RELEASE OF THE CEREBRAL PROTEIN S-100 INTO BLOOD AFTER REPERFUSION DURING CARDIAC OPERATIONS IN INFANTS: IS THERE A RELATION TO OXYGEN RADICAL-INDUCED LIPID PEROXIDATION?

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Global ischemic events occurring during cardiopulmonary bypass (CPB) and particularly after reperfusion have been suggested to initiate alterations in the functional and structural integrity of cerebral endothelium and related brain cells.^{1,2} An important pathogenetic factor involved in the reperfusion syndrome is the generation of oxygen-free radicals.³⁻⁵ Brain capillary endothelial cells, which form the blood-brain barrier under the influence of astrocytes, have been considered an important source of free radicals.^{4,5} Cerebral microvessels and astroglial cells are an important target for free radical-induced deteriorations as well.⁵ Thus measurement of malondialdehyde in the blood, as a marker of radical-induced lipid peroxidation, may provide information on an excessive generation of free radical-related membrane injury. Astrocytes represent a main part (50%-60%) of the brain volume.⁵ The protein S-100 (dimere $\beta\beta$ and $-\beta$) is specific for the cytoplasm of astroglial cells.⁶ In adults, S-100 is not detectable under normal conditions in the blood ($<0.2 \mu\text{g/L}$).⁶ Therefore the aim of this study was to evaluate the serum kinetics of protein S-100 in relation to possible radical-induced lipid peroxidation in infants undergoing corrective cardiac operations.

Methods. Fifty-eight neonates and infants with a median age of 10.1 months (range, 0.1-80 months) were enrolled in this study. Neonates with postnatal preoperative asphyxia (umbilical arterial pH < 0.72) were excluded. Cardiac operation was performed by means of a uniform blood perfusion method with high-flow CPB (120-130 mL/kg/min) and hypothermia, mean minimum rectal temperature 26°C (range, 15°C - 36°C). Total circulatory arrest was not used. Management of acid-base status during CPB was performed according to the alpha-stat method. Blood samples were collected from the same venous line in the superior vena cava at the defined intervals; the blood samples were treated separately

Fig 1. Concentrations of the astroglial protein S-100 and the lipid peroxidation product malondialdehyde in serum at different intervals of corrective cardiac operation in neonates and infants by means of full-flow CPB: *I*, before CPB; *II*, 5 minutes after start of CPB; *III*, 5 minutes after arterial cross-clamping; *IV*, 5 minutes after aortic declamping; and *V*, 5 minutes after the end of ultrafiltration at the end of CPB. Data shown as mean $\pm$ SEM.

immediately after collection. After centrifugation at 2°C for 5 minutes, the serum samples were stored at -70°C until analysis. The serum concentrations of S-100 were analyzed with a commercially available immunoluminometric assay kit (Sangtec Medical AB, Bromma, Sweden). For malondialdehyde,^{3,5} 100 μL of plasma, 750 μL of 150 $\mu\text{mol/L}$ phosphoric acid, 250 μL of 42 $\mu\text{mol/L}$ thiobarbituric acid, and 400 μL water were mixed and boiled in a water bath for 60 minutes. The reaction was stopped by cooling the samples on ice. The analysis of malondialdehyde was performed immediately with high-performance liquid chromatography (fluorometric detector RF-10A, column Supelcosil 150 $\times$ 4 mm LC-18-S; Shimadzu, Duisburg, Germany).

Statistical analysis. The Wilcoxon test was applied to compare differences in serum S-100 and malondialdehyde levels between the time intervals of CPB. The Spearman range-correlation test was used to evaluate possible relationships between the variables.

Results. In comparison to the pre-CPB values, a significant increase in the concentration of protein S-100 was found immediately after the start of CPB ($P = .005$; Fig 1). There was a continuous elevation of the concentration of protein S-100 in the following phases of CPB. However, the main release of protein S-100 was found in the reperfusion phase

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(phase III-V) after the declamping of the aorta ($P = .0051$) and the end of CPB ($P = .0033$; Fig 1). Parallel to the increase of protein S-100 during reperfusion, concomitant elevation of malondialdehyde was also found after the declamping of the aorta ($P = .0012$) and after the end of CPB ($P = .001$; Fig 1).

The maximal serum concentrations of protein S-100 correlated significantly to bypass time ($r = 0.7$; $P = .00073$), cross-clamping time ($r = 0.57$; $P = .0014$), and minimal rectal temperature during CPB ($r = -0.57$; $P = .0015$). The maximal malondialdehyde values correlated significantly to cross-clamping time ($r = 0.46$; $P = .004$) and minimal rectal temperature ($r = 0.40$; $P = .0076$).

Discussion. The wide variation of physiologic parameters during hypothermic nonpulsatile circuit flow followed by rewarming, hyperoxygenation and pulsatile cerebral flow may activate a number of inflammatory cascades including eicosanoid, complement, and kallikrein pathways and upregulation of adhesion molecules on neutrophils and endothelial cells.^{2,3,5,6} All these processes may be accompanied by or may induce the generation of free radicals and radical-induced lipid peroxidation as measured in this study.

Therefore it could be suggested that injured astrocytes may release the cytosolic S-100, which then penetrates through the injured endothelial barrier into the blood. This assumption is supported by the simultaneous increase of the malondialdehyde (Fig 1). The significant release of the protein S-100 into the serum in association with the nonpulsatile extracorporeal circuit and subsequent reperfusion after declamping of the aorta and the start of cardiac ejection (Fig 1) may quite possibly indicate transient increased cell membrane permeability and a possibly altered blood-brain barrier.^{5,6} Although the accumulation of malondialdehyde in the serum may reflect lipid peroxidation in other organs, the con-

comitant release of S-100 from astroglial cells might be due to lipid peroxidation at the cerebral level as well. Moreover, the blood was sampled from the same venous line that mainly drains blood from the brain. Significant contamination of malondialdehyde from other organs is unlikely, because it has a short half-life time of about 10 minutes.³

These observations may have potential therapeutic applications in neuroprotective strategies in pediatric cardiac operations. The neurologic significance of such observation could be, however, assessed only in further follow-up studies, including additional neurophysiologic and neurodevelopmental tests.^{1,2}

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