CNS Neurosciences & Therapeutics

LETTER TO THE EDITOR

CNS Neuroscience & Therapeutics

Plasma Levels of Oxidative Stress Biomarkers and Long-Term Cognitive Performance after Severe Head Injury

Maria Emília Rodrigues de Oliveira Thais,[1] Gisele Cavallazzi,[1] Marcelo Liborio Schwarzbold,[1] Alexandre Paim Diaz,[1] Cristiane Ritter,[2] Fabrícia Petronilho,[2] Alexandre Hohl,[1] Rui D. S. Prediger,[1,3] Marcelo Neves Linhares,[1,4,5] Felipe Dal-Pizzol[2] & Roger Walz[1,4,6]

1 Centro de Neurociências Aplicadas (CeNAp), Hospital Universitário, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
2 Laboratório de Fisiopatologia Experimental, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil
3 Laboratório de Doenças Neurodegenerativas, Departamento de Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
4 Serviço de Neurocirurgia, Hospital Governador Celso Ramos (HGCR), Florianópolis, SC, Brazil
5 Departamento de Cirurgia, Hospital Universitário, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
6 Departamento de Clínica Médica, Hospital Universitário, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

Correspondence
Roger Walz, M.D., Ph.D., Departamento de Clínica Médica, Hospital Universitário, 3º andar, Universidade Federal de Santa Catarina, Campus Universitário, Trindade Florianópolis, SC, Brazil.
Tel.: +55-483-721-9149;
Fax: +55-483-721-9014;
E-mail: rogerwalz@hotmail.com
Received 10 March 2012; revision 4 April 2012; accepted 10 April 2012.

doi: 10.1111/j.1755-5949.2012.00346.x

Traumatic brain injury (TBI) is the leading cause of mortality and morbidity of young people worldwide [1–4]. Oxidative stress has been implicated with the excitotoxic brain injury [5] including TBI [6,7], but the association between plasma markers of oxidative stress and the severe TBI patients' prognosis remains controversial [8]. We have demonstrated recently that the plasma enhancement of thiobarbituric acid-reactive species (TBARS, an index of lipid peroxidation) and carbonyl groups (an index of oxidative o proteins) observed up to 3 days after severe TBI are not independently associated with hospital mortality of patients [8]. To the best of our knowledge, there is no previous study investigating the association between serum biomarker levels during the acute TBI phase and the cognitive prognosis of patients. Here, we correlate the TBARS and carbonyl plasma levels after severe TBI and the cognitive performance of patients evaluated on average 17.3 (SD ± 5) months later.

Included patients hospitalized between May 2006 and September 2009 showed: (1) Glasgow Coma Scale (GCS) score ≤ 8; (2) 18 years of age or older. There were no victims of gunshot injuries. Among the 51 consecutive survivor patients, 22 (43.1%) completed the cognitive assessment. The study was approved by our Ethics Committee, and written informed consent was obtained from relatives (during hospitalization) and patients (chronic period). The TBARS and carbonyl levels determined in the plasma of patients as previously described [6–9] in median 11 h (IQ 6–19.0), 30 h (IQ 24–37), and 70 h (IQ 55–78) after the severe TBI were compared with 10 healthy controls matched for age and sex. The biochemical analysis was blinded for all clinical and cognitive variables of patients.

The variables collected during hospitalization [2,3,8] were gender, age, GCS score, pupillary status, computed tomography (CT) scan classification according to Marshall's classification [3], presence of subarachnoid hemorrhage in the CT, and presence of associated trauma (spine, thorax, or abdomen). The analyzed parameters at the time of the first blood sampling collection were blood pressure, heart and respiratory rate, and positive end-expiratory pressure. The laboratorial variables analyzed in the same sample used for TBARS and carbonyl measurements were arterial blood gases, sodium, potassium, urea, creatinine, hematocrit, hemoglobin, leukocytes, and platelets.

The neuropsychological evaluation was blinded for all the hospitalization variables and includes [10]: Letters and Category Fluency, Rey Auditory Verbal Learning Test (RAVLT), Wechsler Memory Scale III (WMS-III) subtests Logical Memory First Recall (LM 1st), Logical Memory I (LM I), Logical Memory II (LM II), and Visual Reproduction I (VR I), Visual Reproduction II (VR II), Visual Reproduction Recognition (VR Rec), Wechsler Adult Intelligence Scale III (WAIS-III) subtests Digit Span, Similarities, Vocabulary and Block Design. For comparison, healthy control subjects (n = 23) without previous history of neurological or psy-

Table 1 Clinical, demographic, laboratory, radiological, neurosurgical, and oxidative stress of patients with severe TBI

