J Musculoskelet Neuronal Interact 2016; 16(4):369-376

Journal of Musculoskeletal and Neuronal Interactions JMNI

Original Article

Sodium selenate treatment mitigates reduction of bone volume following traumatic brain injury in rats

R.D. Brady[1], B.L. Grills[1], T. Romano[1], J.D. Wark[2], T.J. O'Brien[2], S.R. Shultz[2+], S.J. McDonald[1+]

[1]Department of Physiology, Anatomy and Microbiology, La Trobe University, VIC, 3086; [2]Department of Medicine, The Royal Melbourne Hospital, The University of Melbourne, VIC, 3010, Australia
[+]Joint senior authors

Abstract

Objectives: Administration of sodium selenate to rats given traumatic brain injury (TBI) attenuates brain damage and improves long-term behavioural outcomes. We have previously provided evidence that TBI causes bone loss in rats, however the effect of sodium selenate treatment on bone quantity following TBI is unknown. **Methods:** Rats were randomly assigned into sham injury or fluid percussion injury (FPI) groups and administered saline or sodium selenate for 12 weeks post-injury. Femora were analysed using histomorphometry, peripheral quantitative computed tomography (pQCT) and biomechanical testing. **Results:** Distal metaphyseal trabecular bone volume fraction of FPI-selenate rats was higher than FPI-vehicle rats (41.8%; $p<0.01$), however, femora from selenate-treated groups were shorter in length (4.3%; $p<0.01$) and had increased growth plate width (22.1%; $p<0.01$), indicating that selenate impaired long bone growth. pQCT analysis demonstrated that distal metaphyseal cortical thickness was decreased in TBI rats compared to shams (11.7%; $p<0.05$), however selenate treatment to TBI animals offset this reduction ($p<0.05$). At the midshaft we observed no differences in biomechanical measures. **Conclusion:** These are the first findings to indicate that mitigating TBI-induced neuropathology may have the added benefit of preventing osteoporosis and associated fracture risk following TBI.

Keywords: Neurotrauma, Bone Metabolism, Endochondral Ossification, Bone Growth, Osteoporosis

Introduction

Traumatic brain injury (TBI) is a neurodegenerative condition induced by an external mechanical force to the brain and is a leading cause of mortality and disability worldwide[1]. Brain damage caused by mechanical forces at the moment of impact is often categorized as the primary injury and may involve necrotic cell death, neurovascular damage, axonal injury, oedema and ischaemia[1]. These primary injuries initiate a complex cascade of secondary injury pathways that may develop over the minutes, days and months that follow[2]. In addition to these central effects, TBI can also result in systemic changes in the immune, endocrine and sympathetic systems[2-4], all of which influence bone homeostasis[5-7]. As such, we previously assessed the effect of experimental TBI on bone in the rat. We found that bone mineral density, trabecular bone volume ratio, cortical bone thickness and cortical bone content, were decreased in femora of rats subjected to TBI at 12 weeks post-injury compared to sham-injured rats[8]. These findings, along with those from previous studies in rodents and humans[9-11], suggest that the systemic influence of TBI may also include detrimental effects on bone mass and that brain-injured patients may have an increased risk of developing osteoporosis and be more susceptible to bone fracture. Specifically, human studies indicate that brain-injured patients have an elevated risk of hip fracture which has been associated with increased morbidity and mortality[11].

There is currently no clinical pharmaceutical intervention available that improves long-term outcome following TBI[12]. However, the delayed and progressive nature of the neurodegenerative aftermath of TBI provides an opportunity for a pharmacological intervention to mitigate these effects[12]. Sodium selenate is a potent PP2A/PR55 activator that reduces

The authors have no conflict of interest.

Corresponding author: Stuart McDonald, Department of Physiology, Anatomy and Microbiology, La Trobe University, VIC, 3086, Australia
E-mail: stuart.mcdonald@latrobe.edu.au

Edited by: F. Rauch
Accepted 19 August 2016

the hyperphosphorylation of tau[13-15], and we previously assessed a 12-week sodium selenate treatment regimen as a novel TBI therapy in rats[16]. Sodium selenate treatment significantly increased brain tissue PP2A/PR55, and reduced tau phosphorylation, brain damage and behavioral impairments following TBI compared to saline-vehicle treatment[16]. These findings demonstrate that sodium selenate may be a novel therapeutic approach to improve outcome following TBI. Importantly, sodium selenate is highly water-soluble, readily crosses the blood brain barrier, and a treatment regimen similar to that used in this study has already been demonstrated to be safe in a six month Phase I trial in patients with prostate cancer[15]. Sodium selenate is also currently being assessed in a Phase II study for Alzheimer's disease[14]. Considering the lack of an effective pharmaceutical intervention for TBI patients, sodium selenate has the potential to be translated into clinical TBI trials.

