Original article

Serum melatonin levels in patients with traumatic brain injury-induced coma

Wusi Qiu, Qizhou Jiang, Guoming Xiao

Department of Neurosurgery, Hangzhou Second Hospital, College of Medicine, Hangzhou Normal University, China

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Address for correspondence:
Prof. Wusi Qiu
Department of Neurosurgery
Hangzhou Second Hospital
College of Medicine, Hangzhou Normal University
e-mail: shihai954@163.com

Abstract

Introduction: Melatonin (MLT) is a known antioxidant. We present here the changes of serum melatonin levels in adult patients with traumatic brain injury (TBI)-induced coma.
Material and methods: Sixty-one adult patients with TBI-induced coma, assessed according to Glasgow Coma Scale (GCS) scores on admission, were divided into very severe injury, severe injury and moderate injury groups. Thirty-one adult healthy volunteers were selected as the control group. Blood samples were collected twice at day 1, day 3 and day 7 after admission. The prognosis of these patients was evaluated 3 months after TBI.
Results: The serum MLT levels of each TBI group at each time point were significantly lower than those of the control group ($p < 0.05$). The MLT levels of very severe and severe TBI groups were significantly lower than those of the moderate TBI group ($p < 0.05$). The MLT level of the very severe group was significantly lower than that of the severe group ($p < 0.05$). The lower the serum MLT level, the more severe was the condition of TBI (by GCS scoring), and the lower were the Glasgow Outcome Scale (GOS) scores.
Conclusions: Lower serum MLT levels may indicate worse condition, and serum melatonin levels may correlate with the severity and prognosis of patients with TBI.

Key words: melatonin, traumatic brain injury, coma, GOS, prognosis.

Introduction

Melatonin (5-methoxy-N-acetyltryptamine, MLT) is a natural product of the pineal gland. It plays a variety of physiological roles such as the adaptation of the day and night cycle, acclimation, and participation in immune reactions (Alonso-Alconada *et al.* 2013; Barlow *et al.* 2014; Bhattacharya *et al.* 2014; Bouslama *et al.* 2007; Dehghan *et al.* 2013; Ding *et al.* 2015; Erol *et al.* 2004; Giusti *et al.* 1996; Li *et al.* 2009; Naseem and Parvez 2014; Sarrafzadeh *et al.* 2000; Zhao *et al.* 2015). Its protective effect in many neurodegenerative disorders (e.g., Alzheimer's disease, Parkinson's disease, ischemia-reperfusion injury, mental disorders) has also been implicated, such as protecting ischemic brain tissues through a variety of ways (Koh 2012). Melatonin and its metabolites are potent antioxidants and free radical scavenger agents with physiological activity to reduce DNA damage and infarct volume after ischemic injury (Alonso-Alconada *et al.* 2013; Sarrafzadeh *et al.* 2000; Yürüker *et al.* 2015). The poor prognosis of severe traumatic brain injury (TBI) is often associated with the ischemia and hypoxia in traumatized brain tissue, and serum MLT may be related to changes in the medical condition of patients with severe TBI. In this study, we investigated the changes in serum MLT levels in patients with moderate or severe TBI, and aimed to evaluate the clinical significance and possible relationship with prognosis.

Material and methods

Study population

This retrospective study reviewed data from the three levels of a first-class comprehensive hospital at the Affiliated Hospital of Hangzhou Normal University. According to Good Clinical Practice standards, the research protocol was approved by the Institutional Review Board and by the ethical committees of the Clinical Medical College of Hangzhou, and Declaration of Helsinki principles were strictly adhered to.

Written consent was obtained from the local Ethics Committee (Ethics Committee reference number: 20080125).

Clinical data were collected from 61 adult patients (37 males and 24 females) aged 18 to 59 years old between January 2008 and January 2014, who were comatose due to a traumatic brain injury. According to their Glasgow Coma Scale (GCS) scores when they were admitted to the hospital, the patients were divided into three groups: 19 subjects with very severe TBI (GCS score 3–5), 21 subjects with severe TBI (GCS score 6–8), and 21 with moderate TBI (GCS score 9–12). Upon admission, all subjects were definitively diagnosed by the patient's history, physical examinations, and such radiographies as computed tomography (CT) (Qiu *et al.* 2009). All the comatose states were as confirmed and immediately classified according to GCS scoring. Selected subjects did not have severe compound injuries or hemorrhagic shock, were not pregnant, and did not undergo various types of hormone therapy. A control group was composed of 31 healthy volunteers. Written informed consent was signed by the patients' legal guardians or by a healthy proxy.

