Intracerebral microdialysis in neurointensive care: the use of urea as an endogenous reference compound

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Object. When evaluating the results of intracerebral microdialysis, the in vivo performance of the microdialysis probe must be considered, because this determines the fraction of the interstitial concentration obtained in the microdialysis samples. The in vivo performance is dependent on several factors, for example, the interstitial compartment’s diffusion characteristics, which may vary during the course of the acute brain injury process. In the present study the authors investigated the method of controlling the in vivo performance by using urea, which is evenly distributed in all body fluid compartments, as an endogenous reference compound and by comparing the urea levels in three compartments: the brain (CNS), abdominal subcutaneous tissue (SC), and blood serum (BS).

Methods. Sixty-nine patients with traumatic brain injury or cerebrovascular disease were included in the study. In 63 of these patients a CNS probe was used, an SC probe was used in 40, and both were used in 34. Urea was measured by enzymatic methods, at bedside for the microdialysis samples and in routine clinical laboratory studies for the BS samples, with the probe calibrated to give identical results. The correlation coefficient for CNS/SC urea was 0.88 (2414 samples), for CNS/BS urea it was 0.89 (180 samples), and for SC/BS urea it was 0.98 (112 samples).

Conclusions. Urea levels in the CNS, SC, and BS were highly correlated, which supports the assumption that urea is evenly distributed. The CNS/SC urea ratio can therefore be used for monitoring the CNS probe’s in vivo performance. Fluctuations in other substances measured with microdialysis are probably caused by biological changes in the brain, as long as the CNS/SC urea ratio remains constant.

KEY WORDS • microdialysis • urea • traumatic brain injury • cerebrovascular disease

Abbreviations used in this paper: BS = blood serum; CNS = brain; ICP = intracranial pressure; NICU = neurointensive care unit; SAH = subarachnoid hemorrhage; SC = subcutaneous; TBI = traumatic brain injury.
limiting, from which we infer that in vitro recovery is not a good measure of in vivo performance. In addition, the brain injury process itself may affect in vivo performance through edema formation, and alterations of temperature and blood flow, or by stimulating gliosis. This may necessitate repeated calibrations to ensure that the probe is performing consistently. Several methods of in vivo calibration have been described; for a review see Gardner, et al., and Kehr. However, the reported methods are too tedious for repeated calibrations in the NICU setting.

Ideally, a continuous monitoring of the CNS probe performance in the clinical setting should include no extra procedures. One way to achieve this is to measure the dialysate levels of an endogenous reference compound. Such a substance should have stable levels independent of the specific brain injury and treatment, and be evenly distributed in all body fluid compartments.

In the present study we tested urea as an endogenous reference compound for bedside monitoring of the CNS probe’s in vivo performance. The bulk of nitrogen produced after protein degradation is excreted in the form of urea, which is almost exclusively synthesized in the liver and exits the body via the urine. The synthesis and breakdown of proteins is normally in balance, giving stable levels of BS urea. Because urea rapidly passes through biological membranes, concentration gradients between body fluid compartments are normally not present.

We compared the levels of urea acquired from the CNS (CNS urea), SC adipose tissue (SC urea), and blood serum (BS urea). Furthermore, the two different analytical methods were compared for urea.

Our hypothesis was that the CNS/SC urea ratio could be used for continuous monitoring of the CNS probe’s in vivo performance.

Clinical Material and Methods

The Uppsala University Hospital’s ethics committee for human research approved the use of microdialysis in NICU patients after informed consent was received from either the patients or their relatives.

Patient Population

Sixty-nine patients were included in the study (37 men and 32 women). Their mean age was 52.5 ± 14 years (range 16–76 years). Thirty-four patients had SAH, 29 had TBI, four had intracerebral hematoma, and two had intracerebral arterial occlusion. The majority of the patients were intubated on admission to the NICU, and therefore their neurological assessment is presented as the best motor response measured with the Glasgow Coma Scale. The mean of the best motor scores on admission was 4.7. Nine patients died, and the mean of the best motor scores at discharge was 5.2 for the rest. The patients were monitored and treated according to standard NICU protocols targeting control of ICP, cerebral perfusion pressure, and early detection and treatment of avoidable factors to limit secondary brain injury. Patients with focal lesions and uncontrollable ICP were treated surgically, whereas the remaining patients, who had severe ICP disruptions or recurrent seizures, were treated with barbiturate-induced coma. Finally, during severe delayed ischemia after SAH moderate hypothermia, that is, a body temperature of 33 to 35°C, was used.

