Hypolipoproteinemia and hyperinflammatory cytokines in serum of severe and moderate traumatic brain injury (TBI) patients

Kyriaki Venetsanou[1] , Konstantinos Vlachos[2], Athanassios Moles[1], Gerassimos Fragakis[2], George Fildissis[1], George Baltopoulos[1]

[1] Athens University School of Nursing Intensive Care and Research Unit, KAT General Hospital, Athens 14561, Greece
[2] Neurosurgical Clinic, KAT General Hospital, Athens 14561, Greece

ABSTRACT. Traumatic brain injury (TBI) acts as an inducer of the inflammatory reaction expressed by the release of pro-inflammatory cytokines [interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8)], and causes metabolic alterations in the early, post-traumatic state, either in the brain or/and the systemic circulation. The metabolic changes involve carbohydrates, proteins and lipids. We focused on the serum lipid profile, the impact of trauma on lipoproteins, and their subsequent effects, on inflammation. We investigated the role of cytokines and serum lipids, in patient outcome, reviewing 30-day mortality and the Glasgow Coma Scale (GCS). A total of 75 patients with severe or moderate TBI (GCS ≤ 13) were allocated to two groups (group 1 non-survivors and group 2 survivors). One blood sample was collected from each patient within 24h of admission. Cytokines were measured in serum by ELISA and serum lipids using an enzymatic method. We found significantly decreased serum lipid levels and increased cytokines levels for all patients compared with healthy volunteers. Comparing the two groups, IL-6 and IL-8 levels were higher (p < 0.0001) and LDL levels lower (p = 0.003) in non-survivors than in survivors. We observed a significant inverse correlation between IL-8 and LDL (p = 0.04) in patients with an unfavorable outcome. Our results suggest that LDL alone, or in combination with IL-6 and IL-8, could be a possible prognostic factor for outcome in patients with TBI, as regards 30- day mortality.

Keywords: low-density lipoprotein cholesterol, traumatic brain injury, cytokines

TBI is one of the major causes of morbidity and mortality in trauma patients, and is characterized by a complex pathophysiology. Unfavorable outcome is associated with features such as hypoxia, hypercapnia, hypotension, hypovolemia, hypothermia or coagulopathy [1], and biochemical disorders including overwhelming inflammatory reactions and metabolic alterations [2].

Several studies have reported the induction of an inflammatory reaction in the brain and systemic circulation after TBI. Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, tumor necrosis factor-α [TNF-α] and interferon-γ [IFN –γ]) are involved as mediators in the post-TBI inflammatory cascade. Studies based on various animal models have shown that IL-6 is overexpressed in brain tissue. Similar results have been obtained for IL-6 in human cerebrospinal fluid (CSF) and serum from patients with severe head trauma [3]. The anti-inflammatory cytokine IL-10 has only been detected in CSF [4]. Recently, Gopcevic *et al.* [5] proposed IL-8 as a potential factor that is predictive of mortality in patients with severe TBI (GCS ≤ 8).

In parallel, metabolic consequences of the stress that follows trauma, burns or surgery, include effects upon carbohydrate, protein and lipid metabolism. Wilson *et al.* [6] reported that hypocholesterolemia after trauma in critically ill patients was an indicator of a poorer prognosis, and suggested the investigation of the post-traumatic role of other lipoproteins and cytokines. HDL is reduced in sepsis/septic shock [7] and has been found to be involved in the inflammatory process [8]. LDL is implicated in the development of inflammation and oxidative stress in certain diseases such as atherosclerosis, diabetes and heart disease [7, 8].

The aim of this study was to evaluate the levels of serum pro-inflammatory cytokines and serum lipids in patients with severe (GCS ≤ 8) or moderate (eight < GCS ≤ 13) TBI, within the first 24 hours following trauma, and to investigate their contribution to patient outcome.

PATIENTS AND METHODS

Blood collection and treatment

Seventy five patients with TBI were admitted to the emergency department of our hospital; 50 males and 25 females, with a mean age of 50 years (range 18 to 75). Patient

outcome, assessed 30 days after injury, was based on death or survival. TBI was the cause of death in 80% of cases, and events secondary to the TBI in 20%.

We did not consider factors that affect serum lipids such as dietary intake and hormonal parameters.

A single blood sample (10 mL) was drawn from an intravenous line within 24 hours of the TBI. Serum was obtained from each blood sample by centrifugation at 1 500 rpm, for 10 min at room temperature and was stored at -80°C until analysis.

The Scientific Council and Ethics Committee of "KAT" Hospital approved the study protocol.

