Side chain oxidized oxysterols in cerebrospinal fluid and the integrity of blood-brain and blood-cerebrospinal fluid barriers

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Abstract The side chain oxidized oxysterol 24*S*-hydroxycholesterol (24-OH-chol) is formed almost exclusively in the brain, and there is a continuous passage of this oxysterol through the circulation to the liver. 27-Hydroxycholesterol (27-OH-chol) is produced in most organs and is also taken up by the liver. The 27-OH-chol-24-OH-chol ratio is about 0.1 in the brain and about 2 in the circulation. This ratio was found to be about 0.4 in cerebrospinal fluid (CSF) of asymptomatic patients, consistent with a major contribution from the circulation in the case of 27-OH-chol. In accordance with this, we demonstrated a significant flux of deuterium labeled 27-OH-chol from plasma to the CSF in a healthy volunteer. Patients with a defective blood-brain barrier were found to have markedly increased absolute levels (up to 10-fold) of both 27-OH-chol and 24-OH-chol in CSF, with a ratio between the two sterols reaching up to 2. There was a significant positive correlation between the levels of both oxysterols in CSF and the albumin$_{CSF}$-albumin$_{plasma}$ ratio. The 27-OH-chol$_{CSF}$-24-OH-chol$_{CSF}$ ratio was found to be about normal in patients with active multiple sclerosis and significantly increased in patients with meningitis, polyneuropathy, or hemorrhages. Results are discussed in relation to the possible use of 24-OH-chol$_{CSF}$ as a surrogate marker of central nervous system demyelination and/or neuronal death.—Leoni, V., T. Masterman, P. Patel, S. Meaney, U. Diczfalusy, and I. Björkhem. **Side chain oxidized oxysterols in cerebrospinal fluid and the integrity of blood-brain and blood-cerebrospinal fluid barriers. *J. Lipid Res.* 2003. 44: 793–799.**

Supplementary key words 24*S*-hydroxycholesterol • 27-hydroxycholesterol • neurodegeneration • demyelination

24*S*-Hydroxycholesterol (24-OH-chol) is almost exclusively formed in the brain, where it is present in greater amounts than in any other organ (8.6–15.1 ng/mg wet weight) (1). There is a daily flux of about 7 mg of this oxysterol from the brain to the circulation, with the majority of this efflux apparently occurring as direct transport across the blood-brain barrier (BBB) (2). It has been estimated that less than 1% of the 24-OH-chol produced by the brain is transported to the circulation via passage through the cerebrospinal fluid (CSF) (1). The enzyme responsible for the 24*S*-hydroxylation of cholesterol is a member of the cytochrome P450 superfamily (designated CYP46), and has been mainly localized to neurons (3). Assuming that the expression of this enzyme across the neuronal population is relatively stable, any loss of these cells would result in a decreased production of this oxysterol. In accordance with this, reduced levels of plasma 24-OH-chol have been observed in connection with several chronic neurological conditions known to affect the number of neurons (4).

In contrast to the above oxysterol, 27-hydroxycholesterol (27-OH-chol) is formed in most cells, and there is a constant flux of this oxysterol from extrahaepatic tissues to the liver (5, 6). A noteworthy exception is the brain (no net flux has been observed from this organ) and the levels of 27-OH-chol are about 10-fold lower than those of 24-OH-chol (1). However, the plasma levels of 27-OH-chol are about twice those of 24-OH-chol.

The term BBB is commonly used to describe a whole range of mechanisms that regulate and protect the internal environment in the brain (7–11). More specifically, this term is used to indicate a relative restriction of the entry of the plasma proteins into the brain. This barrier acts as a diffusion restraint to an extent that depends on molecular size and lipid solubility. Contributing to this selec-

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Abbreviations: BBB, blood-brain barrier; CSF, cerebrospinal fluid; MS, multiple sclerosis; 24-OH-chol, 24*S*-hydroxycholesterol; 27-OH-chol, 27-hydroxycholesterol; Q_{Alb}, albumin$_{CSF}$-albumin$_{plasma}$ ratio.