Whether sodium selenate treatment and/or attenuating the brain injury affects bone mass after TBI, however, has yet to be determined. Furthermore, although we found no overt negative effects of sodium selenate treatment in rats in our initial TBI study[16], and there were no documented serious side-effects in sodium selenate trials in Alzheimer's patients[14], the potential for selenium toxicity is an important issue to consider. Though no studies have characterized the effect of prolonged sodium selenate treatment on bone, there is preliminary evidence that sodium selenate or sodium selenate metabolites may have toxic effects on skeletal cells[17], and this is an important factor to consider as sodium selenate progresses towards clinical trials in TBI patients. For these reasons, here we investigated the effect of sodium selenate treatment on the quantity of bone within the femur of rats subjected to TBI. Male rats were randomly assigned into either sham injury or fluid percussion injury (FPI) groups and administered either saline or sodium selenate for 12 weeks post-injury. Open-field testing to assess locomotion was conducted at 4 and 12 weeks post-injury, and rats were killed at 12 weeks post-injury for analysis of femoral structural and mechanical properties.

Materials and methods

Subjects

Fifty-four male Long-Evans hooded rats obtained from Monash animal research services (Melbourne, Australia) were 12 weeks of age and weighed 250-300 g at the time of injury. Rats were housed individually under a 12 hour light/dark cycle, and given access to food and water *ad libitum* for the duration of the experiment. Animal procedures were approved by The University of Melbourne animal ethics committee (#1112173), and all animal experiments were carried out in accordance with the guidelines of the Australian code of practice for the care and use of animals for scientific purposes by the Australian National Health and Medical Research Council.

Experimental groups

Rats were randomly assigned to one of four experimental conditions: sham-injury + saline-vehicle treatment (SHAM + VEH; n=13), sham-injury + sodium selenate treatment (SHAM + SS; n=15), fluid percussion injury (FPI) + saline-vehicle treatment (FPI + VEH; n=13), or FPI + sodium selenate treatment (FPI + SS; n=13). The assigned treatment began immediately post-injury, selenate treatment groups received 1 mg/kg/day[16], delivered continuously via subcutaneously implanted mini-osmotic pump (Model 2006, Alzet, CA, USA) for the entire duration of the study.

Surgery and FPI

FPI and sham-injury procedures were based on a standard protocol as previously described and used by our group[16]. Briefly, rats were placed in a sealed Plexiglas box into which 4% isoflurane and 2 L/min oxygen flow was introduced for anaesthesia. Anaesthesia was maintained throughout surgery using a nose cone with a standard stereotaxic device and 2% isoflurane + 500 mL/min oxygen flow. Under aseptic conditions rats underwent a craniotomy (5 mm diameter) centred -3.0 mm posterior and 4.0 mm to the right of bregma to expose the intact dura mater of the brain. A hollow plastic injury cap was sealed over the craniotomy with cyanoacrylate and dental cement. A horizontal incision was made between the shoulder blades to allow for the rapid insertion of the subcutaneous mini-osmotic pump immediately post-injury. The rat was then removed from anaesthesia and attached to the FPI device via the head cap. At the first response of hind-limb withdrawal, rats received a FPI pulse to the brain with a force of 3 atmospheres. Upon resumption of spontaneous breathing the head cap was removed, the treatment pump was inserted, and the incisions were sutured. Sham-injury rats underwent the same procedures, except the fluid pulse was not administered. Six weeks post-injury, rats were anesthetized, the initial treatment pump was removed, and a replacement treatment pump was implanted as described above.

Acute injury severity

Apnea, unconsciousness, and self-righting reflex times were monitored in all rats immediately after injury as indicators of acute injury severity[18-20]. Apnea was the time from injury to spontaneous breathing. Loss of consciousness was the time from injury to a hind-limb withdrawal response to a toe pinch. Self-righting reflex was the time from injury to the return of an upright position.

Open-field testing

Locomotion was assessed using an open-field as previously described[16,21]. Rats were tested at 4 weeks and 12 weeks post-injury. Rats were placed in the centre of a circular open-field arena (100 cm diameter) enclosed by walls 20 cm high, and allowed to freely explore for 5 min. Behaviour in the open-field was recorded by an overhead camera, and *Ethovi-*

Table 1. Acute injury severity measures, FPI resulted in worsened acute injury severity outcomes as indicated by increased apnea, hind-limb withdrawal, and self-righting reflex times compared to the sham-injured group regardless of the assigned treatment. *=FPI significantly greater than sham-injured group, $p<0.001$. Values are mean±SEM.

