S100B and its relation to intravascular bubbles following decompression

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Key words
Biomarkers, decompression sickness, bubbles, Doppler, diving research

Abstract
(Havnes MB, Hjelde A, Brubakk AO, Møllerløkken A. S100B and its relation to intravascular bubbles following decompression. *Diving and Hyperbaric Medicine*. 2010;40(4):210-2.)
Introduction: When neurological damage occurs in divers, it is considered to be caused by gas bubbles. Entrapment of these bubbles may lead to cellular injury and cerebral oedema. S100B is a protein biomarker that is released in CNS injuries and the concentration is related to the amount of brain damage.
Methods: A total of 27 rats were randomly assigned to one of three groups. Group I served as controls (n = 9). Group II (n = 7) underwent a simulated dive to 400 kPa and Group III to 700 kPa (n = 11). In groups II and III, venous gas bubble scores were evaluated by ultrasound during the first hour after surfacing. The amount of S100B in serum after the dives was tested using a commercial ELISA kit. Bubble grades were compared to S100B protein concentrations.
Results: The average level of S100B was significantly higher in rats compressed to 700 kPa compared to the control rats, (P = 0.038) and the rats compressed to 400 kPa, (P = 0.003). There was no difference in S100B concentration between groups I and II. Following the dive to 700 kPa, there were significantly higher bubble grades observed than following the dive to 400 kPa (P = 0.001).
Conclusion: The correlation between bubble grade and an increase in serum protein level of S100B indicates that this protein may be useful as a biomarker for neurological damage caused by decompression.

Introduction

When neurological damage occurs in divers, the prime suspects are vascular gas bubbles. Most bubbles are filtered out by the pulmonary capillaries but may also break through the lung filter or enter the arterial circulation via shunts to mediate bubble-induced tissue injury.[1,2] Entrapment of these bubbles may lead to cellular injury and cerebral oedema.[3] Under normal conditions, central nervous system (CNS) tissue is separated from plasma by the blood-brain-barrier (BBB), formed by endothelial cells of the brain microvessels and their underlying basement membrane.[4] The BBB acts as a sieve to restrict passage of large molecules, including most plasma proteins, into the CNS. Increased permeability of the BBB has been demonstrated after decompression, resulting in oedema.[3,5,6] Increased permeability of the cerebral vasculature could be due to changes induced by chemical factors released or activated by microbubbles or to direct mechanical injury of blood vessels from these bubbles.[7]

S100B is a protein biomarker that is released in CNS injury, the concentration being related to the amount of brain damage.[8] Elevated S100B concentrations have been reported in patients with stroke compared to control subjects and also in elite breath-hold divers after prolonged apnea.[9,10]

Signs and symptoms of decompression sickness (DCS) differ with the pressure profile and the breathing gas, but have a common first step, namely the formation of gas bubbles. The emerging evidence of the effects of venous gas emboli on the endothelium has led to the hypothesis that these are a main cause for neurologic DCS through its adverse effects on the CNS.[11] This experiment examines whether S100B could be used as a biological marker of decompression stress in the CNS and to examine a possible correlation between bubble grade and serum S100B concentration.

Methods

All experimental procedures and the care of experimental animals conformed to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and the protocol was approved by the Norwegian Council for Animal Research.

A total of 27 rats (female Sprague Dawley, Scanbur, Denmark) were randomly divided into three groups. Group I was a surface control group (100 kPa) breathing air (n = 9). Groups II and III underwent simulated dives for 45 min to 400 kPa (group II, n = 7) or 700 kPa (group III, n = 11) in a 20 L hyperbaric chamber. The compression and decompression rates were similar in both groups, 200 kPa min^{-1} and 50 kPa min^{-1} respectively. During the simulated dive, the rats were awake and observed through a window in the hyperbaric chamber.

Immediately after surfacing, the rats were anaesthetised with a subcutaneous injection of a mixture of haloperidol 0.33 mg, fentanyl 0.05 mg and midazolam 0.5 mg; 0.4 ml per 100 g body weight. The pulmonary artery was monitored at discrete intervals (15, 30, 45 and 60 min after surfacing) for gas bubbles using a 10 MHz transducer connected to a FiVe ultrasound scanner (GE Vingmed Ultrasound AS, Norway). Bubbles are seen on the monitor screen as bright

spots in the pulmonary artery, and verified with Doppler. Bubble quantity was graded on a 0 to 5 scale according to a previously described method.[12] Results are presented as maximum bubble grade during the observation period.

S100B levels in serum drawn one hour after the dives were tested using a commercial ELISA kit (BioVendor-Laboratorní medicína, Brno-Modice, Czech Republic). Bubble grades were compared to S100B protein concentrations.