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tivity are the tight junctions between the endothelial cells (BBB) and the tight junctions at the apices of the epithelial cells of the choroid plexuses (blood-CSF barrier). Many diseases and injuries of the central nervous system (such as stroke, tumors, autoimmunity, infection, and traumatic brain injury) are accompanied by BBB disruption, resulting in secondary damage to neurons (12, 13).

Due to the distinct cerebral and extracerebral origins of the majority of 24-OH-chol and 27-OH-chol, respectively, the ratio of these oxysterols in the CSF may reflect the functionality of BBB and blood-CSF barriers. If this ratio is similar to that in the brain, then it is unlikely that there is a significant passage of 27-OH-chol into the CSF. Conversely, a compromised functionality of BBB or blood-CSF barriers could probably result in an increase in this ratio, as 27-OH-chol is "trapped" in the CSF.

In the present work, we have measured the absolute levels of these side chain oxidized oxysterols in the circulation and CSF of patients with a documented dysfunction of the BBB and blood-CSF barriers. It is demonstrated that the levels of both oxysterols are increased in direct proportion to the severity of the dysfunction of the two barriers. Evidence is also presented that most of the 27-OH-chol present in CSF under normal conditions is derived from the circulation.

MATERIALS AND METHODS

Infusion of hexadeuterated cholesterol to a healthy volunteer

The experimental conditions for this infusion have been previously described (14). Briefly, phospholipid liposomes enriched with 10 g of [26,26,26,27,27,27-$^2H_6$]cholesterol were slowly infused into a healthy, normolipidemic male (age, 58; weight, 84 kg; BMI, 25). Infusion of the mixture did not produce any obvious adverse effects, and there was no effect on plasma levels of transaminases or alkaline phosphatase during the infusions. A single CSF was taken by lumbar puncture 3 days after the start of the infusion, and analyzed for the deuterium enrichment as previously described (14).

Selection of patient groups

Plasma and CSF samples were collected for routine diagnostic purposes by the Division of Neurology, Huddinge University Hospital. After determination of albumin and immunoglobulin G (IgG) levels, the excess sample material was frozen at −20°C or lower. Samples were stored for not longer than 3 years before analysis. These storage conditions have not been shown to affect the levels of the side chain oxidized oxysterols under investigation.

In a previous study (15), we investigated the plasma levels of 24-OH-chol in a heterogeneous group of multiple sclerosis (MS) patients (n = 118) at various stages of the disease. From this a subgroup (n = 49; mean age, 41 years; female-male, 33:15) was selected based on the fulfilment of the following criteria for inactive disease: *i*) "normal" $albumin_{CSF}$-$albumin_{plasma}$ ratio (Q_{Alb}) ($<8 \times 10^{-3}$); *ii*) normal levels of 24-OH-chol in the plasma ($\leq$120 ng/ml) and CSF ($\leq$3.5 ng/ml), as defined by ref. 15; *iii*) age $\leq$60 years; *iv*) absence of gadolinium-enhancing lesions on the MRI investigation immediately preceding or following plasma and CSF collection. This group was designated Controls.

A second population of patients (n = 92; mean age, 50 years; female-male, 60:32) were selected primarily on the basis of an increased Q_{Alb}, greater than 8×10^{-3}, which is strongly suggestive of a BBB and blood-CSF barrier dysfunction (16, 17). These patients were designated BBB group, collectively. They were subgrouped subsequently according to diagnosis upon release from the hospital as follows: the subgroup of patients with demyelinanting polyneuropathy (i.e., Guillain-Barré syndrome or chronic inflammatory demyelinanting polyneuropathy) (n = 17) was designated Polyneuropathy; the subgroup of patients with headaches of uncertain etiology and an increased Q_{Alb}, (n = 12) was designated Headache; the subgroup of patients with confirmed subarachnoid hemorrhage (n = 6) was designated SAH; while those with confirmed meningitis (n = 13) were designated Meningitis. Patients with relapsing remitting (n = 20) and primary progressive (n = 1) MS and with an increased Q_{Alb} comprised the group designated Alb-MS. The BBB group also included patients with the following diagnoses: nondemyelinating polyneuropathies of heterogeneous origins (n = 10), motor neuron disease (n = 3), Bell's palsy (n = 4), fatigue syndrome (n = 1), Wegener's granulomatosis (n = 1), general myalgia (n = 1), vitamin-B_{12} deficiency n = 1), cervical myelitis (n = 1), and cranial mononeuropathy (n = 1).