Sampling and Analyses

Sixty-three patients were monitored with intracerebral microdialysis, 40 with subcutaneous microdialysis, and 34 with both. In most cases the CNS probe was inserted into the frontal cortex through a separate burr hole located close to the ICP monitoring device. In some patients the CNS probe was inserted under a bone flap used for surgical procedures. The SC probe was inserted in the lower right quadrant of the abdomen, in the SC adipose tissue.

The microdialysis probes used were CMA/70, which has a 10-mm membrane length, for the CNS compartment and CMA/60, which has a 30-mm membrane length, for the SC tissue (CMA Microdialysis, Stockholm, Sweden). They were perfused with artificial cerebrospinal fluid (in mM): Na+ 148, Ca ++ 1.2, Mg ++ 0.9, K + 2.7, and Cl – 155 at a rate of 0.3 µl/minute. Hourly dialysate samples were analyzed for urea at bedside on a CMA/600 microdialysate analyzer with an enzymatic colorimetric method (CMA Microdialysis). The CMA/600 microdialysate analyzer was also used to measure CNS glucose, CNS lactate, and CNS pyruvate in one patient. Blood samples were obtained daily and were analyzed enzymatically for urea by using a Hitachi 717 automatic analyzer (Boehringer Mannheim GmbH, Mannheim, Germany) at the Uppsala University Hospital Department of Clinical Chemistry.

Comparison of the Analytical Methods

Because the CNS and SC urea were analyzed with the

![Graph showing relative changes in BS urea (S-urea) calculated as the actual value/initial value ratio, plotted day by day for all patients, for the 1st week. There was a slow increase in the BS urea levels in all patients, which rose to approximately twice the initial value after 6 days. The same pattern was seen in CNS and SC urea. CVL = cerebrovascular lesion.](image-url)
Intracerebral microdialysis: urea as an endogenous reference compound

CMA/600 and the BS urea with the Hitachi 717, as a consequence the CNS/BS and SC/BS ratios were calculated from values obtained with two different analytical methods. We therefore compared these methods. Standard urea solutions of 11 different concentrations, three samples of each, were prepared. Each sample was divided into two aliquots. One of these was sent to the laboratory for routine analysis in the Hitachi 717 and the other was analyzed in the CMA/600. The values were then compared pairwise.

Imprecision data were also generated for the two analytical methods. This was done for CMA/600 by running 24 aliquots of a stock solution of urea in saturated benzoic acid (13.3 ± 0.1 mM) on six different occasions. In our tests the urea method on the CMA/600 analyzer had a total imprecision coefficient of variation of 4.9% and a within-series imprecision of 4.1%. Imprecision data for the Hitachi 717 method was 2.2 to 2.8%, obtained from routine studies conducted in the hospital laboratory.

In Vitro Recovery

After removal of the CNS probe the in vitro recovery was measured for urea in 13 patients. The probe was placed in a standard urea solution and perfused with the same perfusion fluid and at the same rate as when obtaining samples in the patients. Two dialysate samples and two samples from the standard solution were obtained and analyzed with the CMA/600. The in vitro recovery was calculated as the ratio between the urea concentrations in the dialysate and in the standard solution.

Statistical Analysis

Comparisons of urea levels in different compartments and between the two analytical methods were made using the Pearson linear correlation (Statistica for Windows; StatSoft, Inc., Tulsa, OK). The Statistica software was also used for graphic presentation of the results.

Results

Urea Levels in Serum

Four hundred fifty blood samples were analyzed for urea. There was a gradual increase in BS urea to approximately twice the basal level by the 6th day postadmission (Fig. 1). The same pattern was seen for CNS and SC urea.

Comparisons Among CNS, SC, and BS Urea

Samples obtained at the same time were compared pairwise by using the Pearson linear correlation. There was a high degree of correlation between urea levels in the different compartments (Table 1). The correlation coefficient was 0.98 for SC/BS.

Temporal Changes in the CNS/SC Ratio

Based on the similarity between SC and BS urea levels, the in vivo performance of the CNS probe was estimated by plotting the ratios for all the samples. This showed that there were no significant changes in ratios over time. Figure 2 shows examples from two patients.

Fig. 2. Graphs showing the CNS/SC urea ratios over time from two different patients. Upper: The CNS/SC urea ratio is quite stable, except for two values that were considered outliers. Lower: The CNS/SC urea ratio becomes more unstable toward the end of the measurements, and finally microdialysis was discontinued because of impaired function of the CNS probe.