	SHAM + VEH	SHAM + SS	FPI + VEH	FPI + SS
Apnea	0	0	40±4*	40±3.0*
Unconciousness	0	0	320±30*	335±25*
Self-righting	140±11	132±12	512±23*	528±24*

sion Tracking Software (Noldus, Netherlands) quantified the total distance travelled.

Peripheral quantitative computed tomography (pQCT)

Femora (right) were fixed in paraformaldehyde and stored as previously described[22]. Scans were performed using a Stratec XCT-Research SA+ scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany) as previously described[23-25]. Briefly, a 1 mm slice (peel mode 20, contour mode 1) was taken at the distal metaphysis (15% of total femoral length, from upper border of distal condyle) and at the midshaft (50% of total femoral length, from upper border of distal condyle) to quantify cortical bone. Tissue of a density of 710 mg/cm^3 or greater was considered cortical bone. Bone mineral content, density and thickness of femoral bone were analysed.

Histological processing, staining, and histomorphometry

Prior to histological processing, femoral length was measured using digital measuring callipers. Femora were then processed to plastic, sectioned and stained according to previous methods[26]. Briefly, femora were dehydrated in a graded series of ethanols and infiltrated and embedded in LR White resin (London Resin Company limited, Reading, England). Five micron thick longitudinal plastic sections were cut at the midpoint of undecalcified femur on a Leica RM 2155 Rotary Microtome (Leica, Wetzlar, Germany) with a tungsten carbide knife. Sections were stained using Goldner's modification of Masson's trichrome stain. Four sections per femur were examined and photographed on a Leica DMBRE microscope. Sections of femur were assessed both qualitatively and quantitatively. Trabecular bone measurements were obtained from a 5 mm^2 field, positioned 1 mm distal to the lowest point of the epiphyseal cartilage plate in the metaphysis using Leica Qwin software (Leica Microsystems, Wetzlar, Germany).

Mechanical testing

Biomechanical properties of the diaphysis of the left femur (mediolateral bending) were assessed using a three-point bending apparatus[27]. Load and deflection data were recorded continuously using transducers connected to an x-y plotter by preamplifiers. After testing, mechanically-fractured ends were imprinted into dental wax. Using images of each imprint, cross-sectional (CS) areas (mm^2) were measured using a Leica DMRBE microscope linked to a PC with Leica Qwin software (Leica Microsystems, Wetzlar, Germany). The overall cross-sectional area of each bone at its breaking point was then calculated by averaging the measured areas of each mechanically-fractured end. Biomechanical properties measured were: peak force to failure, stiffness, ultimate bending stress and bending modulus. All of these parameters were calculated from the load deflection data.

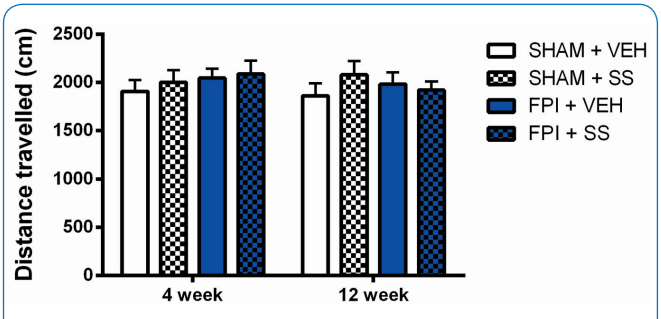

Figure 1. No difference in total distance travelled during open-field session was observed between groups.

Statistical analysis

A two-way ANOVA, with treatment and injury as the between-subjects variables, was used to analyze all measures. Tukey post-hoc comparisons were carried out when appropriate. All data was analyzed using GraphPad prism 6 (GraphPad software, Inc.) with significance defined as $p<0.05$.

Results

Acute injury severity

FPI worsened apnea ($p<0.001$), unconsciousness ($p<0.001$), and self-righting reflex times ($p<0.001$) compared to sham-injury regardless of the assigned treatment group (Table 1).