A third group, also selected from the previously characterized MS population (15), was comprised of MS patients with an active disease (MS active) (n = 20; mean age 37 years; female-male, 13:7), as indicated by fulfilment of one or both of the following criteria: *i*) presence of gadolinium enhancing lesion on MRI; *ii*) plasma levels of 24-OH-chol greater than 2 SD above those of age-matched controls (15, 18).

Analytical methods

Levels of 24-OH-chol and 27-OH-chol in plasma were determined by isotope dilution-mass spectrometry essentially as previously described (19). Minor modifications were introduced in order to reliably analyze these oxysterols in CSF samples. Briefly, 10 ng of [$^2H_3$]24-OH-chol and 10 ng of [$^2H_4$]27-OH-chol were added to 500 μl of CSF together with 10 μl of butylated-hydroxytoluene (5 mg/ ml) and 20 μl of EDTA (10 mg/ ml). Samples were hydrolyzed and extracted as previously described (19).

Statistical calculations

For each sample, the levels of 24-OH-chol and 27-OH-chol were determined, and the 27-OH-$chol_{CSF}$-24-OH-$chol_{CSF}$ ratio (R 27-24 CSF) and Q_{Alb} quotient calculated. Data were evaluated statistically using the two-tailed paired Student's *t*-test, the Mann-Whitney rank-sum test, and parametric and nonparametric ANOVA.

Ethical aspects

All experiments involving the human volunteers were approved by the Ethics Committee at Huddinge University Hospital (approval no. 346/99), and informed consent was obtained. The same committee also approved all the investigations with the patients suffering from neurological diseases (approvals no. 488/98, 303/99, 274/01). The latter studies were performed on plasma and CSF primarily collected for diagnostic purposes.

Excess of this frozen material was allowed to be utilized for the above investigation after first removing patient identity except for information concerning age, gender, and diagnosis.

RESULTS

Evidence for a flux of 27-OH-chol from the circulation to the CSF in a volunteer

The relatively high ratio between 27-OH-chol and 24-OH-chol in CSF (~0.4) compared with that in the brain

TABLE 1. Levels of 24*S*-hydroxycholesterol and 27-hydroxycholesterol in plasma and cerebrospinal fluid, $albumin_{CSF}$-$albumin_{plasma}$ quotients, and 27-hydroxycholesterol$_{CSF}$-24*S*-hydroxycholesterol$_{CSF}$ ratio in Control and blood-brain barrier group

	n	Q_{Alb}	24-OH-Chol Plasma	27-OH-Chol Plasma	24-0H-Chol CSF	27-0H-Chol CSF	R 27-24 CSF
		$\times 10^{-3}$		*ng/ml*			
Control	49	5.5 ± 0.2	75.7 ± 2.4	116 ± 5.4	1.96 ± 0.1	0.76 ± 0.1	0.42 ± 0.1
BBB group	92	17.3 ± 1.4	66.3 ± 2.2	129 ± 5.0	2.79 ± 0.3	1.96 ± 0.2	0.75 ± 0.1

BBB, blood-brain barrier; CSF, cerebrospinal fluid; 24-OH-chol, 24*S*-hydroxycholesterol; 27-OH-chol, 27-hydroxycholesterol; R 24-27 CSF, 27-hydroxycholesterol$_{CSF}$-24*S*-hydroxycholesterol$_{CSF}$ ratio; Q_{Alb}, $albumin_{CSF}$-$albumin_{plasma}$. Data are shown as mean ± SEM.

(~0.1) suggests that there is a flux of 27-OH-chol from the circulation into the CSF (1). In order to confirm this, we used material from a previously described experiment in which a healthy volunteer was infused with hexadeuterated cholesterol (14). Three days after the start of infusion, when the deuterium enrichment of plasma cholesterol was maximal (~13%), a sample of CSF was collected and analyzed in parallel with the plasma sample.

