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Plasma 24S-hydroxycholesterol and other oxysterols in acute closed head injury

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Abstract

Primary objective—To determine if plasma levels of 24S-hydroxycholesterol, the primary catabolite of brain cholesterol, provide a measure of axonal damage in acute brain trauma.

Research design—Determination of plasma 24S-hydroxycholesterol in a series of persons admitted to an intensive care unit for treatment of closed head injury.

Methods and procedures—Levels of 24-S-hydroxycholesterol, 27-hydroxycholesterol, lathosterol and total cholesterol were measured in peripheral blood from 38 persons from 14–55 years of age treated by craniotomy and ventriculostomy for intractable intracerebral hypertension. Severity of brain injury was estimated by the Glasgow Coma Scale (range = 3–13, median = 6 points) and overall injury by the Injury Severity Scale (range = 10–48, median = 29). All subjects were intubated and anaesthetized with intravenous propofol. Plasma sterol levels were compared with those of age-matched control subjects.

Outcomes and results—There was no significant increase in plasma 24-S-hydroxycholesterol in subjects with head injury, but measures of peripheral cholesterol synthesis were markedly reduced as compared with values for age-matched normal control subjects.

Conclusion—Plasma 24S-hydroxycholesterol levels do not change with severe closed head injury.

Keywords

Closed head injury; 24-S-hydroxycholesterol; oxysterols

Introduction

In addition to presently available clinical scales, it would be useful to have a reliable, valid biological marker for severity of closed head injury. A number of potential cerebrospinal fluid (CSF) markers are under consideration, including amyloid β 1–42 and tau [1], proteolytically cleaved tau [2–4] and CSF alpha II spectrin breakdown products [5]. Enolase and glutamate levels have also been considered as markers, but have considerable limitations [2]. On the other hand, concentration of S100B, a calcium-binding neurotrophic protein secreted by astrocytes, is released following a variety of brain insults [6]. Elevated S100B levels have been reported following insults such as middle cerebral artery ligation in rats (CSF) [7], closed head injury in children (serum) [8] and subarachnoid haemorrhage in adults (plasma) [9].

The present study was undertaken to determine the relationship of plasma 24S-hydroxycholesterol (24S-Chol), the water-soluble metabolite of brain-synthesized cholesterol found in plasma, and severity of head injury. It was expected that the ratio of 24S-Chol to 27-hydroxycholesterol (27-OHChol), the water-soluble metabolite of peripherally synthesized cholesterol, would increase in relation to severity of brain injury.

The brain contains $\sim 23 \text{ mg gm}^{-1}$ of unesterified cholesterol (23% of the total human cholesterol pool), largely in the form of myelin, but also in cell membranes [10]. Thus, a brain of 1200 g contains $\sim 27.6 \text{ g}$ of cholesterol. The brain synthesizes virtually all of its cholesterol; there is almost no passage of exogenous cholesterol through the blood–brain barrier. Cholesterol is produced within the central nervous system by astrocytes and oligodendrocytes and shuttled to neurons by apoE and ATP cassette transporters [11]. Total brain cholesterol is relatively stable; the bulk of brain cholesterol has a half-life of 5 years [12]. The small amount of cholesterol that is continuously shed from the outer leaflet of cell plasma membranes is oxidized to 24S-Chol by the cytochrome P450 enzyme cholesterol 24S-hydroxylase (CYP46) [13] and 6–8 mg/day enters the general circulation in this form [12]. The 24S-Chol is then metabolized by the liver and excreted as bile acids. In contrast to other sterols, there seems to be no diurnal variation in plasma 24S-Chol [14].

Plasma 24S-Chol concentration varies considerably over the life cycle. At 1 year of age, mean plasma level is 385 ng mL^{-1} . Concentrations decline in the 3rd decade of life to $\sim 77 \text{ ng mL}^{-1}$ and then rise slowly to an average of 101 ng mL^{-1} in the 8th decade [15]. These changes seem largely due to the relationship between brain weight and liver volume; higher brain : liver volume ratio is associated with higher 24S-Chol levels. Liver volume increases much more than brain size after 3 years of age and diminishes more than brain size late in life. Because plasma 24S-Chol and total cholesterol concentrations correlate highly, a better index of brain 24S-Chol production may be the ratio of plasma 24S-Chol to total cholesterol [15] or to 27-OHChol, the water soluble oxidation product of peripherally synthesized cholesterol, which is conveyed to the liver and also metabolized to bile acids.