Locomotor activity

Locomotor activity was assessed at 4 and 12 weeks post-injury in the open-field. There were no differences in distance travelled in the open-field between sham and TBI rats at

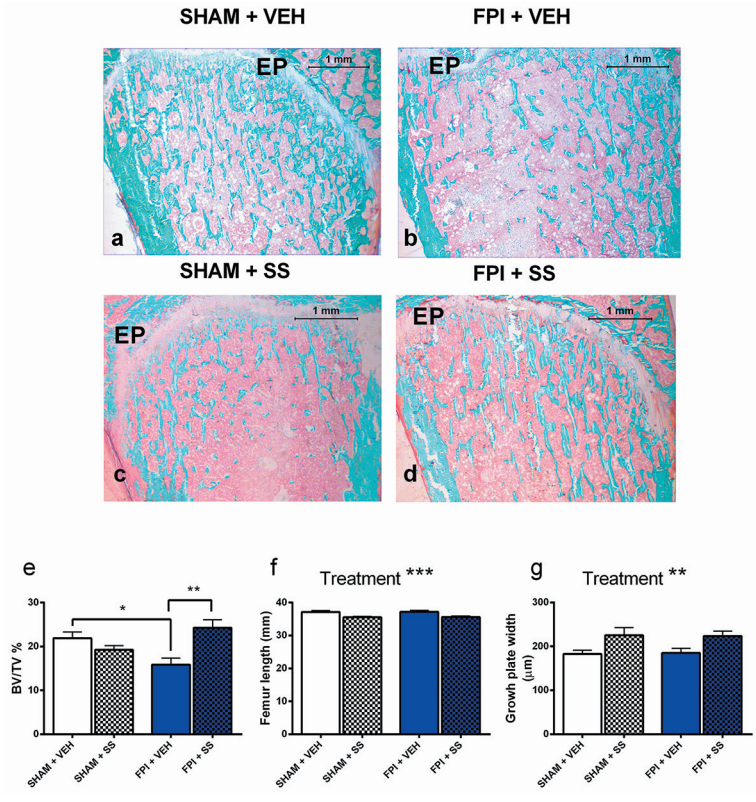

Figure 2 a-g. Longitudinal representative histological sections of the distal metaphyseal region of femora (a-d). Histological assessment shows FPI + VEH rats (a) had a lower fraction of trabecular bone compared to SHAM + VEH rats (b). Whereas, there was a larger proportion of trabecular bone in FPI + SS rats (d) when compared to FPI + VEH rats (c; Goldner's trichrome stain; original magnification 25 x). Histomorphometric analysis (e) shows a decreased trabecular bone volume fraction in FPI + VEH rats compared to SHAM + VEH rats however trabecular bone volume fraction was greater in FPI + SS rats compared to FPI + VEH rats. SS-treated rats had shorter femora (f) and wider growth plates (g) compared to VEH-treated rats. Treatment** indicates a significant main treatment effect. *p<0.05, **p<0.01, ***p<0.001.

any of the time-points regardless of the assigned treatment group (Figure 1).

Histologic and histomorphometric analysis

There was a decreased proportion of trabecular bone in FPI + VEH rats compared to SHAM + VEH rats, however there was a larger proportion of trabecular bone in FPI + SS rats when compared to FPI + VEH rats (p<0.01; Figure 2a-d). Data obtained from histomorphometric analysis (Figure 2e) reflected the qualitative histological assessment; as two-way ANOVA revealed a significant injury x treatment interaction (p<0.001) on the trabecular bone volume ratio (BV/TV). Post-hoc analyses confirmed a significant decrease in the percentage of trabecular bone in FPI + VEH rats compared to both SHAM + VEH and FPI + SS groups (p<0.01; Figure 2e).

Two-way ANOVA also revealed a significant treatment effect on femoral length (p<0.001), with femoral length of SS-treated rats decreased by 4.3% compared to VEH-treated rats (p<0.001; Figure 2f). Growth plate width was increased by 22.1% in SS-treated rats compared to VEH-treated rats (p<0.01; Figure 2g).

pQCT

Two-way ANOVA revealed a significant injury x treatment interaction (p<0.005) on the measure of cortical thickness. Post-hoc analysis indicated that FPI + VEH rats had significantly reduced cortical thickness compared to both SHAM + VEH and FPI + SS groups (p<0.05; Figure 3a), whereas the SHAM + VEH and FPI + SS groups did not differ. Analysis of the distal metaphyseal region of femora revealed no difference in total bone density or cortical content (Figure 3b, c). No differences were observed at the midshaft in any measured parameter (Figure 3d-f).

Mechanical testing

There were no statistically significant differences in biomechanical measures (Table. 2).