In accordance with the previous work (14), there was no significant incorporation of ^2H in 24-OH-chol, suggesting that this oxysterol is formed from brain cholesterol.

The observed deuterium enrichment of CSF 27-OH-chol was about 4%. A similar degree of enrichment was found in the free fraction of 27-OH-chol in a matching plasma sample (S. Meaney, unpublished observations). At this time point, the deuterium enrichment of total plasma 27-OH-chol was about 3% (14).

Attempts to define the CSF levels of 24-OH-chol and 27-OH-chol in a normal population

Table 1 summarizes the results of measurements of plasma and CSF levels of 24-OH-chol and 27-OH-chol in a selected population of MS patients (n = 49) with inactive disease designated Controls. The levels of 24-hydroxycholesterol were 76 ± 2 ng/ml in plasma and 1.96 ± 0.1 ng/ml in CSF. The levels of 27-OH-chol were 116 ± 5 ng/ml in plasma, and 0.76 ± 0.1 ng/ ml in CSF. No statistically significant differences with regards to age or sex were found. There was, however, a low but statistically significant correlation between plasma and CSF levels of both 24-OH-chol ($P < 0.05$) and 27-OH-chol ($P < 0.01$) ($P <$ 0.05 was statistically significant).

Levels of 24-OH-chol and 27-OH-chol in plasma and CSF of patients with a compromised BBB

Table 1 summarizes results of measurements of plasma and CSF levels of 24-OH-chol and 27-OH-chol in patients with varying defects in the BBB (BBB group). Interestingly, no statistically significant differences were found when plasma levels of 24-OH-chol and 27-OH-chol in the group with a defective BBB were compared with those in Controls (Student's *t*-test); however, CSF levels of both oxysterols were significantly higher in the BBB group ($P <$ 0.01 and $P < 0.001$, respectively, Student's *t*-test). Furthermore, there was a highly significant correlation between 24-OH-chol and 27-OH-chol in CSF of the BBB group ($r =$ 0.74; $P < 0.001$; **Fig. 1**). As shown in **Fig. 2A** and **B**, there was a highly significant correlation between the levels of both oxysterols in CSF and the Q_{Alb}. No statistically significant correlations were found between the R 27/24 CSF and Q_{Alb} in either the Control or the BBB group.

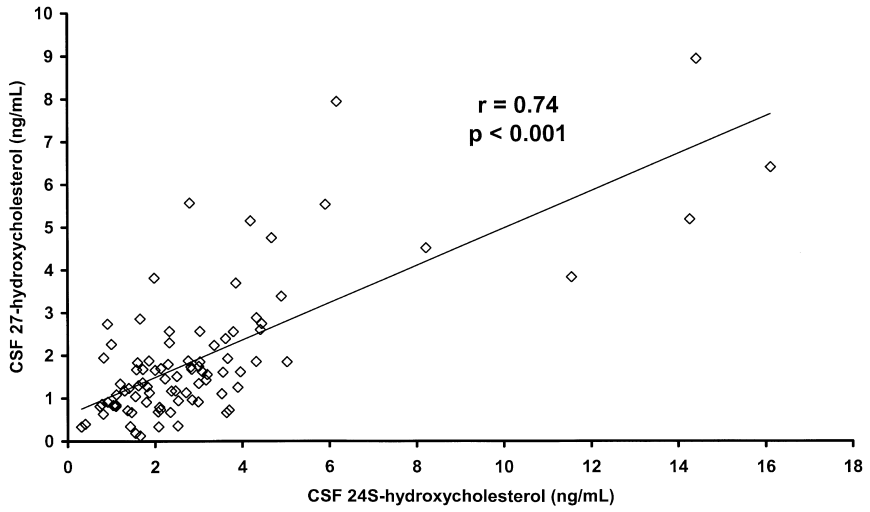

Fig. 1. Correlation between cerebrospinal fluid (CSF) levels of 24*S*-hydroxycholesterol (24-OH-chol) and 27-hydroxycholesterol (27-OH-chol) in the patients with increased $albumin_{CSF}$-$albumin_{plasma}$ quotient (Q_{Alb}) ($>8 \times 10^{-3}$).

