The early response to major trauma and intramedullary nailing

The stress response to trauma is the summation of the physiological response to the injury (the ‘first hit’) and by the response to any on-going physiological disturbance or subsequent trauma surgery (the ‘second hit’).

Our animal model was developed in order to allow the study of each of these components of the stress response to major trauma. High-energy, comminuted fracture of the long bones and severe soft-tissue injuries in this model resulted in a significant tropotropic (depressor) cardiovascular response, transcerebral embolism of medullary contents and activation of the coagulation system. Subsequent stabilisation of the fractures using intramedullary nails did not significantly exacerbate any of these responses.

Major trauma with fracture of long bones results in tissue injury, the intravasation of medullary fat and marrow into the systemic circulation and the subsequent excitation of the coagulation and inflammatory pathways, resulting in the ‘stress’ response. Although the exact nature and sequence of these events are unknown, they are likely to be critically important in the later development of life-threatening complications.

Existing animal models either do not incorporate high-energy forces or use fixation methods which do not model normal osteosynthetic biomechanics.

We report a new ovine model in which reproducible high-energy injuries to bone and the soft tissues replicate the physiological sequelae of trauma. We investigated the haemodynamic, embolic and coagulation responses to high-energy fracture of the femur and tibia in this ovine model and determined how this response was modified by intramedullary reaming and nailing.

Materials and Methods

The study was performed under Home Office Licence after approval by the University of Edinburgh Ethical Review Committee.

We selected 21 one-year-old Scottish Blackface sheep weighing 35 kg to 50 kg and divided them into three groups of seven animals. The entire study was performed under general anaesthesia. Group 1 (the control group) underwent placement of monitoring cannulae and a femoral stabilising cable, but received no further intervention. Group 2 (the ‘fracture’ group) had fractures of both femora and both tibiae. Group 3 (the ‘fracture and nailing’ group) had the same fractures as group 2, followed by stabilisation by reamed intramedullary nailing.

General anaesthesia was induced with etomidate (Hypnomidate; Janssen-Cilag, Sandton, United Kingdom; 0.5 mg kg\(^{-1}\)) and midazolam (Hypnovel; Roche, Welwyn Garden City, United Kingdom). After intubation and positive-pressure ventilation using a mechanical ventilator (Manley Pulmovent MPP, Harlow, United Kingdom) with halothane (Rhodia Organique, Bristol, United Kingdom), neuromuscular blockade was established using intravenous rocuronium (88 µg/kg; Esmeron, Organon-Technika, Boxtel, The Netherlands).

A pulmonary artery catheter (Swan-Ganz CCOmboV; Edwards Lifesciences, Irvine, California) allowed measurement of the central venous pressure, pulmonary artery pressure, continuous cardiac output and venous oxygen saturation. A cannula in the right auricular artery allowed for continuous monitoring of arterial pressures and sampling for arterial blood-gas measurements. All continuous haemodynamic and respiratory data were saved at intervals of five seconds using dedicated software (Datex-Ohmeda Collect, Hoevelaken, The Netherlands). A venous cannula in the right jugular vein was used for the administration of drugs, maintenance fluids (0.9% saline at 10 ml kg\(^{-1}\) hour\(^{-1}\)) and for obtaining venous blood samples. Aliquots of 6 ml were withdrawn into citrate collecting tubes at the base-
line, 15 minutes after each fracture, 15 minutes after each nailing procedure and at the end of the experiment. Samples were centrifuged at 3000 rpm for 30 minutes; the supernatant was aspirated and then stored at -80˚C. The prothrombin and activated partial prothrombin times were measured in batches. Plasma levels of fibrinogen and anti-thrombin II (AT III) were assayed using Enzyme-linked immunosorbent assay (ELISA) techniques.

A transoesophageal echocardiography probe was used to obtain screen images of the pulmonary artery (Hewlett Packard Sonus 2000 scanner, Philips Medical Systems, Reigate, United Kingdom). VHS video recordings were made for 30 s before each procedure, and continued for at least two minutes, or until all embolism had ceased. Embolism was graded by two observers blinded to the experimental group and the mean score taken. A modified Mayo scoring system was used (Table I).