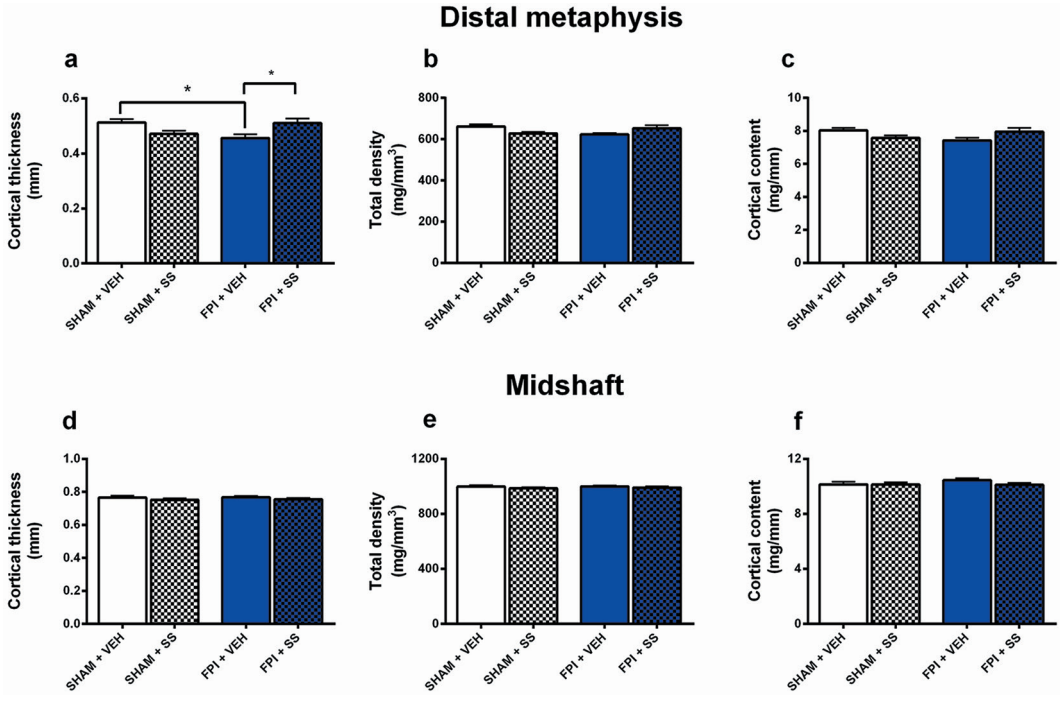

Figure 3 a-f. pQCT analysis of bone parameters of the distal metaphyseal and midshaft region of femora. The distal metaphyseal region of femora from FPI + VEH rats had reduced cortical thickness compared to both SHAM + VEH and FPI + SS groups (a). No difference in total bone density or cortical content were observed (b and c) at the distal metaphysis. Nor were there any differences observed at the midshaft in measured parameter (3d-f). * $p<0.05$.

Table 2. Biomechanical characteristics of sham-vehicle, sham-selenate, FPI-vehicle and FPI-selenate femoral samples. There were no differences in any measured mechanical properties between any groups. Values are mean ± SEM.

	SHAM + VEH (n=15)	SHAM + SS (n=15)	FPI + VEH (n=14)	FPI + SS (n=14)
Peak force (N)	222±6	221±7	230±10.7	225±11
Stiffness (x 10^4 Nm^2)	29.5±1.0	30.1±1.1	29.7±1.0	29.2±1.1
Cross sectional area (x 10^{-6} μm^2)	6.8±0.2	6.8±0.2	6.2±0.6	6.8±0.3
Ultimate bending stress (x 10^7 Nm^{-2})	6.9±0.2	6.8±0.2	6.8±0.2	7.0±0.3
Bending modulus (x 10^9 Nm^{-2})	3.4±0.2	3.4±0.2	3.2±0.2	3.4±0.1

Discussion

We recently demonstrated that TBI is detrimental to bone microstructure in rats[8], which is consistent with human findings that link brain injury to the later onset of osteopenia, osteoporosis and long-term elevated risk of fracture[28,29]. We have also previously shown that sodium selenate treatment attenuates brain damage and reduces behavioural impairments following experimental TBI in rats[16]. The effect sodium selenate treatment has on TBI-induced reduction in bone mass is unknown, but its effect on bone is important to consider if its potential therapeutic use is to be translated to the clinical TBI setting. Therefore, here we assessed the effect of continuous sodium selenate treatment on the quantity of bone within the femur of rats subjected to TBI 12 weeks post-injury. Histomorphometric analysis demonstrated that there was a larger proportion of trabecular bone in TBI rats treated with selenate compared to their vehicle treated counterparts. Furthermore, pQCT analysis revealed that cortical thickness was increased in brain-injured rats that were treated with selenate compared to brain-injured rats treated with vehicle. Interestingly, femora from selenate-treated rats were shorter in length and had increased growth plate width compared to vehicle-treated rats. Taken together, these findings suggest

that selenate treatment mitigated TBI-induced reductions in bone volume, however it concomitantly reduced femoral growth. We also found that there were no differences between groups in distance travelled in the open-field which suggests that the effects of TBI and selenate treatment on bone were not confounded by immobilization.