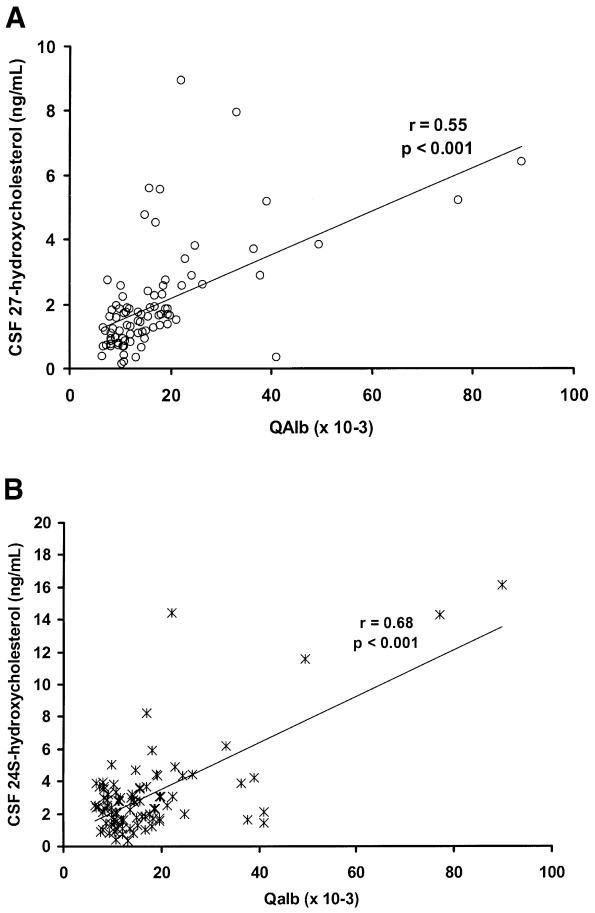

Fig. 2. Correlation between Q_{Alb} and CSF 27-OH-chol (A), and Q_{Alb} and CSF 24-OH-chol (B). Q_{Alb} is an accepted marker for blood-brain barrier dysfunction.

Application of the Mann-Whitney rank-sum test, as required by the nonparametric distribution of the values, revealed a statistically significant increase ($P < 0.001$) in the case of the R 27/24 CSF in the BBB group.

Levels of 24-OH-chol and 27-OH-chol in CSF in the diagnostic subgroups

The levels of 24-OH-chol and 27-OH-chol in the different diagnostic groups are reported in **Table 2**. No statistically significant differences were found between plasma levels of 24-OH-chol and 27-OH-chol in each group (one-way ANOVA). However, the CSF levels of 24-OH-chol were significantly higher in members of the MS-Alb, MS active, Meningitis, and Polyneuropathy subgroups than in the Controls, Headache, and SAH groups. The CSF levels of 27-OH-chol were higher in the Meningitis, Polyneuropathy, and SAH subgroups than in both Controls and the MS subgroups (one-way ANOVA). Probability values for comparison of levels of 24-OH-chol and 27-OH-chol in Controls and the various disease subgroups are also illustrated in Table 2.

Statistically significant correlations ($P < 0.05$) between 24-OH-chol and 27-OH-chol CSF levels were found in some of the different diagnostic subgroups. Interestingly, no such correlation was found in case of the Meningitis, MS active, and Headache subgroups.

The 27-OH-chol$_{CSF}$-24-OH-chol$_{CSF}$ ratio in the different diagnostic subgroups

The R 27/24 CSF was calculated in all the diagnostic groups. Results expressed as a box-and-whisker plot are shown in **Fig. 3**. The change in the ratio between the different groups is readily apparent.

Table 2 also shows probability values for comparison of the R 27/24 CSF in Controls and the various disease subgroups. After application of Kruskal-Wallis one-way ANOVA with pair-wise multiple comparison procedures (Dunn's Method) for the not-parametrical distribution of the values, a significant increase ($P < 0.05$) was observed in the ratios of patients of SAH, Meningitis, Headache, and Polyneuropathy subgroups compared with the ratio in Controls. Otherwise, no significant differences were found between controls and the two different MS groups.