	Cognitive examination	
Variables	Nonevaluated n = 29 (56.9%)	Evaluated n = 22 (43.1%)
Sex		
Male	25 (85.2)	20 (90.9)
Marshall CT classification		
Type I injury	01 (3.4)	02 (9.1)
Type II injury	10 (34.5)	07 (31.8
Type III injury	03 (10.3	02 (9.1)
Type IV injury	03 (10.3)	03 (13.6)
Evacuated mass lesion	06 (20.7)	04 (18.2)
Nonevacuated lesion	06 (20.7)	04 (18.2)
SAH on admission cranial CT	13 (44.8)	14 (63.6)
Brain surgery		
Yes	14 (48.3)	12 (54.5)
Associated trauma	17 (56.6)	16 (72.7)
Admission Glasgow Coma Scale		
7 or 8	13 (44.8)	11 (50.0)
5 or 6	08 (27.6)	04 (18.2)
3 or 4	8 (27.6)	07 (31.8)
Admission Pupils		
Isocorics	17 (58.6)	14 (63.6)
Anisocorics	10 (34.5)	08 (36/4)
Mydriatics	2 (6.9)	0
Age in years	34.8 ± 16.2	31.7 ± 11.1
Days in the ICU	12 ± 8	11 ± 5
Days of Hospitalization	30 (19)	28 (23)
Systolic blood pressure, mmHg	127 (27)	128 (35)
Diastolic blood pressure, mmHg	76 (16)	75 (23)
Heart rate/min	90 (15)	90 (25)
Respiratory rate/min	22 (18)	18 (14)
Glucose levels, mg/dL	158 (74)	149 (39)
Hematocrit, %	34.2 (5.3)	33.1 (87.9)
Hemoglobin, g/100 mL	11.7 (1.8)	13.2 (7.9)
Leukocytes/mm^3	15,578 (6978)	15,346 (8024)
Carbonyl levels, nmol/mg protein 10^{-14}		
First blood sample (n = 51)	25.3 (IQ 9.0–86.6)	26.2 (IQ 9.5–95.0)
Second blood sample (n = 42)	32.6 (IQ 10.3–110.8)	52.7 (IQ 16.9–145.3)
Third blood sample (n = 42)	33.2 (IQ 14.6–142.0)	46.3 (IQ 18.8–145.0)
TABARS levels, nmol/mg protein 10^{-5}		
First blood sample (n = 51)	38.9 (IQ 13.4–196.9)	48.7 (IQ 8.1–199.5)
Second blood sample (n = 42)	23.1 (IQ 14.7–242.9)	44.6 (IQ 33.0–236.0)
Third blood sample (n = 42)	74.5 (IQ 53.2–417.3)	72.9 (IQ 59.2–321.3)

TBI, traumatic brain injury; CT, computed tomography; SAH, Subarachnoid hemorrhage. The laboratorial variables were determined in the first blood sample, and the clinical data were collected at the time of the first blood sample collection. The first, second, and third blood samples were collected respectively 11 (IQ 6–19.0), 30 (IQ 24–37), and 70 h (IQ 55–78) after TBI. There were no statistically significant differences between the evaluated and nonevaluated patients among the studied variables ($P > 0.20$). Analysis was carried out by Fisher's exact test, Student's "*t*" test or Mann–Whitney *U*-test.

chiatric disorders, matched for gender, age, and education level, were recruited during the same period.

As previously demonstrated [8], plasma levels of TBARS of patients were significantly higher than controls ($P < 0.001$), showing a time-dependent enhancement at 11, 30, and 70 h after the TBI. The plasma carbonyls also were significantly higher than controls ($P < 0.01$) at 30 and 70 h after the TBI (data not shown).

There were no significant differences between the cognitively evaluated (n = 22) and nonevaluated (n = 29) patients according to the hospitalization variables ($P \geq 0.26$), plasma TBARS levels ($P \geq 0.12$), and plasma carbonyl levels ($P \geq 0.33$; see Table 1).

Patients showed significant lower scores in Letters and Category Fluency, RAVLT, LM 1st, LM I, LM II, VR I, VR II, Vocabulary and Block Design ($P < 0.001$). Patients also showed a trend for lower scores than controls in the Digit Span ($P = 0.08$) and similarities ($P = 0.06$), and a normal VR Rec test performance. The findings were not because of gender, age, education level, and hand dominance imbalances between patients and controls (data not shown).

Table 2 shows the correlation between plasma levels of TBARS, carbonyl, and cognitive tests. There was no significant correlation between the serum level of TBARS and carbonyl and the cognitive performance of patients ($P > 0.25$). Among the 15 evaluated cognitive tests, only the vocabulary scores correlate significantly (Spearman coefficient 0.50 and $P = 0.04$) with TBARS levels (third collected blood sample), but this result was not confirmed after the correction for age and education ($P = 0.23$, data not shown).

