Effects of Therapeutic Hypothermia on Activity of Some Enzymes in Cerebrospinal Fluid of Patients with Anoxic–Ischemic Brain Injury

Per Vaagenes

I assessed the effect of therapeutic hypothermia on the activity in cerebrospinal fluid of creatine kinase (EC 2.7.3.2) and its brain isoenzyme (CK-BB), lactate dehydrogenase (EC 1.1.1.27), and aspartate aminotransferase (EC 2.6.1.1) as markers of cerebral damage in patients with transient anoxic–ischemic brain injury. Moderate hypothermia (30–32 °C) lasting more than 24 h resulted in disproportionately greater activity of creatine kinase during the post-insult period than in patients not treated with hypothermia but having similar insults and outcome (p < .01 for survivors, and p < .005 for nonsurvivors). No differences were observed for the thermostable enzymes lactate dehydrogenase and aspartate aminotransferase, which demonstrates that the effect of hypothermia must be taken into account when thermolabile enzymes are used as sole markers of brain damage in such patients.

Additional Keyphrases: creatine kinase and its isoenzymes, lactate dehydrogenase, aspartate aminotransferase, blood–brain barrier, enzyme thermostability, assessing cerebral damage.

Although changes in temperature significantly alter the stability of enzymes and affect their activity in vitro (1–3), little attention has been given to the effects of clinical hypothermia on the patterns of enzyme activity in body-fluid compartments in vivo (4–9). Spuriously high activity may be encountered when thermolabile enzymes such as creatine kinase (CK, EC 2.7.3.2) and its brain isoenzyme (CK-BB) are used as markers of brain damage in patients with hypothermia associated with global or focal anoxic–ischemic insults (6, 10, 11). Here I report studies of some enzymes in cerebrospinal fluid (CSF) of patients with anoxic–ischemic insult who were treated with hypothermia for its possible brain-damage-ameliorating effect (12). Also examined were possible CSF enzyme changes in patients who were comatose from exposure to cold at the time of admission, but without anoxic–ischemic insult.

Materials and Methods

In 21 patients with anoxic–ischemic brain insult, prolonged hypothermia was instituted to minimize brain damage. Eleven patients with asphyxial cardiac arrest due to near-drowning were hypothermic on admission, with rectal temperature ranging from 26 to 31 °C. In 10 patients with anoxic–ischemic insult owing to sudden ventricular fibrillation or asystole (n = 9), or massive cerebral air-embolism (n = 1), hypothermia was instituted within 1 to 3 h after the

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In the hypothermic group, 12 patients had severe brain damage and died. Seven survived, with or without minimal or moderate cerebral defects. Two patients who were regarded as potential survivors (none or only slight histological change in the brain at autopsy) died during the hypothermic period of extracerebral causes (cardiac failure). Also in this group, the mean values for maximum enzyme activity were significantly lower for the survivors than for the nonsurvivors, with p values of <.005, <.001, and <.005, for CK, LD, and ASAT, respectively.

From Figure 1, however, it is evident that CK values were significantly higher in the hypothermic group of both survivors (p < .01) and nonsurvivors (p < .005) than in the normothermic group, although the outcome was comparable. CK ranged from 3 to 10 U/L in the normothermic survivors, whereas activities up to 84 U/L were recorded for the hypothermic survivors. In the nonsurvivors, peak CK activity ranged from 12 to 714 U/L in the normothermic group, and from 75 to 6120 U/L in the hypothermic group, although the clinical outcome was comparable for the two groups.

For LD and ASAT there were no differences in activity values between normothermic and hypothermic patients, regardless of outcome.

Figure 2 illustrates the relationship between maximum CK and maximum LD and ASAT in CSF from the hypothermic and normothermic groups. Hypothermia affects the intercepts of the regression lines, but does not alter their slopes or significantly change the r (Pearson's correlation coefficient) values.

Table 1 shows the time-to-peak activities for the three enzymes (a definite peak of activity could not always be determined in those hypothermic patients who died). In the normothermic group, CK activity peaked significantly earlier than LD (p < .001) or ASAT (p < .001). This was not true for the hypothermic group.

Figure 3 shows the temporal CK changes in CSF of 21 normothermic and 14 hypothermic patients, and illustrates its protracted increase in patients with hypothermia.

Figure 4 shows the temporal enzyme changes in CSF in relation to body temperature for five nonsurvivors with hypothermia lasting for approximately 120 h. CK decreased dramatically concomitantly with the restoration of normal body temperature; there was no similar temperature-related decrease in LD or ASAT. The same pattern was seen in 13 of the 15 patients in whom peak enzyme values could be identified. Enzyme decay was determined for CK in seven normothermic and six hypothermic patients. The average biological half-life in the normothermic group was 41.1 (SD 19.3) h, which agrees with previous observations (10). In the hypothermic group, the average half-life was 20.6 (SD 6.6) h, significantly shorter (p < .0025) than in the normothermic group. One would have expected a slower decay during hypothermia, and the calculated half-life merely reflects the rapidity by which CK is inactivated when normal body temperature is restored. Half-lives could not be determined for LD or ASAT owing to the inadequate number of points on the decay curves.

In blood, CK activity was maximum at 28.3 (SD 17.2) h in the normothermic group, and at 50 (SD 21.6) h in the hypothermic group. This significant (p < .01) difference, although most likely caused by the difference in temperature per se, could also be associated with the high proportion of patients with acute myocardial injury in the normothermic group, because myocardial infarction causes a prompt increase in CK in blood (18). Peak CK values occurred earlier in blood than in CSF, but the difference was significant only in the normothermic group (p < .001). There was no correlation between enzyme activities in CSF and in blood (r was always < .32).

