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BONE FRACTURE

The effect of anti-retroviral therapy on fracture healing

AN IN VIVO ANIMAL MODEL

S. M. Graham,
M. M. K. Jalal,
D. G. Lalloo,
A. Hamish R. W. Simpson

From Royal Infirmary of Edinburgh, The University of Edinburgh, Edinburgh, UK

Aims

A number of anti-retroviral therapies (ART) have been implicated in potentially contributing to HIV-associated bone disease. The aim of this study was to evaluate the effect of combination ART on the fracture healing process.

Methods

A total of 16 adult male Wistar rats were randomly divided into two groups (n = eight each): Group 1 was given a combination of Tenfovir 30 mg, Lamivudine 30 mg, and Efavirenz 60 mg per day orally, whereas Group 2 was used as a control. After one week of medication preload, all rats underwent a standardized surgical procedure of mid-shaft tibial osteotomy fixed by intramedullary nail with no gap at the fracture site. Progress in fracture healing was monitored regularly for eight weeks. Further evaluations were carried out after euthanasia by micro-CT, mechanically and histologically. Two blinded orthopaedic surgeons used the Radiological Union Scoring system for the Tibia (RUST) to determine fracture healing.

Results

The fracture healing process was different between the two groups at week 4 after surgery; only two out of eight rats showed full healing in Group 1 (ART-treated), while seven out of eight rats had bone union in Group 2 (control) (p = 0.040). However, at week eight postoperatively, there was no statistical difference in bone healing; seven out of eight progressed to full union in both groups.

Conclusion

This study demonstrated that combination ART resulted in delayed fracture healing at week 4 after surgery in rats, but did not result in the development of nonunion.

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Keywords: HIV, Anti-retroviral therapy, Bone disease, Fracture healing, Nonunion

Article focus

- Our study focuses on the effect of anti-retroviral therapy (ART) in the fracture repair process.

Key messages

- ART does not result in the development of a nonunion following a fracture, but does appear to slow the fracture-healing process.

Strengths and limitations

- This the first study of its type investigating the effect of ART on fracture healing in vivo.
- With emerging evidence of the use of ART in the treatment of COVID-19, an awareness of any potential sequalae of this therapy will rapidly become a major public health focus.

Correspondence should be sent to Simon M. Graham; email: simon.graham@ndorms.ox.ac.uk

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- The ART medication was given in combination, therefore the individual effect of each therapy on fracture healing was not investigated in this study.

Introduction

Approximately 35.3 million people are HIV-positive, with the highest prevalence seen in Sub-Saharan Africa.[1] The treatment of HIV has evolved significantly since the first approval of Zidovudine (ZDV) in 1987. The initial aim of anti-retroviral therapy (ART) was to slow the HIV pandemic. As mortality in patients with HIV began declining in 1996 following the introduction of combination ART treatment programmes, there has been a shift in treatment goals from preventing death to prolonging life and improving health of individuals living with HIV.[2] Development of new ART drugs, and administration of them in combination, with the aim of reducing resistance and improving efficacy, has resulted in the life expectancy of individuals living with HIV to be the same as HIV-negative members of the population.[3] However, a number of ART drugs have been implicated in the emerging evidence that the therapy plays a role in HIV-associated bone disease, resulting in a reduction in bone mineral density (BMD), bone mineralization, and bone turnover.[4–11]

As well as their benefit in the treatment of HIV, ART have been used in the treatment of other viruses (herpes, hepatitis, and influenza). Additionally, recently they have been used in the treatment of SARS-CoV-2 (COVID-19) based on virtual screening and in vitro studies.[12] The World Health Organization (WHO) does not currently recommend the use of ART as treatment or prevention of COVID-19, outside of the context of clinical trials. However, with the recent positive results from a clinical trial of the benefit of ritonavir, a protease inhibitor (PI), in reducing hospitalization or death by 89% compared to placebo in non-hospitalized high-risk adults with COVID-19, this is a rapidly evolving area of public health medicine.

There are currently six different classes of ART drugs, all of which have been used in the treatment of HIV and other medical conditions. Evidence appears to implicate Tenofovir (TDF)[13–18] as having the clearest detrimental effect on BMD, closely followed by various protease inhibitors (PIs).[4,19–22] Other drugs in the Nucleoside reverse transcriptase inhibitors (NRTI) class may have similar but less severe effects.[23–27] However, the true effect of ART on the fracture repair process has not been previously investigated in in vivo or clinical studies. Yet given the devastating morbidity of nonunion,[28,29] there is a pressing need to identify developing nonunion before it becomes established,[30,31] remove any inhibitors to fracture repair,[32-34] and potentially intervene early with surgery or novel treatments such as stem cells, exosomes, or growth factors.[35-37] The aim of this study was to analyze how a combination ART regimen affects the fracture repair process.

