (1968) studying GOT, LDH, and GPT in the CSF and serum in 30 patients with head injuries only found GOT levels raised in the serum, but this rise correlated in the first place with peripheral contusion and no definite correlation was observed between the GOT activity in serum and CSF and the clinical severity of the lesion. Lindblom and Åberg (1972) found no correlation between the clinical severity of brain

injury in man and the occurrence of enzyme abnormality.

Rabow *et al.* (1971) showed raised levels of LDH 1 more often in patients with cerebral contusion than in those with cerebral concussion. Rabow (1972) demonstrated, in a series of 70 patients, that the maximal serum LDH 1 activity in the acute phase after head injury, shows a very good correlation with the prognosis in the individual patient. Klun (1974) showed increasing levels of GOT and GPT in the serum of patients dying after head injury, while in the surviving patients decreasing values were noted. Thomas and Rowan (1976) found an increase of the LDH isoenzymes 1 and 2 in the serum after head injury, but also remark that prognostic application of serum LDH isoenzyme estimations is not definite as a wide range of LDH isoenzymes existed within the head injured group, so that there was overlap of findings between those who died and those who were left disabled.

These conflicting results may be explained by the high incidence of peripheral contusions and muscle damage in patients with severe head injuries causing raised levels of serum enzyme activity. Recent research of Somer *et al.* (1975) into the brain isoenzyme of CPK in the serum of these patients, in addition to LDH isoenzyme studies as conducted by Thomas and Rowan (1976) and Rabow (1972) seems to be a more specific line of research. The CSF is in far more intimate contact with the extracellular fluid of the brain than is the blood. CSF may, in fact, serve as the lymph of the brain. Previous studies have demonstrated high levels of especially CPK, HBDH, LDH, and GOT in the CSF of cats in which a cold lesion of the brain had been induced. Isoenzyme studies on CPK and LDH confirmed that these enzymes were of cerebral origin (Maas, 1977).

Other workers have reported results indicating that enzyme changes in CSF after severe brain injuries would yield more consistent results than serum enzyme analysis. Smith *et al.* (1960) found that patients with either persisting or greatly raised values of GOT in the CSF also had the most extensive brain damage. In these patients it was predictable that neurological defects would persist. These authors stated that the GOT level in the CSF is indicative of the severity of a craniocerebral injury and may be helpful in predicting end results in the early stages of injury to the head.

Kaltiala *et al.* (1968) found the most pronounced increase of GOT in the CSF of patients with cerebral contusion, not in those with cerebral con-

cussion alone. Klun (1974) however, found no difference in GOT or GPT activity of the CSF in patients dying or surviving after head injury. Florez *et al.* (1975), reporting on CSF and serum enzyme studies conducted on 35 patients with head injuries, showed the GOT activity of both serum and CSF to correlate well with the severity of the brain lesion, but they could find no such correlation for the CPK activity. They conclude that the prognostic value of the changes in CSF and serum enzyme activity is limited. Del Villar *et al.* (1973) demonstrated a benign prognosis in the majority of 37 patients with craniocerebral injuries without CPK activity in the CSF, and a bad prognosis when CPK was very elevated. However, low CPK values in the CSF were also seen in three of eight patients with serious neurological symptoms. They do not mention at what time after injury the specimens of spinal fluid were obtained, and our studies have shown that rapid changes of CSF enzyme activity can occur, especially within the first seven hours after trauma. Nevertheless, the observation that some patients can be clinically in a very serious condition and still have low activities of the enzymes in the CSF also struck us during preliminary clinical studies (Maas, 1977). Although it seems logical that the CSF enzymes would be related to the extent of brain damage, the fact that low enzyme activities may also occur in the CSF of patients with severe head injuries decreases the prognostic value.

Very little experimental work on the relation between the enzyme activity of the CSF and the extent of brain damage has been done (Smith *et al.*, 1960; Wakim and Fleischer, 1956). Smith *et al.* (1960) established this relation in a limited number of dog experiments.

The present studies also establish a definite relation between CSF enzyme activity and the extent of brain damage. This is evidenced by the higher levels of CSF enzyme activity and more extensive histological lesion occurring in the series with severe injuries (series 2) than in the two groups with less severe injuries (3a and 3b). CSF enzyme estimations should also offer more information than the VFP alone, being more discriminative as to the severity of the injury. CSF enzyme estimations provide information on the extent of brain damage, while ventricular fluid pressure measurement does not clearly do so. The height of the intracranial pressure is not necessarily related to the extent of brain damage. Brain oedema can be out of proportion compared to the actual brain damage.

It must be remembered that, even in the standardised experimental model described, great

variations in the response of CSF enzyme activity to brain trauma were demonstrated in the individual animals. Further research into the cause of this variability of individual response is required.

Low enzyme activities in the CSF of patients in the acute stage after brain injury in combination with a high intracranial pressure, could, after exclusion of a haematoma, indicate severe brain oedema in a not so severely damaged brain, with the possibility of a potentially treatable syndrome. The results reported here indicate that the CSF enzyme activity is related to the extent of cerebral damage, and estimation of CSF enzymes should, therefore, be of value in estimating the outcome in individual patients with severe head injuries. It must, however, be borne in mind that prognosis is not only determined by the degree, but also by the site of brain damage, while pre-traumatic factors and the presence of extra-cerebral lesions must also be taken into account.

A definite prognosis for the individual patient cannot be established on the basis of the enzyme activity of the CSF alone. The prognostic value of CSF enzyme estimations depends on the consistency with which enzymes released into the extracellular fluid will be transported towards CSF. It is conceivable that this transport may be influenced by a number of factors, for instance the height of the blood pressure. More research into this aspect is in progress.

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