Sample collection and measurements

For all TBI groups in which serum MLT levels were used to monitor brain injury, 3 ml blood samples from the median cubital vein were collected at 6 a.m. and 4 p.m. on day (D) 1, D 3, and D 7 after patients were admitted to the hospital. Samples were drawn over a period of 3 months. By the end of this period, 48 patients had survived, 10 had recovered sufficiently for discharge, and 13 died. Blood samples from the control group were also collected in the same period. After blood samples were collected, sera were immediately separated using centrifugation, then stored at –20°C for future measurement. Serum melatonin enzyme linked immunosorbent assay (ELISA) kits were purchased from Immuno-Biological Laboratories GmbH, Hamburg, Germany. All measurements were performed by closely following the manufacturer's instructions.

Standardized treatment of TBI was based on Clinical Practice Guidelines for Traumatic Brain Injury, and none of the patients were taking hormone therapy.

Statistical analyses

The MLT levels were expressed as mean ± standard deviation, and the statistical analysis of the data in this study was performed using SPSS 13.0. The differences between any two groups were compared using one-way analysis of variance (ANOVA) and the least significant difference (LSD).

Results

Samples were drawn over a period of 3 months. By the end of this period, 48 patients had survived, 10 had recovered sufficiently for discharge, and 13 died.

The mean serum MLT levels of the dead patients (when they died) was 19.1 ±9.8 pg/ml. During the statistical analysis of the MLT levels in all TBI groups, the dead patients in each time period were not included.

The changes of MLT levels and GCS scores of the subjects in all TBI groups on D 1, D 3, and D 7 after their injuries are shown in Table 1.

A summary of the data showed that: 1) the serum MLT levels of each TBI group at each time point were significantly lower than those of the control group ($p < 0.05$); 2) the MLT levels of very severe and severe TBI groups were

Table 1. Comparison of the serum melatonin levels and GOS scores between patients with TBI and healthy volunteers

	Time point		Very severe TBI	Severe TBI	Moderate TBI	Control subjects
Serum melatonin levels (pg/ml, $\bar{x}$ ± SD)	1 day after TBI	6 am	23.9 ±8.9[a]	43.5 ±27.4[a,c]	68.1 ±17.3[a,b]	121.1 ±26.3 (6 am)
		4 pm	24.7 ±9.6[a]	45.4 ±31.6[a,c]	75.2 ±28.7[a,b]	
	3 days after TBI	6 am	21.7 ±7.9[a]	40.4 ±23.1[a,c]	63.1 ±16.2[a,b]	
		4 pm	22.7 ±9.4[a]	44.5 ±22.6[a,c]	73.2 ±23.5[a,b]	106.6 ±14.3 (4 pm)
	7 days after TBI	6 am	24.5 ±8.3[a]	48.4 ±22.4[a,c]	98.7 ±16.9[a,b]	
		4 pm	25.6 ±9.7[a]	51.9 ±24.6[a,c]	95.6 ±18.6[a,b]	
GOS scores	1 month after TBI		3.0	3.5	4.3	–
	3 months after TBI		3.5	4.0	4.8	–

Comparison of any two groups using the LSD method. TBI – traumatic brain injury, GOS – Glasgow Outcome Scale [a]$p < 0.05$; comparison of each TBI group with the control group at each time point; [b]$p < 0.05$, comparison of the moderate TBI group with severe and very severe TBI groups at each time point, respectively; [c]$p < 0.05$, comparison of the severe TBI group with the very severe TBI group at each time point.

significantly lower than those of the moderate TBI group ($p < 0.05$); and 3) the MLT level of the very severe group was significantly lower than that of the severe group ($p < 0.05$). This indicates that the lower the serum MLT level, the more severe was the condition of TBI (by GCS scoring), and the lower were the Glasgow Outcome Scale (GOS) scores.

Discussion

Since 1960 when Lerner *et al.* first purified MLT from pineal gland extracts, scholars in all fields of life sciences have developed a strong interest in it (Barlow *et al.* 2014; Ding *et al.* 2015; Erol *et al.* 2004; Gorgulu *et al.* 2001; Naseem and Parvez 2014; Yürüker *et al.* 2015; Zhao *et al.* 2015). Especially in recent years, with the development of new research methods, significant progress has been made in studying the physiological and pharmacological effects of MLT. Currently, people believe that the main physiological functions of MLT are to regulate the body's biological rhythms, regulate the neuroendocrine-immune system, and serve as an analgesic and sedative/hypnotic agent. In addition, animal studies demonstrated that MLT is a strong free radical scavenger and indirect antioxidant, and that it has a protective effect on ischemic brain injury (Bhattacharya *et al.* 2014; Bouslama *et al.* 2007; Dehghan *et al.* 2013).