Methods

Experimental design. A total of 16 adult male Wistar rats (450 to 550 g) were used to test the effect of ART on fracture healing in vivo. One week of acclimatization was allowed before commencing any medication. All of the rats were kept under similar circumstances. Rats were blindly randomized into two groups (n = 8 per group): Group 1 was given daily oral ART therapy, whereas Group 2 was used as a control. Three weeks after the start of the therapy, surgery was carried out by creating a controlled fracture of one tibial shaft under anaesthesia and fixing the bone using intramedullary nailing. Fracture healing was evaluated radiologically (including micro-CT) and biomechanically.

Anti-retroviral therapy. At the time this study was undertaken, the WHO recommended a combination of two nucleoside reverse transcriptase inhibitors (NRTIs), with one non-nucleoside reverse transcriptase inhibitor (NNTRI) (Tenfovir/TDF + Lamivudine / 3 TC (or emtricitabine/FTC)+ Efavirenz / EFV) for the first-line management of HIV.[38] These guidelines are continuously evolving and are updated on an annual basis by the WHO. A combination of Tenfovir 30 mg, Lamivudine 30 mg, and Efavirenz 60 mg per day was administered orally to Group 1. The rats were 'pre-loaded' with ART three weeks before surgery, and the therapy was continued for eight weeks until the end of the experiment. The rats were pre-loaded since any effect ART has on bone metabolism has been shown to take several weeks to occur.[13–15] Therefore, at the time that the rats sustained a fracture, they had already been on ART for a number of weeks.

Medicated jelly was used for oral administration of ART and the compliance was checked closely; the controls were not given medicated jelly. Medication was administrated by a single individual (MJ) and they were the only person aware of which jelly was medicated and which was a control.

Surgical procedure. The surgical procedures were approved by the local Research Ethics Committee at the University of Edinburgh and then the UK's Home Office. Procedures were done under sterile conditions; isoflurane inhalational anaesthesia was used for induction at 5% followed by maintenance at 2.5% to 3%. The skin was prepped using chlorhexidine and the operative field was draped with sterile towels. Skin was incised and subcutaneous tissues over the medial aspect of the tibia were dissected and the tibia was exposed. The tibia was divided at the mid-shaft using a circular burr under saline irrigation. Then, an 18 G hypodermic needle was initially inserted through the fracture site and tapped in a retrograde direction until it protruded out of the tibial tuberosity. This needle was used as a guidewire for another 18 G needle which was inserted in an antegrade direction until it reached the narrowest part of the lower tibia, then the upper part was cut at the level of tibial tuberosity and tapped to impact it into the distal tibia. This ensured that rotational stability was obtained as the triangular tip of