Sample groups

Patients were divided into two groups according to outcome.
– Group 1: non-survivors (n = 30).
GCS on admission: GCS ≤ 8 (n = 21), and GCS >8 (n = 9).
– Group 2: survivors (n = 45).
GCS on admission: GCS ≤ 8 (n = 14), and GCS > 8 (n = 31).

Cytokine assays

Cytokines levels were measured using commercially available human specific enzyme-linked immunoassays kits: IL-6 and IL-10 (DIACLONE, France), IL-1β (Quantikine HS, R&D, USA) and IL-8 (BD Opt EIA, BD, USA). All samples were tested in duplicate, in accordance with the manufacturers' instructions, and standard recombinant proteins were used as internal controls in each assay. All cytokines kits were human specific, with a sensitivity for detecting levels < 2 pg/mL, < 0.8 pg/mL, < 0.1 pg/mL and < 10 pg/mL for IL-6, IL-8, IL-1β and IL-10 respectively.

Serum lipid determination

TC, LDL, HDL, TG were measured using standard laboratory techniques (Liquicolor, Human, Germany).

Statistical analysis

Experimental data were analyzed with GRAPHPAD 4.0. Values were expressed as mean ± SEM and compared using one-way analysis of variance (ANOVA), adjusted for multiple comparisons by the Tukey test. A p < 0.05 was considered to be statistically significant.

RESULTS

Analysis of our experimental data revealed elevated levels for all cytokines tested (IL-6, IL-8, IL-10, IL-1β), taking into account all patients with TBI as a single group (n = 75), compared with those levels found in healthy subjects (non-detectable levels [N.D.]), p < 0.001 (*table 1*).

Conversely, serum lipids levels (TC, HDL, LDL) were found to be reduced following TBI, compared with those from healthy donors (*table 2*). Statistical differences were found for TC (p < 0.001), HAL (p < 0.001) and LDL (p < 0.01), but not TRIG (p > 0.05).

We classified patients as a function of outcome, survivors (n = 45) and non- survivors (n = 30).

Table 1
Cytokine profile in serum from patients with severe or moderate TBI GCS ≤ 13, upon admission

Cytokines (pg/mL)	Patients with TBI (n = 75)	Healthy volunteers (n = 16)
IL-8***	57.2 ± 9.4	N.D.
IL-6***	88 ± 16.3	N.D.
IL-10***	35.9 ± 7.5	N.D.
IL-1b***	2.4 ± 0.2	N.D.

IL-8, IL-6, IL-10 and IL-1b are shown as mean ± SEM. N.D.: non-detectable levels. *** p < 0.001 for cytokine levels in patients with severe or moderate TBI and healthy individuals.

We found: a) statistically significant, higher levels of IL-6 and IL-8 (p < 0.001) in non-survivors versus survivors, while no differences were observed for IL-10 and IL-1β (*figure 1*); b) a significantly lower baseline LDL for group 1 *versus* group 2 (p =0.03), but no statistical differences for TC, TRIG and HDL. Mean concentrations of LDL were 83.1 ± 9.3 mg/dL in non- survivors and 97 ± 5.8 mg/dL in survivors (*figure 2*); c) a significant inverse correlation between IL-8 and LDL levels (p = 0.04). Patients with lower LDL appeared to have significantly higher levels of IL-8 and an unfavorable outcome.

We found no statistically significant differences in any of the parameters classifying patients according to their GCS (GCS ≤ 8 [n = 35] and GCS > 8 [n = 40]) as determined upon admission (data not shown).

Table 2
Lipid profile in serum of patients with severe or moderate TBI, GCS ≤ 13, upon admission

Serum lipids (mg/dL)	Patients with TBI (n = 75)	Healthy volunteers (n = 16)
TC***	159 ± 3.1	200 ± 25
HDL***	39.9 ± 2	51 ± 6
LDL**	93.3 ± 5.3	140 ± 30
TRIG	117 ± 8.3	120 ± 40

