Oxidized Low-Density Lipoprotein and Paraoxonase Levels in Matched Serum and Cerebrospinal Fluid from Patients with Aneurysmal Subarachnoid Hemorrhage: Preliminary Results

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The role of oxidative modification of low-density lipoprotein (Ox-LDL) and protective features of paraoxonase (PON1) in a variety of the central nervous system diseases have been extensively investigated; however, levels of molecules mentioned above in either serum or cerebrospinal fluid (CSF) in patients with aneurysmal subarachnoid hemorrhage (SAH) have not been studied.

The levels of Ox-LDL and PON1 in both serum and CSF were measured within the first three days and on day seven of post-SAH in 10 consecutive patients and the results were compared to ten patients with normal pressure hydrocephalus without any other known central nervous system diseases.

Statistically significant difference was noted in CSF levels of both Ox-LDL and PON1 between the patients and control.

Increased levels of both Ox-LDL and PON1 measured during the acute stage of SAH might have a role in the development of vascular complications seen after SAH.

Key Words: aneurysms, oxidized LDL, paraoxonase, subarachnoid hemorrhage, vasospasm.

Several lines of evidence during the last 10 years have suggested that oxidized low-density lipoprotein (Ox-LDL) has a central role in the initiation and acceleration of atherosclerosis and some other diseases.[34,37,39] This molecule exerts its pathogenic effect on the vascular structures by increasing synthesis and/or secretion of adhesion molecules, monocyte chemotaxis, cytotoxicity of endothelial cells, and proliferation of smooth muscle cells.[4,33] Ox-LDL has been detected in atherosclerotic lesions of human and rabbits.[41]

Paraoxonase (PON1) is a high-density lipoprotein (HDL)-related enzyme and its activities are reduced in some inflammatory-based diseases, including hypercholesterolemia, diabetes, and cardiovascular diseases[19,20,37]. It is strongly believed that PON1 protects LDL, as well as HDL from oxidation that is induced by free radical generator or reactive oxygen species (ROS) that are also produced after subarachnoid hemorrhage (SAH).[3,9,22] Protection against LDL oxidation by PON1 may be the result of an interaction between PON1's free sulfhydryl group and specific oxidized lipids in Ox-LDL.[3]

It has been demonstrated that ROS is increased in SAH patients due to erythrocyte lyses in the subarachnoid

space and is important for the development of cerebral vasospasm (VS).[16,27] Therefore, after SAH increased ROS may cause the formation of Ox-LDL, which in turn may play role in the development of VS. In the present study, we measured Ox-LDL and PON1 in both CSF and serum of patients with 10 consecutive SAH after an aneurysm rupture and the levels compared to 10 patients with normal pressure hydrocephalus without any other known central nervous system diseases. This is the first study demonstrating preliminary results of Ox-LDL and PON1 in patients with SAH.

Materials and Methods

Patients

The study had approval from the Human Investigations Committee at Istanbul University and written informed consent (consent obtained from next of kin of patients incapable of giving informed consent) was required for participation in this study. We studied the patients referred to our neurosurgical unit with SAH established by computerized tomography (CT). We excluded the patients if who had any kind of infection, in which Ox-LDL and/or PON1 may play a part, at the time of CSF and serum collection. The inclusion criterion was the admission of the patients to our unit within the first three days of SAH.

Demographics of Patients and Control Group

This study included 10 consecutive patients with aneurysmal SAH and 10 control patients, seven of whom had normal pressure hydrocephalus and three had hydrocephalus caused by aqueductal stenosis without any other known central nervous system diseases. The average age was 51.7 ± 16.7 and 50.0 ± 24.4 years in patients with SAH and control group, respectively. A summary of demographic data of the SAH patients is provided in Table 1.

Specimen Handling

For each patient, serial blood and CSF samples at the same time were collected within three days, and on day seven of SAH. Blood and CSF samples were collected via venipuncture and lumbar puncture, respectively. From the control group, blood samples were collected via venipuncture, and CSF samples were obtained while the ventriculoperitoneal shunting was performed. The samples from the control group were obtained once. As soon as possible, each 10 mL CSF and blood sample was centrifuged at 10,000 rpm for 15 minutes and the supernatant was stored at —70 °C until assayed.

Ox-LDL Measurements

A sandwich ELISA technique measured ox-LDL levels in both serum and CSF (Mercodia AB, Uppsala, Sweden) with a captured antibody, mAB-4E6, directed against a conformational epitope in the apoB-100 moiety of LDL according to the Manufacturer's instructions. Measurements were performed at 450 nm. Results were calculated by multiplying the concentration of the controls and the unknown samples with the dilution factor. The detection limit was less than one mU/L of Ox-LDL. Results were expressed as U/L.