DISCUSSION

Methodological commentary

A general concern with studies on CSF is obtaining suitable control material. In the present study, we used material from MS patients with clinically quiescent disease as defined by the criteria described in Materials and Meth-

TABLE 2. Absolute levels of 24-OH-chol and 27-OH-chol and the different ratios in the different diagnostic subgroups

Group	n	Q_{Alb}	24-OH-Chol Plasma	24-OH-Chol CSF	CSF 24-OH-Chol ANOVA[a]	27-OH-Chol Plasma	27-OH-Chol CSF	CSF 27-OH-Chol ANOVA[a]	R 27/24 CSF	CSF R 27/24 ANOVA[b]
		$\times 10^{-3}$	*ng/ml*		*P*	*ng/ml*		*P*		*P*
Headache	12	13.4 ± 1	60.9 ± 5.0	1.79 ± 0.2	NA	143 ± 11.5	1.16 ± 0.1	↑	0.72 ± 0.07	↑
Meningitis	11	15.2 ± 1.4	64.6 ± 5.2	3.21 ± 0.6	↑↑	129 ± 10.2	2.64 ± 0.5	↑↑	0.88 ± 0.17	↑
Hemorrhage	6	17.4 ± 5.3	62.9 ± 6.7	1.34 ± 0.2	NA	218 ± 42.9	1.79 ± 0.4	↑	1.43 ± 0.34	↑
Polyneuropathy	17	28.3 ± 5.6	69.4 ± 4.9	5.9 ± 1.3	↑↑	126 ± 10	3.3 ± 0.6	↑↑	0.68 ± 0.07	↑
MS-albumin	21	13.3 ± 2.2	79.4 ± 6.0	2.71 ± 0.2	↑↑	125 ± 9.2	1.08 ± 0.2	NA	0.39 ± 0.06	NA
MS active	20	5.2 ± 0.3	88.9 ± 6.3	2.82 ± 0.2	↑↑	100 ± 5.1	0.74 ± 0.1	NA	0.28 ± 0.05	NA

MS, multiple sclerosis; NA, no statistically significant difference. Arrows indicate statistically significant increases compared with Controls (↑, $P < 0.05$; ↑↑, $P < 0.001$).

[a] One-way ANOVA.

[b] Kruskal-Wallis one-way ANOVA on ranks.

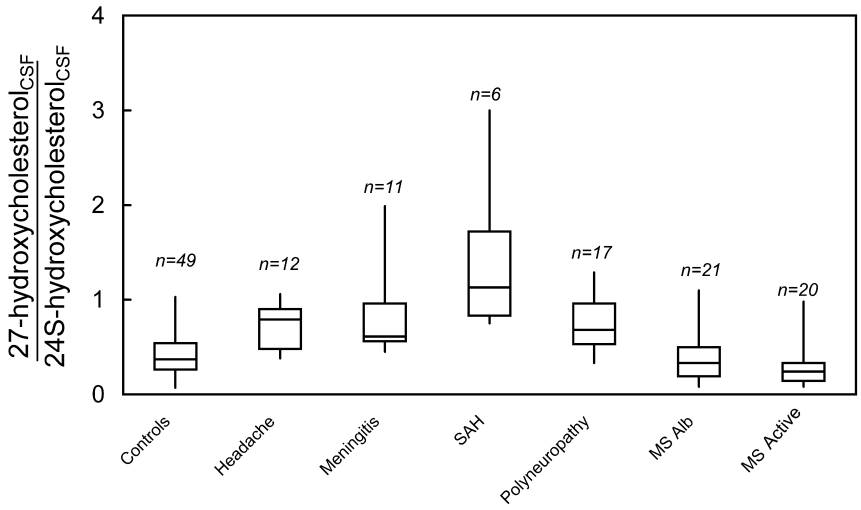

Fig. 3. Ratio 27-OH-chol-24-OH-chol in CSF of patients with different neurological diseases. A nonparametric approach was adopted. The boxes correspond to the first and third quartiles with the median indicated. The vertical lines correspond to the highest and lowest value. Significantly increased levels of the ratio ($P < 0.05$) were observed in case of SAH, Meningitis, Headache, and Polyneuropathy subgroup patients compared with the Controls. Otherwise, no significant differences were found between Controls and the two different multiple sclerosis groups. (Kruskal-Wallis one-way ANOVA on ranks).