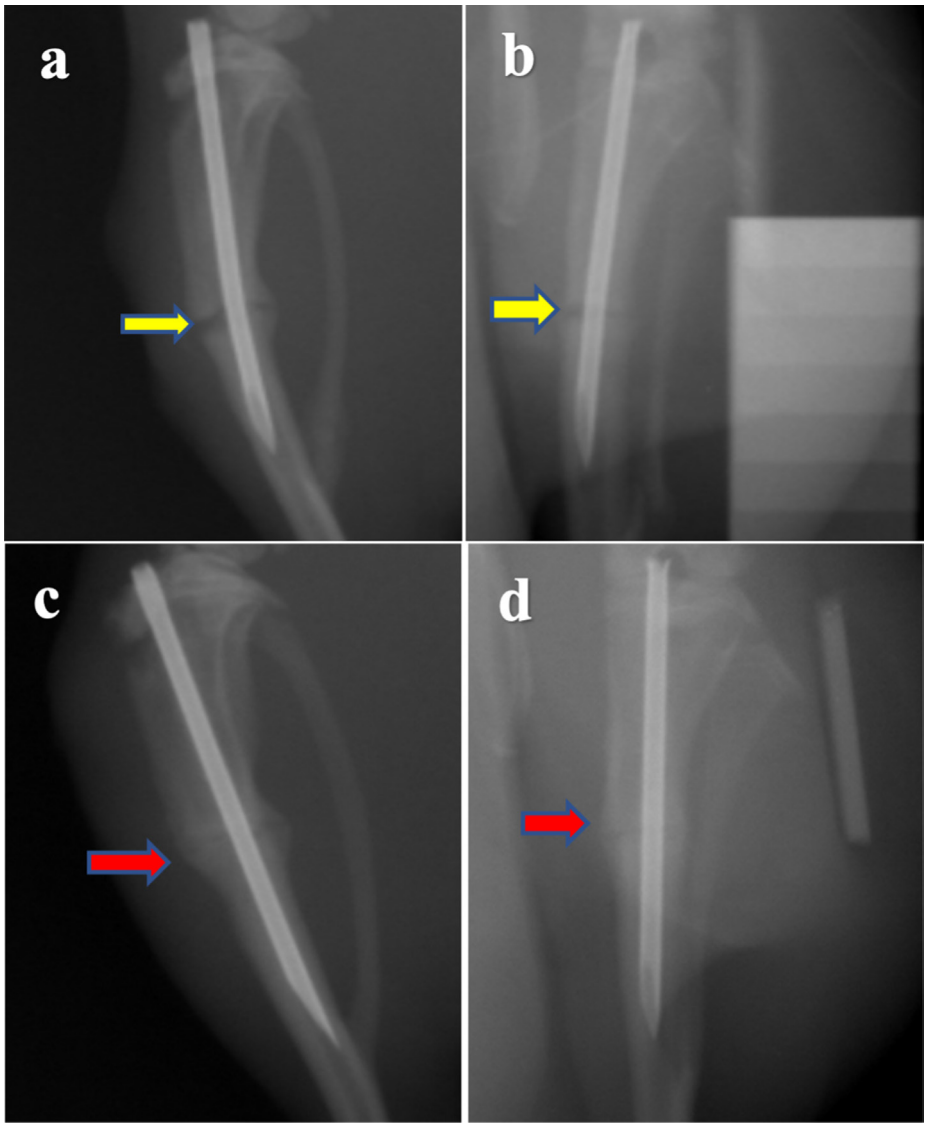

Fig. 1

Lateral and anteroposterior radiographs showing a) and b) a nonunion tibia (yellow arrows) in Group 1, and c) and d) a united tibia (red arrows) in Group 2, four weeks after surgery.

the needle lay in the distal metaphysis making a press-fit against the narrowest part of the lower tibia, while the proximal end was buried beneath the proximal cortex. In addition, the fibula was left intact to provide further stability. The fracture was stabilized without a gap and the stability of the fixation was checked manually. Finally, the wound was washed with saline and the skin was sutured by 4-0 Monocryl using a subcuticular technique.

Health monitoring. After surgery, recovery was allowed for 30 minutes, during which regular monitoring was carried out. The body weight was measured before and after surgery, then daily for one week, followed by weekly recordings until the end of the experiment. General activity, limb movement, and the ability to bear weight were observed closely. To control pain, buprenorphine was given subcutaneously during surgery and for 24 hours postoperatively. Throughout the experiments, the ARRIVE checklist was followed; this has been included in the Supplementary Material to show that the ARRIVE guidelines were adhered to in this study.

Radiological assessment of fracture healing. Progression of bone healing was checked throughout the experiment via serial radiographs every two weeks. Two orthogonal views (lateral and posterior-anterior) were obtained and a diagnosis of union/nonunion was made by two blinded orthopaedic surgeons (SMG and MJ) by scoring them according to the Radiological Union Scale in Tibia (RUST) scoring system.[39] When there was any controversy, a third blinded orthopaedic surgeon (AHRWS) was prepared to score the radiographs and provide a consensus, but this was not required.

The outcomes of the study were defined as follows:

Table I. Fracture healing at week 4 postoperatively.

Treatment group	Union*	Nonunion*	Total
Group 1 (ART)	2	6	8
Group 2 (Controls)	7	1	8
Total	9	7	16

*p-value = 0.0406.
ART, anti-retroviral therapy.

Table II. Fracture healing at week 8 postoperatively.

Treatment group	Union*	Nonunion*	Total
Group 1 (ART)	7	1	8
Group 2 (Controls)	7	1	8
Total	14	2	16

*p-value > 0.05, independent-samples *t*-test.
ART, anti-retroviral therapy.

Union. Radiological union on RUST score (score of three on at least three cortices out of the anteroposterior (AP), lateral, medial, and posterior cortex – yielding a total of nine or more) within eight weeks of surgery.[39–42]

Delayed bone union. Impaired bone healing at four weeks on RUST score (RUST score < 9).[39–42]

Nonunion. Impaired bone healing at eight weeks on RUST score (RUST score < 9).[39–42]

After eight weeks, rats were euthanized under the UK's Home Office regulations (Schedule 1), and fractures were examined clinically by gentle manipulation around the fracture site. Samples were collected, the intramedullary nails were extracted, and tissues were put in sterile tubes containing phosphate-buffered saline (PBS), and were transferred immediately into the laboratory for further investigations.