PON1 Measurements

PON1 concentrations were determined by using competitive ELISA with rabbit anti-human PON1 monospecific antibodies used as described previously.[1] The results were expressed as μg/mL.

Statistical Analysis

Data were analyzed by using the SPSS statistical program (SPSS, Chicago, Illinois, USA). Due to the less number of the samples in this study, Mann-Whitney U test was chosen to evaluate significant differences between the patient and the control group. Differences within each group were tested by "Student t" test. Results are given as mean ± standard deviation. Two tailed "*p*" values were used, and values < 0.05 were considered statistically significant.

Results

Twenty CSF and 20 serum samples from the patients with SAH and 10 CSF and 10 serum samples from the control group were obtained for this prospective clinical study. The samples were tested for Ox-LDL, and PON1. There was no statistically significant difference in age or sex between the two groups ($p>0.05$).

PON1 Concentrations in Serum and CSF

The average serum PON1 level in control group was 72.2 ± 6.8 μg/mL. In contrast, the average level in SAH patients was 77.1 ± 8.1 μg/mL within the first three days and 51.7 ± 5.6 μg/mL on day 7 after SAH. The difference from control was extremely significant related to day seven of SAH ($p=0.00001$), but no such difference was found regarding the level measured within the first three days of SAH ($p=0.18$). There was a significant decrease in concentration during post-SAH days ($p =0.00001$).

On the other hand, there was extremely statistically significant difference in CSF of SAH patients and control. The average level for control was 4.3 ± 2.2. In contrast, average levels were 10.5 ± 2.2 μg/mL ($p=0.00001$), and 6.5 ± 2.0 μg/mL ($p=0.034$) within first three days and day seven of SAH, respectively. There was also a significant decrease in concentration during post-SAH days ($p=0.00001$).

Ox-LDL Concentrations in Serum and CSF

Serum Ox-LDL levels in SAH patients was 87.5 ± 7.9 and 64.2 ± 4.8 U/L within the first three days and on day seven of SAH, respectively. There was statically significant difference between the levels measured within the first three days and the control ($p=0.00001$), while no difference was found the levels measured on day seven of SAH and control ($p=0.64$). There was significant decrease in concentration during post-SAH days ($p=0.00001$).

When regarding the CSF, we found levels of Ox-LDL were markedly different in patients with SAH than control. In the control group, the mean concentration was 2.7 ± 1.9

U/L and two had non-detectable Ox-LDL. In contrast, the average levels in SAH patients were 7.9 ± 1.2 U/L within the first three days and 5.0 ± 1.2 U/L on day 7 after SAH. These differences from control were extremely significant (p=0.00001). Statistically significant difference was also noted between the levels in first three days and day seven of SAH (p =0.00001).

Discussion

The role(s) of oxidative stress and its resulting LDL oxidation have been documented in neurodegenerative, cardiovascular diseases, and diabetes mellitus as well. [32,34,37] As the literature stated, Ox-LDL is mainly involved in the initiation and progression of atherosclerosis.[6,7,24] Detrimental effects of lipid peroxidation (LP) have been implicated in the development of VS after an aneurysmal SAH.[16,27,28,42] Oxidation of lipoproteins is prevented by either the first line (e.g. vitamin E) or the second line of defense (antioxidative enzymes). A HDL-bound ester, PON1 is one such antioxidative enzyme that was indeed shown to hydrolyze specific oxidized lipids and thus to reduce oxidative stress in lipoproteins such as HDL, LDL, as well as in macrophages and in atherosclerotic lesions.[3,9,18] Low serum PON1 activity is associated with high prevalence of atherosclerosis and cardiovascular diseases.[32,26]

The protective role of PON1 in LDL oxidation is the result of the interaction of oxidized lipids (oxidized cholesteryl arachidonate or oxidized arachidonate phospholipids) in Ox-LDL with PON's free sulfhydryl group at cysteine-284.[2,3]

First, the authors emphasized that this study included rather limited number of SAH patients so that future studies should be designed to include more patient population in order to figure out the exact role(s) of such molecules in SAH and VS, a challenging problem in neurosurgical practice. However, the results may be important when considering the actions of Ox-LDL and PON1 in pathological conditions stated in the literature. We found statistically significant difference in both CSF and serum of patients and control. Both molecules tend to decrease during the acute states of SAH. This may be due to the interaction between Ox-LDL and CSF. As LP, particularly peroxidation of LDL occurs during post-SAH days, there is consumption of PON1.