ods. In the context of this study, these individuals represent a (probably heterogeneous) group with normal plasma levels of 24-OH-chol and a normal Q_{Alb} ratio. In view of the fact that albumin is exclusively derived from the circulation, Q_{Alb} is a widely accepted indicator for the BBB function, including CSF flow rate (16, 17). With regard to the CSF-protein concentration, a blood-CSF dysfunction means a decreased CSF flow rate from: *i*) reduced CSF production rate; *ii*) restricted flow in subarachnoid space; and *iii*) restricted passage through arachnoidal villi (16, 17).

The present study is complicated by the fact that the levels of 27-OH-chol, the dominating oxysterol in human circulation, are extremely low in CSF (~1 ng/ml). However, using the optimized procedure described in the present study, it was possible to measure these levels with sufficient precision and accuracy to enable robust comparisons between the different subject groups.

Evidence for entry of plasma 27-OH-chol into the CSF

The finding that the R 27/24 CSF is increased about 4-fold compared with that in the brain, and that it is decreased by a similar degree compared with the ratio in the circulation, may suggest that most of CSF 27-OH-chol originates from the circulating pool. In accordance with this, a statistically significant correlation ($P < 0.01$) between 27-OH-chol in the plasma and the CSF was observed. Furthermore, it was possible to demonstrate that, after infusion of hexadeuterated cholesterol into a healthy volunteer, the deuterium enrichment of total CSF 27-OH-chol was approximately equal to that of the free fraction of plasma 27-OH-chol. It should be emphasized that it has previously been demonstrated that there is no significant enrichment of CSF 24-OH-chol at this time point, and that the enrichment in CSF-cholesterol is very low, near the detection limit (0.5%) (14). Taken together, these results support the idea that in CSF the major portion of 27-OH-chol, but not of cholesterol or 24-OH-chol, originates from the circulation. It is evident from our previous work (14) that all or almost all of the 24-OH-chol originally comes from the brain. It is not possible to exclude that some of the 24-OH-chol found in CSF may come from the circulation, in particular in patients with increased Q_{Alb}. Interestingly, in the case of a net contamination of CSF by plasma (as in subarachnoid hemorrhage), the 24-OH-chol levels tended to be reduced. Although a return of the 24-OH-chol from blood into CSF may be possible, this is not likely to significantly affect the levels of this oxysterol in CSF.

A consequence of the dominating extracerebral origin of CSF 27-OH-chol is that a defect in the BBB should be expected to lead to an increased entry of plasma 27-OH-chol into the CSF, with a resulting increase in the R 27/24 CSF. A clear increase in the absolute concentration of 27-OH-chol ($r = 0.55$, $P < 0.001$, Fig. 2A), in concert with an increased Q_{Alb}, was observed. Surprisingly, CSF levels of 24-OH-chol were also increased ($r = 0.68$, $P < 0.001$, Fig. 2B) in those subjects. If this increase was a consequence of plasma oxysterols leaking into the CSF, the R 27/24 CSF would be expected to approach that of plasma. However, in the majority of individuals with a defective BBB, this was not the case, indicating that most of the CSF 24-OH-chol originates from the brain.

The highly significant correlations observed between 27-OH-chol, 24-OH-chol, and Q_{Alb} suggest that the BBB dysfunction, and consequently the alteration of the CSF flow rate (16, 17), are important determinants of CSF oxysterols levels. The possibility that the CSF levels of 24-OH-chol may also increase in patients with an intact blood-CSF barrier (i.e., normal Q_{Alb}) was illustrated here

in the MS patients with normal Q_{Alb} (MS active). Our findings suggest that CSF 24-OH-chol levels are affected by: *i*) CSF-flow rate; *ii*) massive release from damaged neuronal cells and/or myelin; and *iii*) increased permeability of the BBB as consequence of inflammation or direct barrier damage.