Micro-CT assessment. All samples were scanned by micro-CT. The micro-CT machine used in this study was Skyscan 1172 radiograph scanner (SkyScan, Belgium). The radiograph beam was set at 54 kVp, 185µA, and a 16 µm isometric voxel size with 0.5 mm aluminium filter. The rotation step for the micro-CT was 360° scan. Data were processed for reconstruction by Skyscan NRecon software to produce 3D images, while Skyscan DataViewer provided clear 3D images which were vital to confirm diagnosis.

Mechanical testing. Tibiae that had united were tested mechanically by the Zwick/Roell machine (series 4500, Instron, USA), using a four-point bending mode, and the amount of fracture site displacement following force application was measured. The loading in the four-point bending test was transferred to the bone by two jigs, designed and provided by the engineering workshop at The University of Edinburgh. The upper jig had fulcra 8 mm apart and lower jig had fulcra 20 mm apart. The method of using the four-point bending was found to be appropriate, as it applied both tensile and compressive stresses without applying direct force to the fracture site.

Each sample underwent 21 cycles using a pre-set load, which was set to produce the same bending moment at the fracture site as would occur from half body weight applied at the distal end of the bone (such as during walking). The machine cross-head speed was set at 4 mm/min.

Biomechanical analysis was carried out using the machine software (TestXpert; ZwickRoell, Instron), and data were analyzed in Excel (Microsoft, USA). Stiffness was derived for cycle number 1, 11, and 21; cycle 11 was used to compare stiffness of the healed fractures between the ART and control groups, and with the intact un-fractured contralateral tibiae (which were used as an additional control).

Histological examination. Tissue was sectioned through the longitudinal axes of the bone targeting the callus area. Sections were cut in 5 µm thickness by a specialized microtome (Shandon; Thermo Fisher Scientific, USA) and were mounted onto slides (Superfrost Plus; BDH biosciences, UK). Three main stains were used: haematoxylin and eosin (H&E) for general morphology, Masson's trichrome for bony components, and safranin O/fast green for cartilage tissue detection. These were examined under light microscope (Eclipse E800; Nikon, Japan). Images of at least three slides per sample were taken by the microscope camera (Digital Sight DS-Fi2, Nikon). The diagnoses of union/nonunion were made by relying on the presence/absence of bony bridges connecting the two fracture ends.

Statistical analysis. All the data from the quantitative measurements were processed in Excel 2016 software; the values were then transferred to Minitab 17 for analysis and statistics. The normality of data was determined by the Shapiro-Wilk test, which is designed to test normality for small datasets. The decision whether to use parametric or non-parametric statistical tests in this study was determined using this test. When data were tested, they revealed normal disruption in all values, and thus parametric independent-samples *t*-test was used for comparisons between different groups, including biomechanical. Fisher's exact test was used to show the significance of ratios between union and nonunion. A p-value of less than 0.05 was chosen as the threshold for statistical significance.

Results

Fracture healing. At week 4 postoperatively, only two out of eight tibiae developed union in Group 1 (ART). By contrast, in Group 2 (controls without ART) seven tibiae out of eight developed union (Table I). The union rate in the treatment group was statistically significantly lower than that of the control group (p = 0.041, Fisher's exact test) (Figure 1).

However, at postoperative week 8, in Group 1 (with ART), seven out of eight tibiae developed union. Similarly, in Group 2 (controls) seven tibiae out of eight developed union (Table II). The union rates in the two groups were identical (p = 1.000, Fisher's exact test) (Figure 2).

Micro-CT data analysis. Micro-CT data were processed and reconstructed into two modes: 3D and three-sectional