Studies have shown that many systematic physiological and pathological changes can lead to changes in MLT levels (Alonso-Alconada *et al.* 2013; Barlow *et al.* 2014; Berger *et al.* 2015; Bhattacharya *et al.* 2014; Bouslama *et al.* 2007; Ding *et al.* 2015; Kilic *et al.* 2012; Lekic *et al.* 2010). However, the scientific significance and detailed mechanism of these changes are not yet entirely clear (Berger *et al.* 2015; Ding *et al.* 2015; Senol and Naziroglu 2014). Furthermore, most data are based on animal studies, and there are very few clinical reports on the correlation between MLT and TBI (Barlow *et al.* 2014; Sarrafzadeh *et al.* 2000; Senol and Naziroglu 2014; Yuruker *et al.* 2015; Zhao *et al.* 2015). In this study, we focused on the changes of serum MLT levels in patients with TBI, and conducted dynamic observation and analysis in order to explore their clinical significance.

The relationship between MLT secretion and the severity of TBI

Studies have shown that TBI patients exhibit reduced melatonin levels and a circadian secretion profile which is related to the severity of the injury (Berger *et al.* 2015; Ding *et al.* 2015; Giusti *et al.* 1996; Sarrafzadeh *et al.* 2000). Patients with severe TBI exhibit a clearly disrupted pattern of MLT secretion. Furthermore, the diurnal secretion pattern of melatonin appeared to be dissociated from the circadian rhythm. It is indicated that reduced evening melatonin production due to TBI may cause disruption of circadian regulation of sleep and wakefulness (Barlow *et al.* 2014).

Our results showed that the MLT levels of all TBI groups in the morning and afternoon on D 1, D 3, and D 7 after admission to the hospital were significantly decreased compared to those of the control group. Also, the more severe the injury was, the greater the MLT levels declined. Meanwhile, it can also be seen from these results that the MLT levels in each TBI group clearly dropped as early as one day after injury, dropped further to a low level on the third day after injury, and finally started rising on the seventh day.

The MLT secretion pattern changed after TBI

Under normal circumstances, MLT secretion has a fixed pattern and rhythm over one day, i.e., high levels in the morning and low levels in the afternoon (Ding *et al.* 2015). After TBI, this pattern can change (Yuruker *et al.* 2015). In this study, after comparing the MLT peak levels in the moderate and severe TBI groups with the control group, we found that the normal circadian MLT secretion pattern had changed. The "high level in the morning and low level in the afternoon" pattern was disturbed, showing either a day-night reversal or rhythm disorders. This was most apparent in the subjects with severe and very severe TBI, who showed rhythm reversal on D 1, D 3 and D 7 after injury. The subjects with moderate TBI showed a rhythm reversal, i.e., "low level in the morning and high level in the afternoon", on D 1 and D 3 after injury. On D 7, the normal rhythm resumed. This was also consistent with the recovery process after TBI.

The relationship between MLT secretion and the prognosis of TBI

As illustrated in this study, the significant drop of serum MLT levels occurred in most patients with severe TBI. Several reasons can be postulated: (1) Direct damage. Patients with coma after severe TBI often have damaged cerebral midline structures. In particular, the cerebral midline structures of those with diffuse axonal injury are more prone to damage. If

the posterior portion of the third ventricle is injured, it will inevitably lead to direct damage of the pineal gland, causing abnormal secretion of MLT (Yuruker *et al.* 2015); (2) Central inhibition. Severe TBI is often accompanied by severe brain damage. After injury, the central nervous system (CNS) is severely inhibited (the clinical symptom being coma). The subdivisions of the CNS, such as the hypothalamus, pituitary, pineal gland, etc., are also inhibited, thus reducing MLT secretion (Alonso-Alconada *et al.* 2013; Barlow *et al.* 2014; Yuruker *et al.* 2015); (3) Indirect impact. The occurrence of cerebral edema or intracranial hematoma after severe TBI can significantly increase intracranial pressure, and indirectly suppress the pineal gland and other structures, thus affecting secretory functions like that for MLT (Alonso-Alconada *et al.* 2013; Barlow *et al.* 2014; Yuruker *et al.* 2015). The above reasons can also explain the results of this study, in which the more severe the disease condition was, the more clearly the MLT level dropped. In addition, the more the MLT level decreased when the patient was in the hospital, the lower was the GOS score 3 months after TBI.

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