A possible alternative explanation is that the increase in the concentrations of plasma-derived lipoproteins as a result of the decreased CSF flow rate, may result in increased capacity to extract 24-OH-chol from the brain. In view of the relation between molecular size and flux over the BBB, and given the size of a typical lipoprotein particle, this is only likely to occur in situations with a massive loss of BBB integrity and functionality.

However, as the amounts of 24-OH-chol leaving the brain via the CSF are typically only about 0.1% of the total output from the brain (1), it is unlikely that the total flux of oxysterol would be significantly affected by a blood-CSF barrier dysfunction. In accordance with this, the plasma levels of 24-OH-chol were found to be approximately normal, even in individuals with markedly increased CSF levels of this oxysterol.

Very recently, some features of the flux of 24-OH-chol across an in vitro model of the BBB were described (20). Using cultures of porcine brain capillary endothelial cells, Panzenboeck et al. demonstrated that this flux appears to be independent of ABCA1 and influenced to some extent by the scavenger receptor SR-B1. The efflux of 24-OH-chol was found to have a stimulatory effect on the apoA-I mediated secretion of cholesterol from these cells. Whether or not these effects are of importance in vivo is difficult to judge at present.

Is determination of the levels of 24-OH-chol, 27-OH-chol, and 24-OH-chol-27-OH-chol in CSF of diagnostic value?

The significantly higher CSF levels of the oxysterols found in the different diagnostic subgroups may give some important diagnostic information (see Table 2). In case of SAH there is a massive disruption of the BBB with a direct flux of blood into the subarachnoid space. As could be expected, in this subgroup we found significantly increased CSF levels of 27-OH-chol with normal or slightly reduced levels of the 24-OH-chol. Consequently, the 27-OH-chol$_{CSF}$-24*S*-hydroxcholesterol$_{CSF}$ ratio was high, and in some of these patients approached that in plasma. Patients in the Meningitis subgroup would be expected to have an increased flux of 27-OH-chol over the BBB with less effect on the release of 24-OH-chol from the brain into the CSF. In accordance with this, the R 27/24 CSF was higher in the Meningitis subgroups than in Controls. In patients of the Headache subgroup, there was a similar increase in the R 27/24 CSF. In this case, the increase in ratio was almost exclusively due to a selective increase in the levels of 27-OH-chol with normal levels of 24-OH-chol, as predicted from the observed blood-CSF barrier dysfunction. No differences were found with respect to 24-OH-chol levels in MS-Alb and MS active, but both subgroups had significantly higher values than Controls. The increased levels of 24-OH-chol are likely to reflect demyelination. Otherwise, there were no significant differences between levels of 27-OH-chol in these three subgroups. In the patients in the Polyneuropathy subgroup, markedly increased levels of oxysterols in CSF were found, with an increased R 27/24 CSF. These findings are consistent with the fact that these patients had the most severe dysfunction in the blood-CSF barrier in BBB, as reflected in the markedly increased Q_{Alb}. In addition, they may have a massive degeneration of myelin and neurons at the nerve roots, which could contribute to the increased CSF levels of 24-OH-chol.

In a very recent article (21), elevated levels of 24-OH-chol were observed in CSF from patients with Alzheimer's disease. It was suggested that this might be due to increased neurodegeneration with increased leakage of 24-OH-chol into the CSF.

It is evident from the present results, however, that absolute and relative levels of 24*S*- and 27-OH-chol in CSF must be evaluated in relation to presence or absence of a defect in the BBB. At least part of the increased levels may be a result of a defect in this barrier, which seems to be a frequent finding in several neurological diseases (including Alzheimer's disease) (22). The extent of barrier dysfunction must, however, be defined by other parameters, e.g., by evaluating the Q_{Alb}.

Further investigations to explore the diagnostic potential of oxysterols in various neurological diseases are in progress.

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