STATISTICAL ANALYSIS

Because the S100B data in the control group were not normally distributed (confirmed by the Kolmogorov-Smirnov test and Q-Q plot) and the small number of animals studied, the Mann-Whitney test was used to assess differences between the groups. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). The results are presented as medians (25th and 75th percentiles).

Results

There was a significant difference in S100B concentration between the rats compressed to 700 kPa (Group III) and the rats in the control group (Group 1, $P = 0.038$) (Figure 1). There was also a significantly higher S100B concentration in the rats compressed to 700 kPa (Group III) compared to those compressed to 400 kPa (Group II, $P = 0.003$) (Figure 1). There were no significant differences between Groups I and II. Following the dive to 700 kPa, there were significantly higher bubble grades observed than following the dive to 400 kPa ($P = 0.001$) (Table 1).

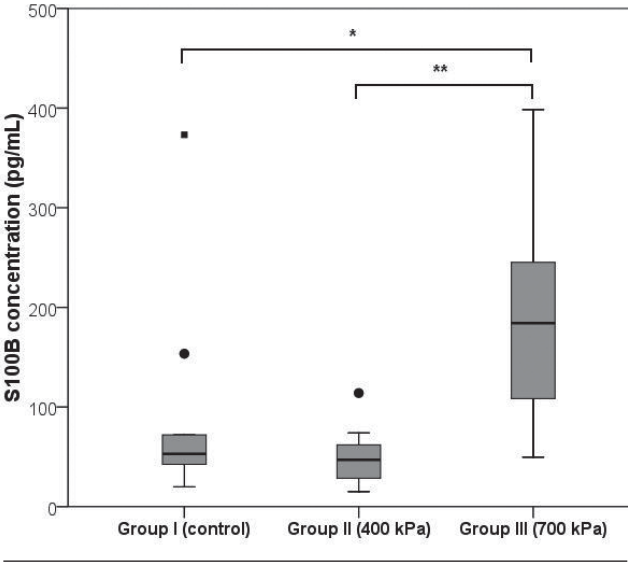
Discussion

We showed an increased concentration of S100B and a higher occurrence and grade of bubbles in rats compressed to 700 kPa compared to both normobaric controls and rats compressed to 400 kPa. The adverse effects of decompression have been discussed for decades, but markers for decompression stress other than the detection of vascular gas bubbles are still lacking. Although intravascular gas bubble scores are related to the risk of DCS and, higher grades with an increased incidence of DCS, there are large inter- and intra-individual differences in the response to bubbles. Thus, the search for other markers of decompression stress has continued. This inter-individual susceptibility to DCS and the fact that

Table 1
Bubble grades after dives to 700kPa and 400kPa (number of animals in each grade; *P = 0.001*)

	Bubble grade					
	0	**1**	**2**	**3**	**4**	**5**
Group II (400 kPa; $n = 7$)	4	3	0	0	0	0
Group III (700 kPa; $n = 11$)	2	0	1	0	0	8

Figure 1
Box plots of serum S100B concentration in undived rats and rats compressed to 400 kPa and 700 kPa, median, 25th and 75th percentiles shown; vertical lines represent the largest and smallest values except:
• outlier values > 1.5 box-lengths away;
■ extreme outlier > three box-lengths away;
(differences between groups: * P <0.05; ** P <0.005)



repetitive dives appear to result in greater tolerance to DCS due to acclimatisation, have given rise to the hypothesis that DCS might have an inflammatory basis.[13,14]

To our knowledge, there are no published data relating bubble formation and S100B concentration. However, S100B has been shown to be increased in goats after deep dives with rapid decompression.[15] These data are supported by our study in rats. On the other hand, a pilot study on S100B in human divers diagnosed and treated for acute DCS did not show an increased concentration of S100B.[16] However, a major difference between that study and ours is that the blood samples were drawn two to three days after the dives, while ours were drawn one hour after the dive. The half-life of S100B in relation to other diseases is estimated to be about 30 minutes, which might explain this difference.[17] In studies of patients with Alzheimer's disease or traumatic head injury, S100B appears to be a useful marker for brain function and, in ischaemic stroke patients, S100B concentrations correlate with infarct volume.[18–20]

Whether a high bubble grade produces brain injury in rats is not known, but it is reasonable to believe so. Injected microbubbles have been shown to affect the BBB in guinea pigs.[21] This method might give better control of the amount of bubbles than in decompressed rats, but is highly invasive and, thus, might alter other variables that could affect biomarker production.

We cannot say for sure that the concentration of S100B in serum is related to neurological DCS since we do not have

a neurological examination of the rats. However, recent experiments suggest that bubbles caused by diving can affect the BBB, and that there is an increased concentration of S100B in human subjects after traumatic brain injury.[22,23] Our preliminary results indicate a correlation between exposure to pressure and the expression of S100B.

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