TC, HDL, LDL, TRIG shown as mean ± SEM; *** p < 0.001, ** p < 001 for serum lipid levels in patients with severe or moderate TBI and healthy individuals.
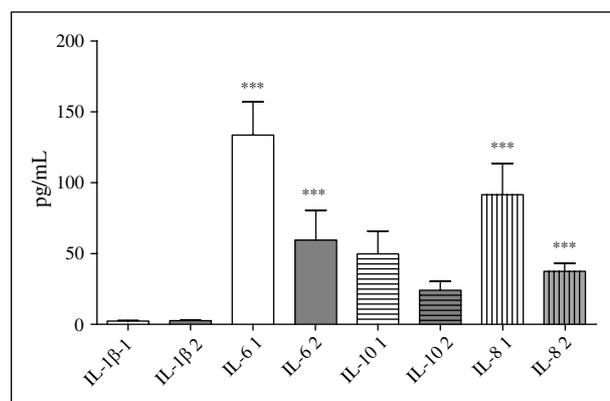
Figure 1
Cytokine concentrations in serum from patients with severe or moderate TBI, GCS ≤ 13, according to patient outcome.
Indexes 1 and 2 refer to patient outcome, group 1 (non- survivors) and group 2 (survivors) respectively. Significant differences were found for IL-6 1 *versus* IL-6 2 (*** p < 0.001) and for IL-8 1 *versus* IL-8 2 (*** p < 0.001).
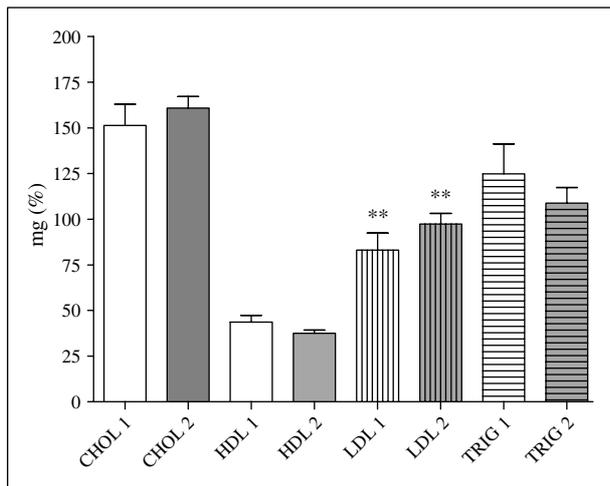
Figure 2

Concentrations of lipid subfractions in serum from patients with severe or moderate TBI, GCS ≤ 13, according to patient outcome. Indexes 1 and 2 refer to patient outcome, group 1 (non- survivors) and group 2 (survivors) respectively. Significant differences found between LDL 1 and LDL 2, **p < 005. No significant differences were found for TC, HDL, TRIG (p > 0.05).

DISCUSSION

TBI is a stress state characterized by local and systemic events, which include initial, clinically determined conditions (hypotension, hypothermia, hypovolemia etc.), and subsequent, stimulation of inflammatory reactions and metabolic changes.

Several investigators have analysed the role of the inflammatory reaction and/or biochemical changes in TBI, in relation to the severity of the TBI and/or patient outcome. We investigated serum cytokine release and lipid subfraction levels in a cohort of patients with severe or moderate TBI (GCS ≤ 13), within 24 h of the incident, considering 30-day mortality. We found all cytokines studied (IL-1β, IL-6, IL-8 and IL-10) to be elevated, and serum lipid subfractions (TC, HDL, LDL) reduced, in both groups, compared to healthy volunteers. Between groups, IL-6 and IL-8 levels were higher in patients with an unfavorable outcome, but no significant differences were found for IL-1β and IL-10. IL-1β was induced early, while IL-10 has a later time frame.

Our results are consistent with those from previous studies. Shohami *et al*. [9] found that circulating TNF-α, IL-1β and IL-6 increased and peaked within a few hours after TBI. Cytokine release is not an effect specific to TBI. Any traumatic injury stimulates the cytokine cascade. Evaluation of the role of cytokines depends on the combination of parallel processes. Maier *et al*. [10] showed that onset of multiple organ failure (MOF) after severe trauma, was associated with a particular plasma cytokine pattern. They also observed a peak within the first 24h. Considering a 10-day curve, they showed a correlation between IL-6 and TNF receptors (TNFRs), and a lethal outcome, but not for IL-8 and IL-10. Recently, Gopcevic *et al*. [4] showed plasma IL-8 to be a predictor of mortality in subjects with severe TBI. In our study, this aspect was confirmed and extended to include both severe and moderate TBI (GCS ≤ 13). We could not regard IL-6 as a predictive factor because of its limited specificity, but in combination with IL-8, it was a valuable index for possible patient outcome. The detection of pro-inflammatory interleukins in CSF, suggested a local inflammatory reaction [4, 11, 12], but the relationship with the systemic inflammatory response is not yet clear.

Trauma is a complex process associated not only with inflammatory response, but also with biochemical compounds.