Because of the tight regulation of total cholesterol content in the brain, it is reasonable to assume that following traumatic brain injury (TBI) with interruption of plasma membranes and myelin sheaths, cholesterol released in the brain would be quickly oxidized, increasing levels of 24S-Chol in blood. Thus, an index of 24S-Chol synthesis might be related to severity of acute TBI. This study determined plasma concentrations of 24S-Chol, total cholesterol, its precursor lathosterol and 27-OHChol.

Methods and procedures

Subjects

Study subjects were persons of either sex with a diagnosis of TBI admitted to the Neurosurgical Intensive Care Unit at Parkland Memorial Hospital in Dallas, Texas who had survived for 24 hours and required ventriculostomy for treatment of refractory intracranial hypertension. All subjects were intubated and sedated with intravenous propofol. Subjects were excluded who were in shock or septic, who had a history of stroke, epilepsy or neurodegenerative disease, anoxic brain injury, concurrent spinal cord injury or central nervous system infection, including human immunodeficiency virus. Persons with extensive liver damage, those taking lipid-lowering drugs and those who had no family member available to sign informed consent if the patient was unable to do so were also excluded. Written informed consent was obtained from a family member or a legally authorized representative on forms approved by the University of Texas Southwestern Medical Center and the Parkland Memorial Hospital Institutional Review Boards. Subject information collected included demographics, past medical history, Glasgow Coma Scale [16] and Injury Severity Scale (ISS) [17] at time of study entry. Control subjects were matched for age and gender and were normal volunteers with no history of head injury with loss of consciousness and no use of lipid-lowering drugs and also signed informed consent.

At the time of ventriculostomy placement, two 7-ml samples of blood were extracted from the patient's central line already in place or from an arm vein. One sample was injected into a vacutainer with serum separating gel and clot activator and the other sample was injected into an ethylenediamine tetraacetate (EDTA) treated vacutainer. The EDTA vacutainer sample was wrapped in foil and placed on ice. Both samples were processed, divided into 1 ml cryovials, some with additives, one continuously covered with foil and all frozen at -80°C within the hour. Blood samples were drawn in a similar manner from controls. One did not attempt to assay sterols in CSF because ventriculostomy fluid was grossly bloody.

Severity measures

Impairment of brain function was quantified by the GCS, which evaluates three domains; eye opening, motor response and verbal response. Administered by ICU nurses, scores range from 3 (no eye, motor or verbal response) to 15 (fully conscious and oriented). The range of 3–8 is considered severe.

The ISS was used as a further index of severity. It is the sum of the squares of patients' three highest Abbreviated Injury Scores (range = 0–6) for injuries regardless of the body region in which they occur (head/neck, face, chest, abdomen/pelvic contents, extremity/pelvic girdle, external). Maximum score is 75 points.

Biochemical methods

Plasma 24S-Chol and 27-OHChol were quantified by isotope dilution with mass spectrometry as described elsewhere [18]. Plasma total cholesterol was measured enzymatically and lathosterol by mass spectrometry [19].

Statistical methods

Sample size calculation was based on a plasma 24S-Chol concentration of 77 ng mL^{-1} derived from age-adjusted values given in Bretillon et al. [15]. A sample size of 20 subjects was needed to detect an 80% difference and 51 subjects to detect a 50% difference in 24S-Chol concentration. A *p*-value of 0.05 was regarded as significant. Means were compared between groups by one-way ANOVA after log transformation of plasma levels of oxysterols.

and sterols. Linear regression analyses were done for the Pearson's correlations. SAS statistical software package (Statview; SAS Institute, Cary, NC) was used for data analysis.

Results

This study was able to acquire plasma samples from 39 head-injured persons and 15 controls in the period of time from May 2005–July 2006. Demographics and outcome data are presented in Table I. Subjects ranged in age from 14–55 years. GCS scores ranged from 3–13 (median = 6). ISS scores ranged from 10–48 (median = 29). All persons required endotracheal ventilation; eight persons died in the Intensive Care Unit.

Mean plasma 24S-Chol levels (72 ± 23 mg dL⁻¹) corresponded to the values ascertained by Bretillon et al. [15] for normal subjects in the age range from 19–50 years, $\sim 77 \pm 4$ mg dL⁻¹. In contrast, plasma cholesterol values were very low (112 ± 34 mg dL⁻¹) and significantly lower than controls (Table I).

Plasma 27-OHChol levels showed a significant inverse correlation with ISS scores ($r = -0.40$, $p = 0.01$), as did ratios of plasma 27-hydroxycholesterol to total plasma cholesterol ($r = -0.42$, $p = 0.009$), while the ratio of plasma 24S- to 27-OHChol increased with ISS scores ($r = 0.41$, $p = 0.01$). On the other hand, there was no significant correlation between plasma 27-OHChol levels, the ratio of plasma 27-Chol to plasma total cholesterol or the ratio of 24S to 27-OHChol and GCS scores.