Sixty minutes after the induction of anaesthesia and after stable haemodynamic conditions for at least 15 minutes, a femoral stabilising cable (Dall-Miles, Stryker, Newbury, United Kingdom) was placed in the subtrochanteric region through a 4 cm incision. The limbs were immobilised using clamps at the knee and the subtrochanteric cables. A pneumatic actuator with a cylindrical head (100 mm bore ram, PRA/182000; Norgren Pneumatics, Lichfield, United Kingdom) was fired at 1.1 MPa, generating a thrust of 8600 N at the mid-diaphyseal level, creating high-energy comminuted fractures with overlying soft-tissue trauma. A total of four fractures were produced in sequence, at intervals of 15 minutes, the femoral fractures occurring first.

In group 3 (the ‘fracture and nailing’ group), stabilisation of all four fractures was performed one hour after the last fracture, through a midline arthrotomy of the knee. Preparation of the canal with sequential AO reamers allowed the introduction of a 10 mm Grosse-Kempf (Stryker) tibial nail into the femur and an 8 mm Seidel (Stryker) humeral nail into the tibia.

At the conclusion of each experiment the sheep was killed, while remaining anaesthetised, using an overdose of intravenous pentobarbital.

The heart, lungs and right kidney were removed for post-mortem analysis. At 24 hours, two tissue blocks were taken from the central, apical and ventral aspects of each lung and from the right renal cortex.

Sections were cut at 4 µm for staining by haematoxylin and eosin, Martius scarlet/blue (MSB) and Oil Red O. Histological analysis was performed by a histopathologist, blinded to the experimental group. The number of regions containing embolic fat, bone marrow or thrombus were counted in each of the standardised sections.

**Statistical analysis.** The mean values of the haemodynamic measurements for each collecting period were calculated. Data were collected from 60 s before the fracture for 55 s, from 2.5 s after the fracture for 10 s, from 50 s after the fracture for 20 s, from 110 s for 20 s, from 290 s for 20 s, from 590 s for 20 s and at 890 s for 20 s. In addition, the results at 5 s and 10 s were recorded.

To reduce multiple testing in the statistical analyses, further mean values were calculated from their measurements to give a short-term (5 s to 1 minute) and long-term (2 to 15 minutes) outcome value, each of which was compared with the pre-fracture levels using the paired t-test. In the control group, data were collected at the same time intervals. Pre-fracture levels and the effect of the serial fractures were then compared between the intervention and control groups by a two-sample t-test. The mean combined embolus scores for the fractures were compared with the mean combined scores for the reaming and nailing procedures, using a paired t-test.

### Results

**Immediate haemodynamic response to fracture.** The heart rate and systolic blood pressure fell immediately in response to both femoral fractures and this response was maintained for at least 15 minutes. Central venous pressure and pulmonary artery pressure also fell in response to a fracture and this response reached greatest significance at between 2 minutes and 15 minutes after injury. The effect of these depressions was incremental, in that the hypotension after one fracture had not recovered before the depressant effect of the subsequent fracture occurred. No significant additional haemodynamic response was seen after nailing and reaming.

**Haemodynamic changes in response to sequential fractures and nailing.** While the heart rate did not change significantly over the experimental period, arterial pressure fell significantly by a mean of 35 mm Hg during the series of four fractures (p < 0.03). No further decrease occurred as a result of the process of reaming and nailing of these fractures. The central venous pressure in the control group rose during the course of the experiment, but fell by approximately 3 mm Hg in response to the fractures and then remained stable. No evidence of spontaneous recovery from this fall in the central venous pressure was seen.

**Embolism.** The median embolic score was calculated for each of the four fractures, the reaming sequences and the nailing procedures (Fig. 1). Pronounced and sustained embolic showers followed each femoral fracture. Smaller showers were detected after the tibial fractures. The embolic scores during the reaming and nailing sequences were...
significantly less than those after fracture (p < 0.01) (Fig. 1).