The patterns of reduced bone volume in animals given TBI in this study were similar to those described in our previous investigation[8], with the effects of TBI most evident at the femoral distal metaphysis. We observed no differences in mechanical properties or bone volumes of the femoral midshaft, which is perhaps not surprising given this region is almost exclusively comprised of cortical bone, which remodels at a slower rate than trabecular bone due to a low surface-to-volume ratio[30]. It is possible that changes at this region take longer to manifest and may have occurred at time-points not featured in this study. In accordance with our previous findings, here we observed significant reductions in bone volume at the distal metaphysis, which is a metabolically active region of bone that is susceptible to bone loss[31].

Brain-injured rats treated with sodium selenate did not have the reductions in distal metaphyseal bone volume of their vehicle-treated counterparts. Therefore it is possible, that the increased bone volumes observed in brain-injured selenate-treated rats was due to the attenuation of the brain injury via selenate treatment. Several of the secondary injury mechanisms of TBI have the potential to have significant catabolic effects on bone. In particular, the neuroinflammatory response that occurs post-TBI can be chronically activated post-TBI[2], and can lead to persistent elevation of circulating cytokines, many of which have been shown to induce osteoclastic bone resorption[2,32]. Therefore, it is possible that sodium selenate prevented loss of bone via inhibition of the neuroinflammatory response post-TBI. In addition, the increased sympathetic outflow following TBI may also contribute to TBI-induced bone loss[4]. Several studies have shown how sympathetic activation of β-adrenergic signalling in osteoblasts inhibits bone formation and triggers osteoclastogenesis and bone resorption[4,33], therefore sodium selenate may have reduced the extent of sympathetic signalling and thus indirectly reduced bone loss.

Though we hypothesise that sodium selenate treatment reduced TBI severity and thereby prevented bone loss, it is possible that sodium selenate had direct influences on bone tissue that may have prevented bone loss following TBI. *In vitro* data suggest that sodium selenite, a major metabolite of sodium selenate[15], can induce apoptosis of osteoclast-like cells via the mitochondrial pathway[34]. Further, intraperitoneal injection of mice with sodium selenite was found to inhibit differentiation of bone marrow-derived monocytes into osteoclasts[34]. Therefore, taking these previous findings into account, it is possible that in the current study, the metabolite sodium selenite reduced bone resorption by decreasing osteoclastic formation and increasing osteoclastic apoptosis, which thereby reduced bone loss in selenate-treated TBI rats compared to vehicle-treated TBI rats. Interestingly, however, we saw no effects of sodium selenate treatment on distal metaphyseal bone volumes in sham animals, which possibly indicates that there was no direct effect of sodium selenate treatment on metaphyseal bone turnover in uninjured animals.

Direct effects of sodium selenate treatment were however apparent at the growth plate, with treated rats displaying reduced femoral length and increased growth plate width. A previous study has reported morphological changes in epiphyseal plates of rats treated with sodium selenate[17]. Furthermore, sodium selenite has been shown to induce growth retardation in rats[35,36]. The precise cellular and molecular mechanisms through which these above changes occur, however are not entirely understood. The reduced longitudinal bone growth and increased epiphyseal growth plate width observed in selenate-treated rats indicates that selenate treatment impaired cartilage-to-bone conversion at the growth plates. Known as endochondral ossification, this process involves the resorption of calcified cartilage matrix at the base of epiphyseal plates by osteoclasts, which then allows blood vessel invasion and bone formation by osteoblasts, which use the calcified cartilage matrix as a template on which to form bone[37]. Several animal studies have shown that pharmacological inhibition of osteoclastic activity with bisphosphonates markedly interferes with the endochondral ossification process, which results in widened growth plates and impaired longitudinal bone growth[38,39]. These findings, along with the aforementioned associations between sodium selenate and reduced numbers of osteoclasts, suggest that the femoral growth plate deficits observed in sodium selenate-treated animals likely occurred due to impaired osteoclastic activity.