The first event that leads to detrimental chain of reactions is the shedding of erythrocytes into the subarachnoid space. There is considerable evidence that hemoglobin from lysed erythrocytes initiates ROS, including superoxide anion, hydrogen peroxide, and hydroxyl radicals. In conjunction with inducible nitric oxide synthase, peroxynitrite may also be produced. Such products finally lead to formation of LP that may cause VS. Therefore, it is clear that attempting to reduce LP in the early period of SAH may diminish VS.

Based on our findings, we can only make some speculations how Ox-LDL may lead to formation of VS. First, during the cellular oxidative modification of LDL, the expression of adhesion molecules, particularly intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) is induced.[27] The role(s) of adhesion molecules in the development of VS after SAH have been well-studied and proved extensively.[21,23,30] The levels of adhesion molecules were found to be elevated in either CSF or serum in patients having recently suffered an aneurysmal SAH and correlated with the severity of VS.[17,23,25] Furthermore, the agents against ICAM-1 showed considerable reduction in VS in animal models of SAH.[8,10] In this respect, we speculate that Ox-LDL by increasing the expression of adhesion molecules may cause VS. Second, several authors have documented the presence of endothelin-1 (ET-1), the most potent vasoconstrictor in human body, in CSF of patients with SAH,[11,12,29,35] and experiments have been performed with ET-1 antagonist showed positive results.[14,43] It has been also shown that Ox-LDL enhances myogenic tone in the rabbit posterior cerebral artery through the release of ET-1 from the endothelium.[40] Taken together, again Ox-LDL may cause VS by increasing the release of ET-1 in CSF. Third, either clinical or animal studies of SAH showed increase in the CSF concentration of nitric oxide (NO) metabolites (nitrite and nitrate) beginning in the acute stage.[36,38] Furthermore, correlation with the patients' condition was also found since the levels of metabolite were higher for those graded in Fisher group 3 than for group 2 in humans.[36] Cultured rat brain endothelial cells treated with Ox-LDL showed significantly increased NO production, decreased membrane fluidity, and increased ROS.[13] Regarding our study, there may be a possible link between Ox-LDL-induced endothelial interaction and increased NO that may cause VS. Finally, It has been suggested that oxyHb from lysed blood in the subarachnoid space is the key molecule, that initiates the production of free radical mechanisms, such as LP which seems to play a major role in the development of VS after SAH.[16,27,28,42] Contribution of extracellular ROS, especially superoxide anion to cerebral VS after SAH has been shown through several mechanisms. Coexistence of superoxide anion with ferrous (Fe^{+2}) or ferric (Fe^{+3}) iron mainly from erythrocyte-derived hemoglobin in the subarachnoid space provides an appropriate milieu, which favors the occurrence of Haber-Weiss or Fenton reactions, leading to generations of harmful ROS attacking the membrane lipids.[31] Increased production of superoxide anions result in elevation of hydrogen peroxide, which may induce vasoconstriction.[5] It has been demonstrated that Ox-LDL caused to increase ROS production and has been suggested that Ox-LDL acts as major risk factor in development of vascular endothelial dysfunction.[13] This is our last speculation that Ox-LDL may cause VS after SAH by increasing ROS.

Limitations of Our Study

This is the first study to show concentrations of Ox-LDL and PON1 in both CSF and serum of patients with aneurysmal SAH. The results are preliminary and we could make only some speculations. The role(s) of both molecules in inflammatory process in SAH cannot be drawn from this study but results may open a different way for understanding of pathophysiology of VS seen after SAH and also this study may will help further clinical studies related to SAH. As a preliminary, the authors want to insist on the limitations of the study. First, the study should have been included large population of patients. Second, CSF and serum samples

would be taken out every day after SAH during the first fourteen days in order to evaluate whether there was a correlation between the levels of molecules tested and VS. Therefore, we were not able to show any correlation with VS and the levels of Ox-LDL and PON1 in this study. Finally, the control group would be included healthy volunteers since hydrocephalus is also a central nervous system disease in which the levels of the molecules measured may also be incresed.

Conclusions

Although there are some important limitations in this study, the results clearly show that after SAH, Ox-LDL and PON1 are increased compared to control and tend to decrease toward day seven owing to the interaction between Ox-LDL and PON1. Since PON1 has been shown to protect LDL, as well as HDL, from oxidation induced ROS, it may be worthy to preserve PON activity during LDL oxidation. This approach may be reached by applying potent antioxidants against LDL oxidation together with PON1 enzyme. Thus, this combination could possibly play a major role in reducing Ox-LDL induced vascular dysfunction after SAH.

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