Levels of CNS Urea During Brain Ischemia

One patient with a TBI developed severe problems with ICP that eventually led to a total brain infarction. During this process the CNS glucose decreased to zero and the CNS lactate/pyruvate ratio increased, indicating severe ischemia.21 However, the CNS urea remained fairly stable (Fig. 3).

Comparison of Methods for Urea Analysis

The results of the comparison between urea analyses in which the CMA/600 analyzer and Hitachi 717 were used are plotted in Fig. 4, and the correlation coefficient between the values obtained with the two methods was 0.996.

In Vitro Recovery

In vitro recovery was measured in 13 patients after removing the CNS probe. It proved to be 0.90 ± 0.13 (mean ± standard deviation).

Discussion

In this study we investigated the use of urea as an en-
had ceased. Changes and remained at similar levels after the cerebral blood flow idly increases. The CNS urea levels did not undergo any major decrease to undetectable levels while the lactate/pyruvate ratio rapidly increases. The CNS urea levels did not undergo any major changes and remained at similar levels after the cerebral blood flow had ceased.

dogenous reference compound and the measurement of the CNS/SC urea ratio for monitoring the in vivo performance of the microdialysis probes. Our main reason for testing this method was to verify that the dramatic fluctuations of energy metabolites and transmitters seen in microdialysis results represent real biological changes and are not artifacts of the microdialysis procedure. For this purpose it was required that we demonstrate that the concentrations of urea in the compartments of interest are highly correlated. Urea is almost exclusively synthesized in the liver and exits the body in the urine. Further-

**Fig. 3.** Graph showing representative CNS glucose (open circles), urea (filled squares), and lactate/pyruvate ratios (filled diamonds) in severe brain ischemia. Intracerebral microdialysis recordings were obtained in a patient with TBI who developed a total brain infarction caused by high ICP. At the time when the cerebral perfusion pressure decreases toward zero, the glucose levels decrease to undetectable levels while the lactate/pyruvate ratio rapidly increases. The CNS urea levels did not undergo any major changes and remained at similar levels after the cerebral blood flow had ceased.

sis, but, as already mentioned, this is not a valid measure of the in vivo recovery. In our material we tried to measure in vitro recovery after removing the CNS probes, but this was only feasible in 13 of 63 CNS probes for practical reasons. The average in vitro recovery of these probes was surprisingly close to the estimated in vivo recovery based on the CNS/SC urea ratio. This finding probably reflects the abundance of urea and the low perfusion flow used. Lönnroth and Jansson used a method in which the in vivo recovery was estimated from dialysate concentrations after using different concentrations of the substance of interest in the perfusion fluid. This method may not be useful for repeated calibrations in the clinical setting because the monitoring will be interrupted for several hours. In retrodialysis, described by Stähle, et al.,24 and Wang, et al.,28 the loss of a substance from the perfusion fluid to the tissue was used to calculate the recovery of the probe. Administration of a substance to an injured brain for calibration purposes is ethically questionable and this method has only been described in animal series. In our center, we have taken the approach of using ratios (for example, the lactate/pyruvate or lactate/glucose ratio) that are thought to be independent of in vivo recovery.10,20,21 A similar approach was taken by Langemann, et al.,14 who compared the levels of glucose and lactate in estimating the validity of the data. However, this approach does not provide any information on the reliability of the concentrations of other substances measured.

The concept of using urea as an endogenous reference compound, as described in the present study, seems so far to be the only method described for continuous in vivo monitoring of the CNS probe performance in which no additional procedures are required.

**Methodological Limitations**

Some of the patients were treated using hypothermia, which may change the diffusion characteristics and alter the urea concentrations in the microdialysis samples. In a study by Boris-Möller and Wieloch, the in vitro recovery of glutamate was studied at different temperatures. In the
Intracerebral microdialysis: urea as an endogenous reference compound

Intracerebral microdialysis used in the NICU often generates data with great fluctuations in the metabolite levels. To ascertain that such fluctuations reflect biological changes rather than methodological problems, there is a need for processes that enable repeated in vitro calibrations of the microdialysis probe performance. The results of our study promote the use of urea as an endogenous reference compound, providing a simple and clinically useful procedure to monitor in vivo microdialysis probe performance at the bedside.

Conclusions

Intracerebral microdialysis used in the NICU often generates data with great fluctuations in the metabolite levels. To ascertain that such fluctuations reflect biological changes rather than methodological problems, there is a need for processes that enable repeated in vitro calibrations of the microdialysis probe performance. The results of our study promote the use of urea as an endogenous reference compound, providing a simple and clinically useful procedure to monitor in vivo microdialysis probe performance at the bedside.

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401