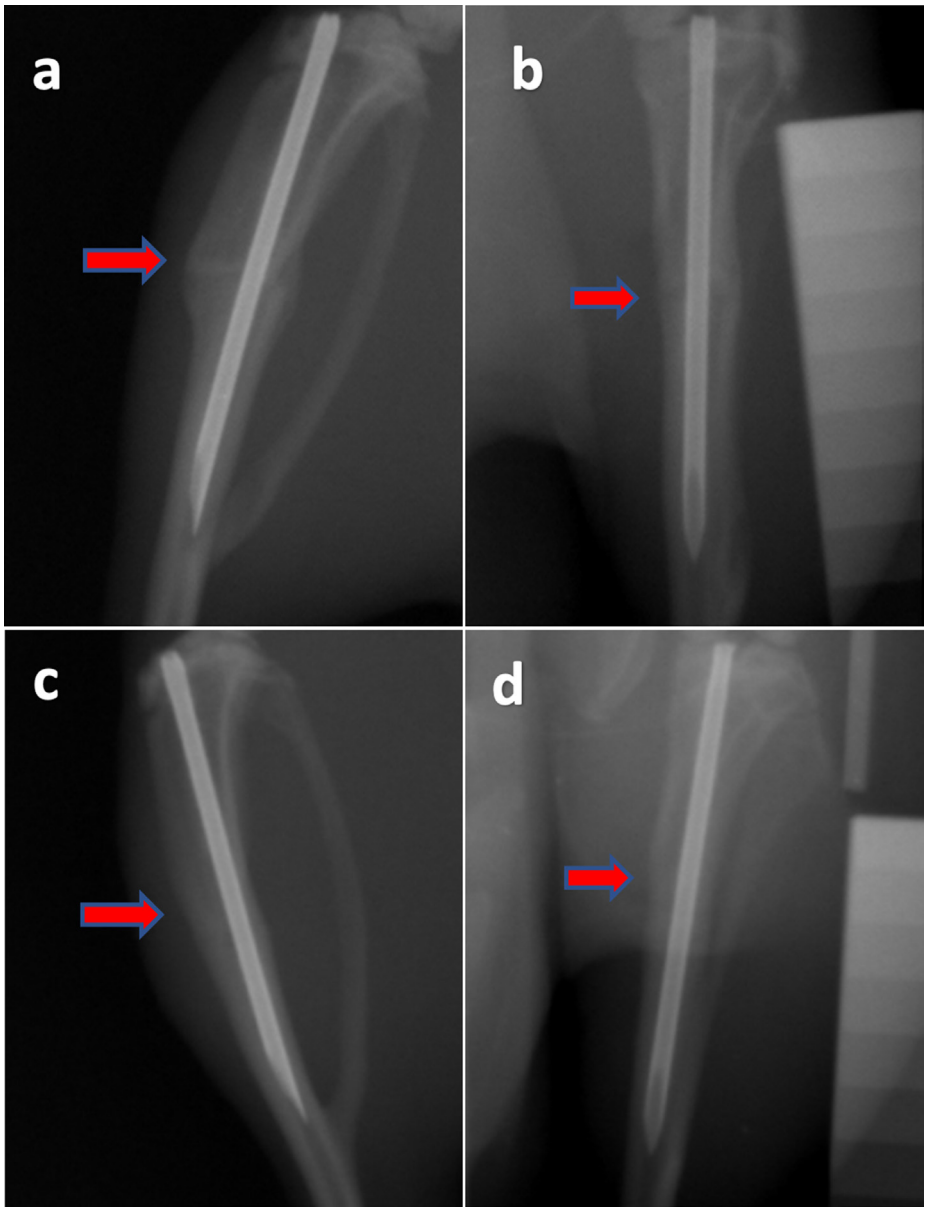

Fig. 2

Lateral and anteroposterior radiographs showing a) and b) united tibias in Group 1, and c) and d) united tibias in Group 2 (red arrows), eight weeks postoperatively.

images. These techniques provided clearer pictures of the status of healing at the fracture site and confirmed the radiograph findings. At week 8 postoperatively, the micro-CT scan showed no difference, and correlated with the RUST scores. Some fractures of the ART group which ended with nonunion appeared as a gap with two separated fracture ends. Conversely, healed tibiae in the control group appeared as full union with complete bridging between the fracture ends (Figure 3).

Biomechanical testing. The biomechanical testing was carried out at the end of the experiment (week eight) when almost all tibiae in both groups were united. There was no significant difference between Group 1 and Group 2 (p >, independent-samples *t*-test). Interestingly, the mean stiffness of the intact contralateral tibiae in the ART group was larger at 25.3 N/mm (standard error of the mean (SEM) = 2.9, n = 8), compared to that of the control group; 19.0 N/mm (SEM = 0.85, n = 8) (p = 0.060, independent-samples *t*-test).