A limitation of this study is the lack of dynamic bone formation parameters. For example, additional data examining bone formation rate, mineral apposition rate and mineralizing surface may have indicated whether the observed changes occurred due to reduced osteoblast activity which would have been informative and should be examined in future studies. Despite the observed deficits at the epiphyseal growth plate, the underlying metaphyseal trabecular and cortical bone volumes were unaffected by sodium selenate treatment, a finding that is similar to that reported in bisphosphonate-treated animals with similarly impaired long bone growth[38,39]. Further studies are necessary to determine the effects of sodium selenate on bone health in humans; however, our findings in rats suggest that sodium selenate treatment inhibits long bone growth in juveniles, but may not have detrimental effects on adult bone.

Conclusion

These are the first findings to indicate that selenate treatment was effective at preventing reductions in bone volume post-TBI. Selenate treatment, however, also decreased long bone growth in rats that had yet to reach skeletal maturity. Therefore, the observed detrimental effects on bone growth in the current study should be carefully considered if sodium selenate treatment is to be translated in a clinical TBI setting

in juveniles who experience a TBI. Further studies are needed to define the exact mechanism through which sodium selenate treatment prevented reductions in bone volume post-TBI, though the findings of this study, along with the previous rodent studies, provide strong preliminary evidence that pharmacological interventions attenuating TBI pathobiology may reduce the risk of osteoporotic development.

Acknowledgements

This work was supported by grants to SRS and TJO from the NHMRC, a fellowship to SRS from CIHR, and funding from La Trobe University to BLG and SJM.

Author Contributions statement

RDB, SJM and SRS drafted the manuscript, and all authors completed manuscript revision. SRS, TJO and SJM conceptualised and designed the experiment. SRS administered the injury methods and conducted open-field analysis. RDB, SJM, BLG and collected tissue. RDB, TR, JDW and SJM completed pQCT analysis. RDB, SJM and BLG completed histomorphometric and biomechanical analyses.

References

1. Xiong Y, Mahmood A and Chopp M. Animal models of traumatic brain injury. Nat Rev Neurosci 2013;14:128-42.
2. Kumar A and Loane DJ. Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain Behav Immun 2012;26:1191-201.
3. Mundy GR. Inflammatory mediators and the destruction of bone. J Periodontal Res 1991;26:213-7.
4. Hinson HE and Sheth KN. Manifestations of the hyperadrenergic state after acute brain injury. Curr Opin Crit Care 2012;18:139-45.
5. Hardy R and Cooper MS. Bone loss in inflammatory disorders. J Endocrinol 2009;201:309-20.
6. Holmes SJ, Economou G, Whitehouse RW, Adams JE and Shalet SM. Reduced bone mineral density in patients with adult onset growth hormone deficiency. J Clin Endocrinol Metab 1994;78:669-74.
7. Giustina A, Mazziotti G and Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev 2008;29: 535-59.
8. Brady RD, Shultz S, Sun M, et al. Experimental traumatic brain injury induces bone loss in rats. J Neurotrauma 2015.
9. Yu H, Watt H and Mohan S. The negative impact of traumatic brain injury (TBI) on bone in a mouse model. Brain Inj 2013.
10. Lee JI, Kim JH, Kim HW, et al. Changes in bone metabolism in a rat model of traumatic brain injury. Brain Inj 2005;19:1207-11.
11. Beaupre GS and Lew HL. Bone-density changes after stroke. Am J Phys Med Rehabil 2006;85:464-72.
12. Blennow K, Hardy J and Zetterberg H. The neuropathology and neurobiology of traumatic brain injury. Neuron 2012;76:886-99.
13. Corcoran NM, Martin D, Hutter-Paier B, et al. Sodium selenate specifically activates PP2A phosphatase, dephosphorylates tau and reverses memory deficits in an Alzheimer's disease model. J Clin Neurosci 2010; 17:1025-33.
14. Zheng P, Shultz SR, Hovens CM, Velakoulis D, Jones NC and O'Brien TJ. Hyperphosphorylated tau is implicated in acquired epilepsy and neuropsychiatric comorbidities. Mol Neurobiol 2014;49:1532-9.
15. Corcoran NM, Hovens CM, Michael M, Rosenthal MA and Costello AJ. Open-label, phase I dose-escalation study of sodium selenate, a novel activator of PP2A, in patients with castration-resistant prostate cancer. Br J Cancer 2010;103:462-8.
16. Shultz SR, Wright DK, Zheng P, et al. Sodium selenate reduces hyperphosphorylated tau and improves outcomes after traumatic brain injury. Brain 2015;138:1297-313.
17. Campo RD and Bielen RJ. Acute toxic effects of sodium selenate on the epiphyseal plate of the rat. Calcif Tissue Res 1971;7:318-30.
18. Shultz SR, Tan XL, Wright DK, et al. Granulocyte-macrophage colony-stimulating factor is neuroprotective in experimental traumatic brain injury. J Neurotrauma 2014;31:976-83.
19. Johnstone VP, Shultz SR, Yan EB, O'Brien TJ, Rajan R. The acute phase of mild traumatic brain injury is characterized by a distance-dependent neuronal hypoactivity. J Neurotrauma 2014;31:1881-95.
20. Shultz SR, Sun M, Wright DK, et al. Tibial fracture exacerbates traumatic brain injury outcomes and neuroinflammation in a novel mouse model of multitrauma. J Cereb Blood Flow Metab 2015;35:1339-47.
21. Johnstone VP, Wright DK, Wong K, O'Brien TJ, Rajan R, Shultz SR. Experimental Traumatic Brain Injury Results in Long-Term Recovery of Functional Responsiveness in Sensory Cortex but Persisting Structural Changes and Sensorimotor, Cognitive, and Emotional Deficits. J Neurotrauma 2015;32:1333-46.
22. McDonald SJ, Dooley PC, McDonald AC, Schuijers JA, Ward AR and Grills BL. Transient expression of myofibroblast-like cells in rat rib fracture callus. Acta Orthop 2012;83:93-8.
23. Romano T, Wark JD and Wlodek ME. Calcium supplementation does not rescue the programmed adult bone deficits associated with perinatal growth restriction. Bone 2010;47:1054-63.
24. Romano T, Wark JD and Wlodek ME. Physiological skeletal gains and losses in rat mothers during pregnancy and lactation are not observed following uteroplacental insufficiency. Reprod Fertil Dev 2014;26:385-94.
25. Anevska K, Gallo LA, Tran M, et al. Pregnant growth restricted female rats have bone gains during late gestation which contributes to second generation adolescent and adult offspring having normal bone health. Bone 2015;74:199-207.
26. Brady RD, Grills BL, Schuijers JA, et al. Thymosin beta4 administration enhances fracture healing in mice. J Orthop Res 2014;32:1277-82.