Less studied have been serum lipid levels following TBI. As we previously mentioned, serum lipid levels were found to be lower than normal values. TC and HDL showed the most significant results, but it seems that they did not correlate with patient outcome. In contrast, LDL was significantly lower for non-survivors. Wilson *et al*. [6] supported the critical role of cholesterol levels and the protective role of lipoproteins in critically ill and injured patients. Hypolipidemia predisposes to a poor clinical outcome in critically ill patients. Chiang *et al*. [13] found that low serum LDL was the best lipid, along with with TC, for predicting all-cause death among maintenance hemodialysis (MHD) patients, but not TRIG or HDL. Kilpatrick *et al*. [14] proved that MHD patients with hypercholesterolemia, high LDL and hypertriglyceridemia had a paradoxical association with better outcome. Our findings suggest a similar serum lipid profile, and a functional role of LDL in patients with severe or moderate TBI. The protective role of LDL may stem from its ability to reduce pro-inflammatory cytokine release (e.g. IL-8 and IL-6), caused by trauma. Lipoproteins are able to bind and neutralize the bacterial endotoxin lipopolysaccharide (LPS). LPS binds to LPS-binding protein (LBP) and stimulates the cytokine cascade, while if LPS is blocked by lipoproteins, this decreases cytokine release. This property could explain the significant, inverse correlation between IL-8 and LDL in non-survivors. Higher IL-8 and lower LDL seem to be adverse indicators for patient prognosis. However, the mechanisms that mediate the inflammatory response after TBI need to be further elucidated.

In conclusion, determination of LDL on admission, alone or with IL-8 and IL-6, could prove to be a factor predictive of poor outcome when considering 30-day mortality in TBI patients. The mechanisms involved in this process are under investigation.

REFERENCES

1. Jeremitsky E, Omert L, Dunham M, *et al*. Harbingers of poor outcome the day after severe brain injury: Hypothermia, hypoxia and hypoperfusion. *J Trauma* 2003; 54: 312-9.

2. Nicholson G. Hormonal and metabolic responses to trauma: anaesthesia and intensive care medicine. **Please, complete the name of the newspaper** 2005; 6: 313-4.

3. Benveniste EN. The role of cytokines in multiple sclerosis/ autoimmune encephalitis and other neurologic disorders. In: Agarwal B, Puri R, eds. *Human Cytokines: Their Role in Research and Therapy*. Boston: Blackwell Science, 1995: 159-80.

4. Csuka E, Morganti-Kossmann MC, Lenzlinger PM, *et al*. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relation to IL-6, TNF-alpha, TGF-beta and blood-brain-barrier function. *J Neuroimmunol* 1999; 101: 211-21.

5. Gopcevic A, Mazul-Sunko B, Marout J, *et al.* Plasma Inter-leukin-8 as a potential predictor of mortality in adult patients with severe Traumatic Brain Injury. *Tohoku J Exp Med* 2007; 211: 387-93.

6. Wilson RF, Barletta JF, Tyburski JG. Hypocholesterolemia in sepsis and critically ill or injured patients. *Crit Care* 2003; 7: 413-4.

7. Wu A, Hinds CJ, Thiemermann C. High-density lipoproteins in sepsis and septic shock: Metabolism, Actions, and Therapeutic Applications. *Shock* 2004; 21: 210-21.

8. Nofler JR, Kehrel B, Fobker M, Levkau B, Assmann G, Von Eckardstcin A. HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis* 2002; 161: 1-16.

9. Shohami E, Novikov M, Bass R, *et al.* Closed head injury triggers early production of TNF-a and IL-6 by brain tissue. *J Cereb Blood Flow Metab* 1994; 14: 615-9.

10. Maier B, Lefering R, Lehnert M, *et al.* Early *versus* late onset of multiple organ failure is associated with differing patterns of plasma cytokine biomarker expression and outcome after severe trauma. *Shock* 2007; (epub ahead of print).

11. Kossmann T, Stahel PF, Lenzlinger PM, *et al.* IL-8 released into the cerebrospinal fluid after brain injury is associated with blood-brain barrier dysfunction and nerve growth factor production. *J Cereb Blood Flow Metab* 1997; 17: 280-9.

12. Chiaretti A, Antonelli A, Riccardi R, *et al.* Nerve growth factor expression correlates with severity and outcome of traumatic brain injury in children. *Eur J Paediat Neurol* 2007; (epub ahead of print).

13. Chih-Kang C, Tai-I H, Shih-Ping H, *et al.* Low-density lipoprotein cholesterol: association with mortality and hospitalization in hemodialysis patients. *Blood Purif* 2005; 23: 134-40.

14. Kilpatrick RD, McAllister CJ, Kovesdy CP, *et al.* Association between serum lipids and survival in hemodialysis patients and impact of race. *J Am Soc Nephrol* 2007; 18: 293-303.