Histological examination. Fracture site histology was assessed and compared between the ART group with nonunion and the control group with union. Tibiae with nonunion revealed a clear gap at the fracture ends filled with fibrous tissue. No woven bone nor cartilaginous callus was found in the inter-fragmentary area (Figure 4). In contrast, connecting bone bridges and an observable

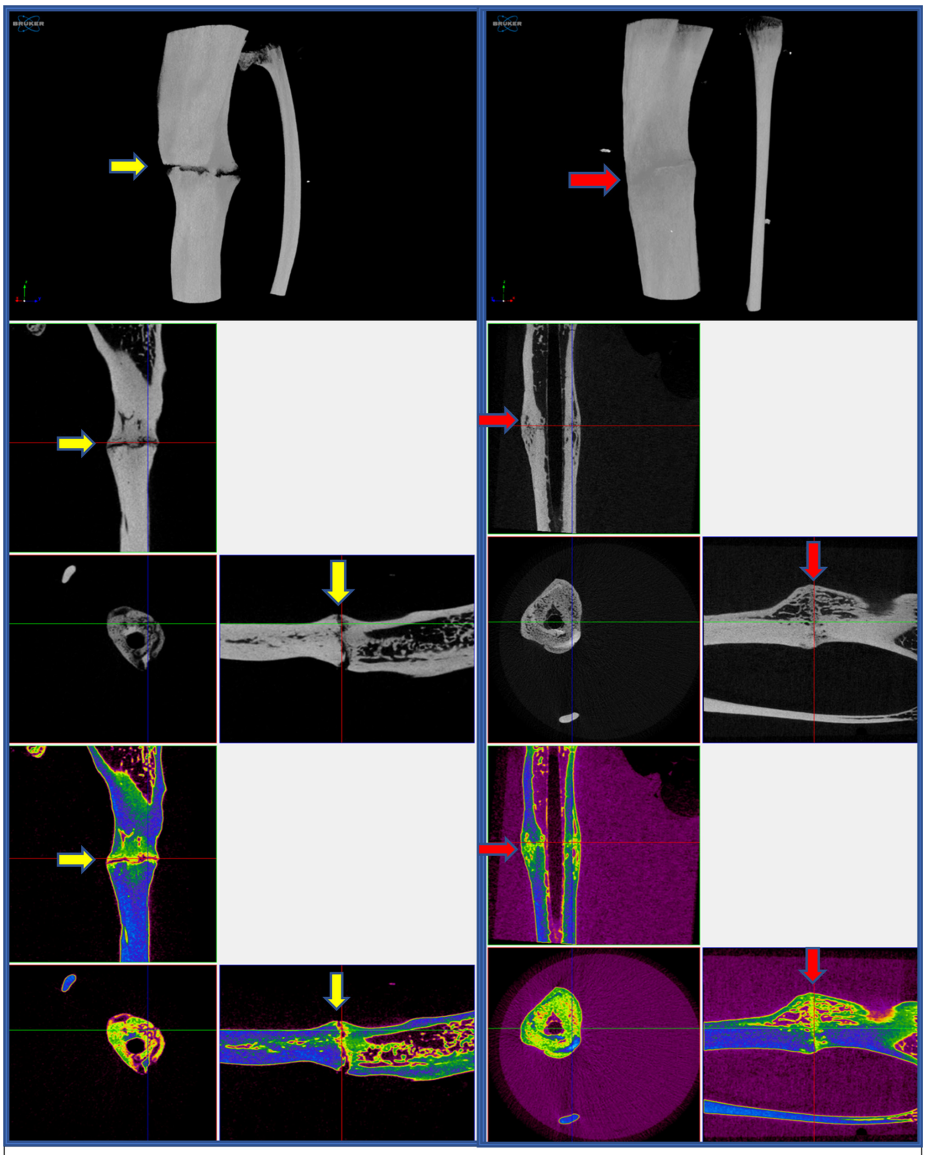

Fig. 3

3D (top) and three-sectional (middle and bottom) images, showing nonunion (yellow arrows) in the anti-retroviral therapy group (left) and union (red arrows) in the control group (right).

callus were found in the tibiae with healed fractures (Figure 4). The presence of new bone and cartilage was also confirmed by Masson's trichrome and safranin O/fast green stains, respectively.

Discussion

This is the first study of its kind to investigate the effect of combination ART on fracture healing in an animal model. It demonstrates that combination ART is associated with an increased risk of delayed fracture healing, but not nonunion, following intramedullary (IM) nailing of tibia fractures in rats. On biomechanical testing, there was no difference in stiffness of the healed fractures between the two groups. However, the tests were carried out at the end of the experiment when almost all tibiae in both groups were united.

The finding that the stiffness of the intact contralateral tibiae in the ART group was larger compared to that of the control group was not expected. This difference could be explained by the radiological findings at week 4, which showed that fracture healing in the ART group was delayed, and thus that rats might put more weight on the contralateral intact tibiae, thereby increasing the biomechanical stiffness. By contrast, in the control group, the fractured tibiae were strong enough at four weeks to bear weight and therefore there was no overloading on the intact tibiae.