The first use of the reamer was associated with maximal embolism, but insufficient embolism was detected to allow the accurate calculation of separate scores for each component of the reaming sequence. The size of the particles tended to increase with time. Only small particles were seen at the time of the first femoral fracture but larger particles were observed during subsequent injuries and treatment. Minor, short-lived, embolic events were observed occasionally when the sheep was moved to prepare for a further fracture.

Fat and thrombus emboli were confirmed histologically in most blocks of lung tissue studied. Insufficient material was detected for meaningful quantitative analysis. Six areas of thromboembolism were detected in group 2 and a further seven in group 3. No emboli were detected in the control animals and no systemic embolism was seen in any block of renal tissue.

Coagulation. The prothrombin time increased over the duration of the experiment in all the animals, but there was no significant difference between the groups. The activated partial prothrombin time did not vary significantly either with time or between groups. There was a progressive decrease in the levels of fibrinogen and antithrombin III. This decrease was significantly greater in groups 2 and 3 than in group 1 (p < 0.01 and p < 0.001, respectively). There was no difference between groups 2 and 3 in the levels of fibrinogen or antithrombin III.

Discussion

We have demonstrated that after major traumatic injury in an ovine model, there was an immediate significant haemodynamic depressant response, followed by demonstrable transcardiac embolism. Subsequently, the coagulation system was activated as demonstrated by the consumption of fibrinogen and antithrombin III. These responses were not seen in the control animals. The response was not significantly altered in the animals that subsequently underwent fracture stabilisation surgery.

Comparison with other animal models. Previous animal research in this field has centred on the effect of stabilisation of the fracture in a variety of models. These have studied fat embolisation during surgery and have concentrated on the instrumentation and pressurisation of intact or osteotomised bones. The variables studied have been; the effect of different stabilisation techniques, the effect of different designs of reamer or nail and the importance of concurrent lung contusion. However, we are not aware of any previously reported studies of the physiological effect of high-energy injuries incorporating fracture of bones.

In order to study the stress response to trauma, it is important to recreate the ‘first-hit’ injury because this causes the activation and priming of neutrophils, and thereby materially alters the nature of the stress response to the subsequent ‘second-hit’ of continuing or surgical stress. Some investigators have approached this issue by creating haemorrhagic shock by venesection and then proceeding to reaming after a period of recovery of 48 hours, or by creating other injuries such as pulmonary contusion. However, there are physiological limitations to this approach. Haemorrhage in the absence of injury has been shown to result in a markedly different and less severe physiological response than that seen when it occurs in the presence of an injury. Moreover, skin itself is an important reservoir of pre-formed cytokines and the soft-tissue component of the injury produces an important stress response which may materially influence outcome. Finally, the use of intact or osteotomised bones in the study of intramedullary instrumentation produces an artificially fat embolism. This is amplified in some models by the additional application of pressurisation using cement.

Thus existing animal models may not be ideally suited to the study of the stress response because they do not recreate the milieu of the ‘first hit’ and because they artificially increase the extent of the embolic component of the ‘second hit’.

Immediate haemodynamic response to fracture. The immediate haemodynamic response to trauma was depression of both the heart rate and blood pressure. This is of interest since nociceptive stimuli are classically associated with sympathetic ‘alerting’ or ‘ergotropic’ responses mediated by the sympathetic nervous system. The most likely aetiology of this response is the von Bezold-Jarisch reflex. This is a neurologically-mediated phenomenon, in which a somatic afferent stimulus from the injury generates an autonomic (parasympathetic) efferent reflex. Although initially described as the response to an intracardiac stimulus, this term has been expanded to include similar responses seen in human subjects after other stimuli including manipulation of a fracture, shoulder arthroscopy, or even emotion. Experimentally, cardiovascular depression resulting in hypotension, vasodilatation and decreased oxygen
delivery has previously been demonstrated in models of skeletal muscle injury and severe haemorrhage.\textsuperscript{11, 26} The response is of importance because its effect is to delay the repayment of the oxygen debt arising from shock, thus prolonging resuscitation, and to worsen gut ischaemia thereby paving the way for later sepsis and multiple organ failure.

