Circulating S100B is increased after bilateral femur fracture without brain injury in the rat

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Background. S100B is an acknowledged marker of brain damage. However, trauma without brain damage also causes an increase in S100B. S100B concentrations are highest in multiple trauma patients with long bone fractures. Clinically, extensive long bone fractures are associated with haemorrhagic shock and haemorrhagic shock per se is associated with increased S100B. The aim of our experimental study was to verify the S100B increase in long bone fracture without haemorrhagic shock.

Methods and results. Bilateral femur fracture was carried out in 10 anaesthetized rats. Blood samples were drawn for immuno-luminometrical S100B measurement 5, 15, 30, 120, and 240 min after fracture. Mean arterial pressure (MAP), heart rate, and body temperature were monitored continuously. S100B increased after bilateral femur fracture and reached a peak 30–120 min after fracture ($P < 0.001$). MAP remained at a level which is not associated with shock in rats. Heart rate and body temperature remained unchanged. Autopsy verified open bilateral femur fracture surrounded only by small zones of clotted blood.

Conclusions. S100B is increased in bilateral femur fracture without haemorrhagic shock in rats. This finding suggests that bone marrow is a potential extracerebral source of S100B.

Keywords: blood, protein S100; brain, traumatic injury; complications, long bone injury

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S100B, a calcium-binding protein found in astroglial and Schwann cells, is an acknowledged marker of brain damage.¹ However, according to recent clinical evidence, the concept that S100B only originates in astroglial and Schwann cells is incorrect.² Following trauma, S100B increases have been reported without brain injury.³,⁴ Clinical studies have linked extracerebral S100B increase to three main sources: fat, muscle, and bone marrow.² The greatest posttraumatic S100B increase in patients without head injuries have been found in long bone fractures.³ Clinically, extensive long bone fractures are often associated with haemorrhagic shock. Experimentally, haemorrhagic-traumatic shock leads to an S100B increase linked to shock severity.⁵ We postulated that the S100B increase found clinically might have been a result of concomitant haemorrhagic shock. Therefore, our aim was to verify the S100B increase in experimental long bone fracture without haemorrhagic shock.

Methods and results

The study followed the requirements defined in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (publication NIH 86-23, revised 1985) and was approved by the Animal Protocol Review Board of Vienna. Male Sprague–Dawley rats ($n=10$, 320–360 g, Animal Research Laboratories, Himberg, Austria) were allowed free access to standard laboratory chow and water during a 7-day adaptation period after delivery to our experimental unit. Before the experiment, the rats were fasted overnight with further free access to water. For the induction of anaesthesia, each rat was placed in a plexiglass box connected to a Rotameter adjusted to 3 vol% isoflurane in air. After the rat had inhaled isoflurane/air for approximately 2 min and ceased to react when the plexiglass box was moved, a mixture of ketamine 100 mg kg⁻¹ and xylazine 10 mg kg⁻¹ was injected intramuscularly into the
right front extremity. Subsequently, the rat was positioned on a temperature-controlled surgical board, where it remained during the entire experiment. Anaesthesia was maintained with isoflurane 0.5% and nitrous oxide 60% in oxygen, the rat breathing spontaneously into a plexiglass head cover connected to a Rotameter. The tail artery was cannulated under aseptic conditions with a polyethylene catheter (PE-50; Fresenius, Germany) to monitor mean arterial pressure (MAP) and heart rate (Cardiosys, Experimetria, Budapest, Hungary), to administer volume replacement, and to sample blood. Body temperature was measured using a rectal temperature line (Fluke 52 Thermometer, Fluke Corporation, Everett, WA, USA) and maintained between 35.8 and 37°C by adjusting the temperature-controlled surgical board as required, and covering each rat with plastic wrap.

A baseline blood sample was drawn after a stabilization phase 20 min following cannulation of the tail artery. Five minutes after the baseline sample had been drawn, bilateral femur fracture was carried out by three-point bending applied to the handle of a custom made fracture apparatus similar to surgical forceps. After femur fracture, the rat was positioned on its right side. Subsequently, continuous volume replacement with pre-warmed Ringer’s solution was started at 30 ml kg⁻¹ h⁻¹ to maintain MAP above a level which could have been associated with shock in rats. Heparinized (10 units ml⁻¹; Baxter Co., Vienna, Austria) blood samples were drawn at the following time points after femur fracture: 5, 15, 30, 60, 120, and 240 min. Of the 10 samples drawn at each time point, four were lost because of technical complications at 5 and 15 min, respectively. All samples were centrifuged immediately and frozen to −72°C for analysis. After the last blood sample had been drawn, the rat was killed while still under anaesthesia and bilateral open femur fracture was surgically verified.

Plasma S100B was measured using a commercially available assay (LIA mat Sangtec 100, Byk-Sangtec Diagnostika, Bromma, Sweden). This is a monoclonal two-site immuno-luminometric assay with a detection limit of 0.1 μg litre⁻¹ for S100B in humans. According to the manufacturer, this test is cross-reactive with rat plasma. As the normal range for rats is not known, baseline values were considered normal and all other S100B values were compared with baseline. Data are presented as median with 25 and 75 percentiles (Q1, Q3). Differences to baseline at the pre-defined time intervals were evaluated by Friedman’s test, followed by Dunn’s Multiple Comparison Test. P<0.05 was considered significant.

S100 increased after bilateral femur fracture, reaching a peak 30–120 min after fracture (P<0.001) (Fig. 1). MAP decreased but remained above a level associated with shock in rats at baseline, 5, 15, 30, 60, 120, and 240 min after bilateral femur fracture (71 (69, 77), 65 (60, 75), 65 (60, 72), 60 (59, 69), 59 (58, 61), 59 (58, 70), and 60 (59, 60)) mm Hg (median (Q1, Q3)), respectively. Heart rate and body temperature remained unchanged. Autopsy verified open bilateral femur fracture in all rats. Fractures were surrounded only by small zones of clotted blood. None of the rats had signs of massive blood loss as a result of femur fracture.

Comment
We found that S100B is increased after bilateral femur fracture without haemorrhagic shock in rats. Increasing attention is being devoted to possible extracerebral sources of the neuromarker S100B. It has been suggested that early increases in S100B are a result of extracerebral rather than cerebral tissue damage, both after cardiac

![Fig 1 S100B concentrations in plasma at baseline and at different time points after bilateral femur fracture in rats. S100B in μg litre⁻¹ expressed as median, 25th and 75th percentiles; ***P<0.001.](https://academic.oup.com/bja/article-abstract/91/4/595/291029)
surgery, though a lack of brain damage was confirmed by physical examination and patient interviews, it was not actually ruled out by neuro-imaging. A clinical study performed during cardiac surgery found the highest extracerebral concentrations of S100B in surgically traumatized fat, muscle, and bone marrow drained or suctioned into mediastinal blood. Although our earlier experimental studies showed no S100B increase after trauma to fat and muscle, we did find that S100B is increased after haemorrhagic shock and is associated with the severity of shock. In previous experimental work, we found that MAP levels between 60 and 70 mm Hg in anaesthetized sham rats similar to those in the present experiment were not associated with any S100B increase. Resuscitation was provided in the present experiment to ensure that the S100B increase was fracture-related and not merely induced by hypovolaemia. The S100B increase within 30 min of fracture is probably attributable to protein release rather than to protein synthesis. In our opinion, bone marrow is the most obvious source of S100B release in our experimental model of bilateral open femur fracture.

From a clinical point of view, these findings indicate that increased S100B within the first hours after multiple trauma may result from long bone fractures and/or haemorrhagic shock and does not necessarily indicate brain damage.

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References