A limitation of this study was that the ART medications were given in combination, which makes it difficult

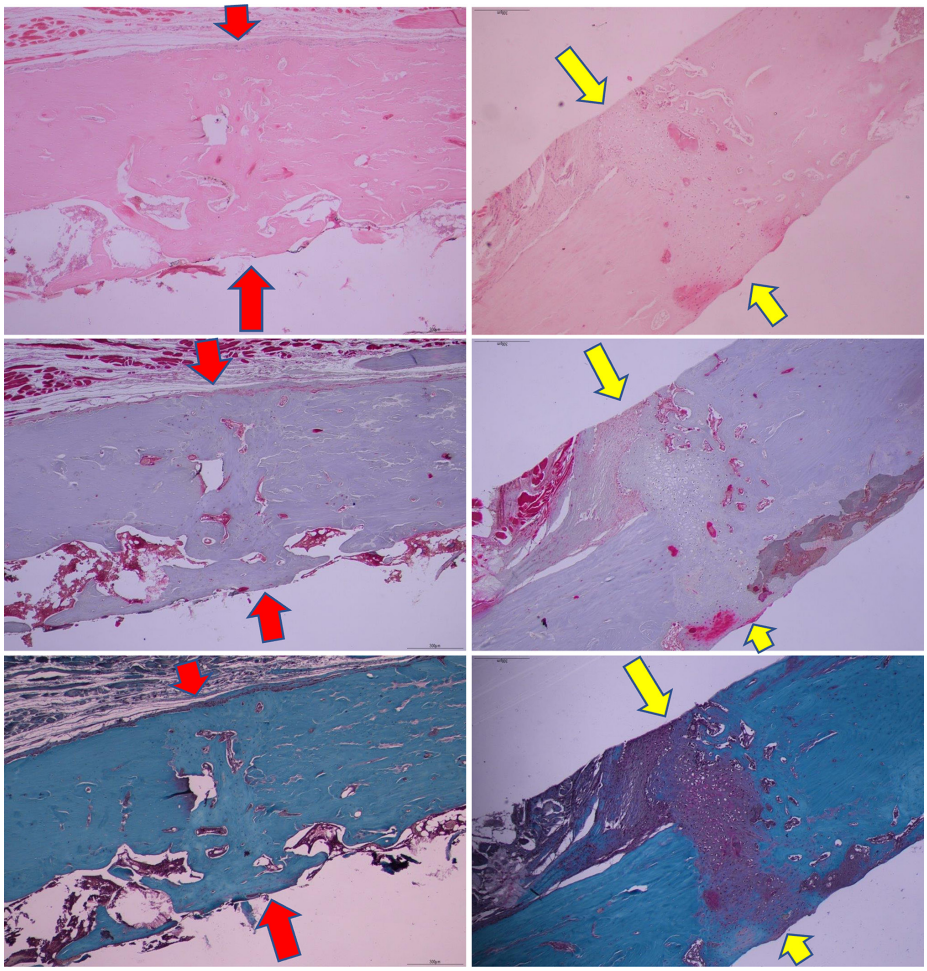

Fig. 4

The histological difference between the control group with union (red arrows) (left) and the anti-retroviral therapy group with nonunion (yellow arrows) (right). Haematoxylin and eosin, Masson's trichrome, and safranin O/fast green stains were used (magnification: 40×).

to draw clear conclusions regarding the clinical effect of individual agents of the ART on bone BMD, bone metabolism, and fracture healing. However, this approach of combination ART mirrors modern day treatment of HIV. Furthermore, the low prevalence of HIV in the global population, the low frequency of those HIV-positive individuals sustaining a fracture, and the low prevalence of those individuals who sustain a fracture developing a delayed union or nonunion mean that recruitment of sufficient participants to power a study is challenging.[43,44]

Our study group recently completed the largest prospective clinical study to assess the association between HIV infection and bone healing following a fracture.[43] We did not find any increased rate of delayed bone healing in HIV-positive patients following an IM nailing of the tibia or femur among trauma patients in the Western Cape, South Africa. In fact, there were lower odds of fracture nonunion in participants with HIV compared to HIV-negative participants. Of the 75 participants with HIV enrolled in the study, 56% (42/75) were taking ART on enrolment. ART regimen at baseline was not associated with a significant risk of delayed union in the study population of participants with HIV. However, a greater number of ART-naïve participants with HIV developed delayed union (23.3%, 7/30) compared to those who were taking ART (12.2%, 5/41; $p = 0.227$).[43] Therefore, although our animal model suggests that some forms of ART may be associated with delayed fracture healing, our clinical research did not confirm this. However, our clinical study was not sufficiently powered to reassure us completely. In combination with evidence from this study, we recommend monitoring patients who sustain a fracture and are on ART, to ensure that certain agents are not slowing the fracture repair process in patients. Future research should focus on the clinical implications of ART on the fracture repair process in a sufficiently powered patient cohort.