Discussion and conclusion

The stimulus for this study was the observation that plasma 24S-Chol is elevated in persons with Alzheimer's disease and vascular dementia as compared with normal controls and persons with depression [20]. In the foregoing study, elevated plasma 24S-cholesterol was seen as a reflection of elevated plasma cholesterol in apolipoprotein E4 carriers, but one was unable to confirm this relationship in a subsequent study [21]. Decreasing plasma concentration of 24S-Chol with increasing dementia severity suggested that elevated 24S-cholesterol levels might accompany the early stage of neurodegenerative disease, when cell membrane damage might be greatest and brain cholesterol turnover the highest [20]. Lower levels of 24S-Chol in later stages might reflect decreased substrate. This led one to postulate that elevated 24S-cholesterol might be elevated early in the course of TBI. It was chosen to sample venous blood rather than CSF because it was thought that outside of a setting in which ventriculostomy provides direct access to CSF in cases of closed head injury, venepuncture would be a less dangerous diagnostic procedure than lumbar puncture. The patient sample was limited to persons admitted to hospital with very severe head injuries, but this seemed the ideal group in which to test the hypothesis and persons with lesser degrees of injury were seldom hospitalized. Despite severe head injury with intracranial hypertension, there was no evidence of increased brain cholesterol catabolism as measured by plasma levels of 24S-chol. Holdenreider et al. [22], who followed six subjects with acute ischemic strokes, also found no increase in plasma 24S-Chol or 27-OHChol in patients followed for 4–10 days. The inverse relationship of plasma 27-Chol with ISS scores parallels the known decrease in persons suffering severe trauma of both plasma total cholesterol [23] and the cholesterol precursor lathosterol [24]. Thus, the finding of an inverse relationship between 27-Chol and severity of injury scores is probably related to the decreased total body cholesterol production in the patients in this time of high metabolic demand. The decreased ratio of 27-Chol to cholesterol might be explained as reflecting a decrease in catabolism of pre-existing cholesterol. The differences in 24S-Chol and 27-OHChol and total cholesterol are explainable by the markedly lower plasma cholesterol in these subjects. Measurement of CSF cholesterol would not have been possible because of

the presence of blood. Plasma cholesterol concentrations would not have been a suitable marker because any increase in direct release of unoxidized cholesterol from the brain into the bloodstream through the damaged blood–brain barrier would have been masked by the dramatic drop in peripheral cholesterol synthesis.

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Table I

Levels of plasma oxysterols in subjects with closed head injury vs. controls.

Number of subjects (F/M)	Cases 12/26		Controls 6/9	
	Mean $\pm$ SD	Median	Mean $\pm$ SD	Median
Age (years)	28 $\pm$ 11	24	29 $\pm$ 7	26
Body Mass Index (kg m ⁻²)	25.0 $\pm$ 3.9	24.6	25.3 $\pm$ 3.8	24.3
Injury Severity Score	27.7 $\pm$ 11.3	29	—	—
Glasgow Coma Scale	5.7 $\pm$ 3.0	6	—	—
<i>Sterol levels</i>				
Total cholesterol (mg dL ⁻¹)	112 $\pm$ 34 ^a	103	179 $\pm$ 25	179
24-S-Hydroxycholesterol (ng ml ⁻¹)	72 $\pm$ 23	70	71 $\pm$ 25	66
27-Hydroxycholesterol (ng ml ⁻¹)	205 $\pm$ 89	178	200 $\pm$ 78	187
Lathosterol (mg dl ⁻¹)	0.13 $\pm$ 0.09	0.11	0.16 $\pm$ 0.07	0.13
<i>Ratios of oxysterols and total cholesterol</i>				
24-S-hydroxycholesterol/total cholesterol	0.68 $\pm$ 0.24 ^a	0.62	0.40 $\pm$ 0.14	0.37
27-hydroxycholesterol/total cholesterol	1.83 $\pm$ 0.57 ^a	1.66	1.12 $\pm$ 0.40	1.05
24-S-hydroxycholesterol/27-hydroxycholesterol	0.39 $\pm$ 0.16	0.36	0.38 $\pm$ 0.13	0.35
Lathosterol/total cholesterol	0.13 $\pm$ 0.09	0.11	0.16 $\pm$ 0.07	0.13

^aSignificantly different from controls ($p < 0.0001$, ANOVA with Fisher's PLSD *post-hoc* test).