Table 2 Correlation between TBARS and carbonyl plasma levels of survivor patients with severe TBI and their long-term cognitive performance

	TBARS			Carbonyl		
	1	2	3	1	2	3
Letter fluency	0.26	0.14	0.14	−0.34	−0.20	0.03
Category fluency	0.12	0.11	0.15	−0.25	−0.21	0.08
Similarities	0.34	0.26	0.30	−0.09	0.24	0.10
Vocabulary	0.27	0.32	0.50	−0.05	0.22	0.24
Block design	0.42	0.26	0.40	0.10	0.06	0.26
RAVLT-total	0.15	0.23	0.26	−0.03	−0.10	0.32
RAVLT-delayed	−0.08	0.23	0.26	−0.03	−0.10	0.32
VP II	0.12	0.23	0.39	−0.06	−0.03	0.27
VP Rec	−0.01	0.14	0.16	0.09	−0.01	0.21
LM 1st	0.24	0.32	0.14	−0.04	0.10	0.08
LM I	0.27	0.34	0.20	−0.05	0.04	0.14
LM II	0.17	0.03	0.08	−0.09	−0.24	−0.02

TBARS, thiobarbituric acid-reactive species; TBI, traumatic brain injury; RAVLT, Rey Auditory Verbal Learning Test; VP, Visual Production; VP Rec, Visual Production Recognition ; LM, Logical Memory. 1, First blood sample; 2, Second blood sample; 3, Third blood sample. TBARS and carbonyl levels were determined the first blood sample of all patients and in second and third blood sample of 21 and 19 patients, respectively. There was no significant correlation between the serum level of TBARS and carbonyl and the cognitive performance of patients ($P > 0.25$). Among the 15 evaluated cognitive tests, only the vocabulary scores correlate significantly (Spearman coefficient 0.50 and $P = 0.04$) with TBARS levels (third collected blood sample), but this result was not confirmed after the correction for age and education ($P = 0.23$, data not shown).

after the correction for age and education ($P = 0.23$, data not shown).

The reader should also be aware of the study limitations. The used biochemical reaction for TBARS determination is not specific [8] for the free radical-induced lipid peroxidation. The wide range of both TBARS and carbonyl levels and the relative small sample size of patients may reduce the study. Missing cases in the follow-up could raise doubt whether the evaluated patients represent the survivor group as a whole. However, we minimized the missing problem by controlling the distribution of the several hospitalization and demographic variables between the evaluated and non-evaluated patients.

In conclusion, the present findings indicate that measurement of plasma TABARS and carbonyl levels up to 70 h after the severe TBI are not useful biomarkers to predict the cognitive morbidity of patients with severe TBI.

Acknowledgments

This work was supported by CPNq (Brazilian Council for Scientific and Technologic Development, Brazil), FAPESC (Foundation for Scientific Research and Technology of the State of Santa Catarina). PRONEX program CNPq/FAPESC (NENASC project).

Conflict of Interest

The authors declare no conflict of interest.

References

1. Diaz AP, Schwarzbold ML, Thais ME, et al. Psychiatric disorders and health-related quality of life after severe traumatic brain injury—A prospective study. *J Neurotrauma* 2012;**29**: 1029–1037.
2. Gullo JD, Bertotti MM, Silva CC, et al. Hospital mortality of patients with severe traumatic brain injury is associated with serum PTX3 levels. *Neurocrit Care* 2010;**14**: 194–199.
3. Martins ET, Linhares MN, Sousa DS, et al. Mortality in severe traumatic brain injury: A multivariated analysis of 748 Brazilian patients from Florianopolis City. *J Trauma* 2009;**67**: 85–90.
4. Schwarzbold M, Diaz A, Martins ET, et al. Psychiatric disorders and traumatic brain injury. *Neuropsychiatr Dis Treat* 2008;**4**: 797–816.
5. Dal-Pizzol F, Klamt F, Vianna MM, et al. Lipid peroxidation in hippocampus early and late after status epilepticus induced by pilocarpine or kainic acid in Wistar rats. *Neurosci Lett* 2000;**291**: 179–182.
6. Petronilho F, Feier G, de Souza B, et al. Oxidative stress in brain according to traumatic brain injury intensity. *J Surg Res* 2010;**164**: 316–320.
7. Schwarzbold ML, Rial D, De Bem TD, et al. Effects of traumatic brain injury of different severity on emotional, cognitive and oxidative stress-related parameters in mice. *J Neurotrauma* 2010;**27**: 1883–1893.
8. Hohl A, Gullo JD, Silva CC. Plasma levels of oxidative stress biomarkers and hospital mortality in severe head injury: A multivariate analysis. *J Crit Care*. Doi: 10.1016/j.jcrc.2011.06.007.
9. Klamt F, Dal-Pizzol F, Conte da Frota ML. Imbalance of antioxidant defense in mice lacking cellular prion protein. *Free Radic Biol Med* 2001;**30**: 1137–1144.
10. Lezak M, Howieson D, Loring D. *Neuropsychological assessment*. New York: Oxford, 2004.