27. Leppanen O, Sievanen H, Jokihaara J, Pajamaki I, Jarvinen TL. Three-point bending of rat femur in the mediolateral direction: introduction and validation of a novel biomechanical testing protocol. J Bone Miner Res 2006;21:1231-7.
28. Lin HL, Lin HC, Tseng YF, et al. Hip fracture after first-ever stroke: a population-based study. Acta Neurol Scand 2015;131:158-63.
29. Smith EM, Comiskey CM, Carroll AM. A study of bone mineral density in adults with disability. Arch Phys Med Rehabil 2009;90:1127-35.
30. Szulc P and Seeman E. Thinking inside and outside the envelopes of bone: dedicated to PDD. Osteoporos Int 2009;20:1281-8.
31. Glatt V, Canalis E, Stadmeyer L and Bouxsein ML. Age-related changes in trabecular architecture differ in female and male C57BL/6J mice. J Bone Miner Res 2007; 22:1197-207.
32. Lee SH, Kim TS, Choi Y and Lorenzo J. Osteoimmunology: cytokines and the skeletal system. BMB reports 2008;41:495-510.
33. Nagao M, Feinstein TN, Ezura Y, et al. Sympathetic control of bone mass regulated by osteopontin. Proc Natl Acad Sci U S A 2011;108:17767-72.
34. Chung YW, Kim TS, Lee SY, et al. Selenite-induced apoptosis of osteoclasts mediated by the mitochondrial pathway. Toxicol Lett 2006;160:143-50.
35. Gronbaek H, Frystyk J, Orskov H and Flyvbjerg A. Effect of sodium selenite on growth, insulin-like growth factor-binding proteins and insulin-like growth factor-I in rats. J Endocrinol 1995;145:105-12.
36. Thorlacius-Ussing O, Flyvbjerg A, Esmann J. Evidence that selenium induces growth retardation through reduced growth hormone and somatomedin C production. Endocrinology 1987;120:659-63.
37. Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS and Mirams M. Endochondral ossification: how cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol 2008;40:46-62.
38. Evans KD, Lau ST, Oberbauer AM, Martin RB. Alendronate affects long bone length and growth plate morphology in the oim mouse model for Osteogenesis Imperfecta. Bone 2003;32:268-74.
39. Evans KD, Sheppard LE, Grossman DI, Rao SH, Martin RB, Oberbauer AM. Long Term Cyclic Pamidronate Reduces Bone Growth by Inhibiting Osteoclast Mediated Cartilage-to-Bone Turnover in the Mouse. Open Orthop J 2008;2:121-5.