There are a number of other possible physiological mechanisms which are known to result in similar cardiopulmonary responses in man and may contribute to this reaction. These include the arterial chemoreceptor reflex, which may in certain circumstances result in bradycardia and hypotension. However, there was no fall in arterial oxygenation before the observed cardiovascular response, making this unlikely. An alternative explanation is cardiovascular depression from the anaesthetic agent. However, this is unlikely to be important when considering the speed of the response and the lack of a significant depressant effect in the control animals. Another explanation is activation of the cardiac C-fibre afferent reflex. Although it is unclear what may have stimulated this response in our study, sudden thoracic movements may have been sufficient to do so.

Under certain circumstances, therefore, an overwhelmingly noxious stimulus in both animals and man invokes a physiological response characterised by sympathetic withdrawal and increased parasympathetic outflow.\textsuperscript{24} It is unclear whether the injury response described in our study is identical to that in man, since the collection of physiological data at the time of injury clearly presents methodological problems and the ergotropic effects of hypotensive haemorrhage, pain and fear may subsequently alter this response to produce the more usual presenting features of hypotension with tachycardia. Patients presenting with hypotension after trauma may not be purely hypovolaemic, particularly when this response is seen in association with bradycardia; there may be an autonomically-mediated cardiovascular depression. The pharmacological manipulation of this response may be a valuable adjunct to fluid resuscitation in these patients and this possibility deserves further research.

Activation of coagulation. This has previously been shown to occur after bone injury in rabbits\textsuperscript{27} and sheep,\textsuperscript{9} as well as in human patients.\textsuperscript{2} Reamed nailing of the intact femur causes a reduction in levels of fibrinogen\textsuperscript{9, 27} and antithrombin III\textsuperscript{27} in both rabbits and sheep. By contrast, unreamed femoral nailing has been shown not to cause a significant decrease in these levels below baseline.\textsuperscript{9} It has been proposed that the degree of reduction is associated with the degree of injury sustained.\textsuperscript{13} We have found a significant decrease in the levels of both fibrinogen and antithrombin III after fracture alone, further suggesting that models incorporating instrumentation of intact bones may not reveal the full extent of the physiological response.

Limitations of the study. Inevitably, the extrapolation of results in experimental animals to human patients requires caution. Nevertheless, the sheep has been used in a number of previous studies providing the basis for comparison. Moreover, implants designed for human patients are suitable for use in sheep. However, the sheep femur is smaller both absolutely and proportionally, compared with the human femur and it has been suggested that this reduces the amount of medullary fat available for embolism.\textsuperscript{8}

The duration of the study period (five hours) allowed the investigation of the early responses to injury and initial treatment, but not that of the later stages of the inflammatory response and other more direct measures of lung injury. The nature of the apparatus, consisting of a single actuator, allowed only one fracture to be produced at a time. It would have been preferable to be able to produce all injuries simultaneously, and modifications are planned for future studies.

Grading of the embolic response is subjective, and previous reports in this field have tended to use scales from 0 to 3.\textsuperscript{28, 29} The more detailed scoring system used by Ereth et al\textsuperscript{10} was modified to provide greater discriminative ability (Table I). High-energy fractures in sheep appear to cause embolism which is of a lower initial intensity but of longer duration than that from experimental models of embolus using instrumentation of intact or osteotomised femora.\textsuperscript{29, 31-33}

We have demonstrated a number of elements of the early stress response to high-energy trauma and have shown that fractures, and fractures treated by intramedullary nailing, have indistinguishable responses in this model. An understanding of this early stress response pathway is desirable, as the ability to measure and manipulate it will potentially allow the response to be attenuated or avoided, thereby reducing the morbidity and mortality from trauma.

Supplementary Material

Tables showing the results of studies on emboli in sheep and other animals are available with the electronic version of this article on our website at www.jbjs.org.uk

We gratefully acknowledge assistance from Professor H. Simpson, Dr R. Elton, Dr A. Lee, and Mr P. Dark. Mrs J. Docherty and Mr B. Hawes provided extensive technical assistance.

The project was financed by a Pump Priming grant from the Royal College of Surgeons of Edinburgh.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References


THE JOURNAL OF BONE AND JOINT SURGERY