Isoenzymes of CK were determined in the CSF of eight patients in the hypothermic and two in the normothermic group. Traces of myocardial CK (CK-MB) or muscle CK (CK-MM) were found late in the course after the insult in five and two patients in the two groups, respectively. Serum isoenzymes were determined in three normothermic and two hypothermic patients. CK-BB was present in both of the hypothermic patients who died with severe brain damage, but in none of the normothermic patients, one of whom died and two survived.

Figure 5 shows the temporal CK changes in CSF and blood of three patients during and after the hypothermic period. A rapid and profound decrease in the total CK activity in CSF was observed concomitantly with restoration of normal body temperature in all the patients, whereas the decrease in blood was not always related to the temperature changes. In cases 2 and 3, only CK-MM activity was observed in blood; in case 1 considerable CK-BB and CK-MB activities were found in blood, and total CK activity was observed to decrease concomitantly with the decrease in CSF, where the activity consisted exclusively of CK-BB.

The total protein concentration was consistently above our normal reference interval, 0.15–0.40 g/L, whenever the CK-M component was observed in CSF, or when CK-BB was found in blood.

In the three patients admitted in profound hypothermia (23–28 °C) owing to cold exposure without cardiac arrest, there was no enzyme increase in CSF during the first 24 h.
after admission, whereas there was a marked increase in blood.

**Hemodynamic findings.** In the hypothermic group, the mean arterial pressure was lower during the hypothermic period than after restoration of normal body temperature, but there was no difference between survivors and nonsurvivors. Heart rate, however, was significantly lower during hypothermia, both in survivors (p < .02) and nonsurvivors (p < .05). In the normothermic group there was no difference in mean arterial pressure or heart rate between survivors and nonsurvivors, but both pressure and heart rate were generally higher than in the hypothermic group after normal body temperature was restored.

**Discussion**

The present study shows that changes in body temperature significantly influence the magnitude of increase and the pattern of CK changes in CSF of patients with anoxic-ischemic brain insults, whereas LD and ASAT, being more thermostable, are not affected. Hypothermia increases the stability of CK by decreasing the effect of thermal inactivation, particularly of CK-BB, which results in a persistent high CK activity in CSF; but as soon as normal body temperature is restored, thermal inactivation causes an abrupt decrease in CK activity, which is also reflected by the short CK half-life observed in this group. The thermal effect was less pronounced in blood, where the more stable CK-M-containing enzyme fractions predominate (2, 3, 10). Therefore, decrease of CK activity in blood is not necessarily related to temperature changes unless significant activity is due to CK-BB (Figure 5).

We have previously shown that CK activities in CSF exceeding 10–12 U/L are associated with a poor prognosis and widespread neuronal necrosis in the brain of patients who have been resuscitated after cardiac arrest (10, 13), whereas activity of <4–5 U/L is associated with full neuro-
logical restitution and no histopathological changes in the brain (13). In the present study, CK activity as high as 84 U/L was measured in the hypothermic group, which was consistent with survival and only minimal to moderate cerebral disturbances. In the normothermic nonsurvivors, CK activity was between 12 and 714 U/L, while in the hypothermic nonsurvivors it ranged from 75 to 6120 U/L.

In the normothermic patient with CK activity of 714 U/L, LD was 3302 U/L and ASAT 1056 U/L, while in the hypothermic patient with CK activity of 6120 U/L, LD was 8485 U/L and ASAT 2320 U/L, which illustrates the high proportion of CK compared with LD or ASAT in this patient, who died three days after resuscitation, in brain death (cerebral circulatory arrest). Severe disturbance of the blood–brain barrier was evidenced by the considerable activity of CK-MM in CSF and the predominance of CK-BB isoenzyme activity in peripheral blood on the third day. On the first day, however, only CK-BB isoenzyme was observed in the CSF. In studies of patients without or with neurological complications associated with open heart surgery (6, 11), moderate hypothermia during bypass did not appear to influence CK activity in CSF, but profound hypothermia (15 °C) did cause a disproportionately high early increase in CK (6). The absence of CSF enzyme increase in the hypothermic patients due to cold exposure indicates that hypothermia per se does not provoke a release of CK from the brain. The massive increase in CK in the blood of such patients has been observed before (19) and has been assumed to be of muscular (30) or cardiac (21) origin.

Although the number of patients in whom CK isoenzymes were evaluated was small, there was suggestive evidence that leakage of the prevalent isoenzyme fractions in blood into the CSF, and vice versa, occurred more often in the hypothermic than in the normothermic group, but that this was a time-dependent diffusion process. An alteration of the blood–brain barrier was also suggested by the increased protein concentrations observed in CSF after arrest, which was more pronounced in the hypothermic group.

In conclusion: the present study shows that prolonged periods of moderate hypothermia (30–32 °C) significantly inhibit the degradation of CK in CSF of patients with anoxic–ischemic brain insults, which results in a disproportionately higher CK than in normothermic patients with the same insult and outcome, whereas the more thermostable enzymes LD and ASAT are not significantly affected by such temperature changes. This effect has to be taken into consideration when CK or CK-BB are to be used as single markers of brain damage in hypothermic patients with brain insults, particularly in those with good potential for cerebral recovery.

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