Ritonavir, a protease inhibitor and ART drug, has very recently been shown to reduce severe illness leading to hospitalization and death following COVID-19 infection. Similar results of other ART benefits are potentially likely to follow. Therefore, ART medication may be used in the future treatment of the current global

health pandemic caused by COVID-19. Evidence is still preliminary regarding the benefit of ART in COVID-19 treatment, but with fast innovation in this area, an awareness of any potential sequalae of these medications will become even more essential.

In summary, in the treatment of HIV, if a specific component of an ART regimen was found to impair fracture healing, changing the ART regimen from the first-line choice would need to be balanced with maintaining the virological efficacy of the alternative ART therapy, which is essentially the primary reason for the treatment itself. This animal model suggests that combination ART is associated with delayed fracture healing, but more research is required to determine the effect of individual medications on the fracture repair process; additionally, a large population-based study is needed to assess if this effect translates into clinical practice.

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Supplementary material

ARRIVE checklist.

References

1. **No authors listed**. Global HIV & AIDS statistics - 2018 fact sheet. UNAIDS. http://www.unaids.org/en/resources/fact-sheet (date last accessed 5 July 2022).
2. **Warriner AH**, **Mugavero MJ**. Bone changes and fracture risk in individuals infected with HIV. *Curr Rheumatol Rep*. 2010;12(3):163–169.
3. **Samji H**, **Cescon A**, **Hogg RS**, **et al**. Closing the gap: increases in life expectancy among treated HIV-positive individuals in the United States and Canada. *PLoS One*. 2013;8(12):e81355.
4. **Brown TT**, **Qaqish RB**. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. *AIDS*. 2006;20(17):2165–2174.
5. **Short C-E**, **Shaw SG**, **Fisher MJ**, **Walker-Bone K**, **Gilleece YC**. Prevalence of and risk factors for osteoporosis and fracture among a male HIV-infected population in the UK. *Int J STD AIDS*. 2014;25(2):113–121.
6. **Kim HS**, **Chin BS**, **Shin HS**. Prevalence and risk factors of low bone mineral density in Korean HIV-infected patients: impact of abacavir and zidovudine. *J Korean Med Sci*. 2013;28(6):827–832.
7. **Aydın OA**, **Karaosmanoglu HK**, **Karahasanoglu R**, **Tahmaz M**, **Nazlıcan O**. Prevalence and risk factors of osteopenia/osteoporosis in Turkish HIV/AIDS patients. *Braz J Infect Dis*. 2013;17(6):707–711.
8. **Grund B**, **Peng G**, **Gibert CL**, **et al**. Continuous antiretroviral therapy decreases bone mineral density. *AIDS*. 2009;23(12):1519–1529.
9. **Hoy J**, **Grund B**, **Roediger M**, **et al**. Interruption or deferral of antiretroviral therapy reduces markers of bone turnover compared with continuous therapy: The SMART body composition substudy. *J Bone Miner Res*. 2013;28(6):1264–1274.
10. **Dubé MP**, **Qian D**, **Edmondson-Melançon H**, **et al**. Prospective, intensive study of metabolic changes associated with 48 weeks of amprenavir-based antiretroviral therapy. *Clin Infect Dis*. 2002;35(4):475–481.
11. **Dolan SE**, **Kanter JR**, **Grinspoon S**. Longitudinal analysis of bone density in human immunodeficiency virus-infected women. *J Clin Endocrinol Metab*. 2006;91(8):2938–2945.
12. **No authors listed**. Coronavirus disease (COVID-19): HIV and antiretrovirals. World Health Organization. 30 November, 2020. https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19-hiv-and-antiretrovirals (date last accessed 5 July 2022).
13. **Gallant JE**, **Staszewski S**, **Pozniak AL**, **et al**. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA*. 2004;292(2):191–201.
14. **Cassetti I**, **Madruga JVR**, **Suleiman JMAH**, **et al**. The safety and efficacy of tenofovir DF in combination with lamivudine and efavirenz through 6 years in antiretroviral-naïve HIV-1-infected patients. *HIV Clin Trials*. 2007;8(3):164–172.
15. **Moyle GJ**, **Stellbrink H-J**, **Compston J**, **et al**. 96-Week results of abacavir/lamivudine versus tenofovir/emtricitabine, plus efavirenz, in antiretroviral-naive, HIV-1-infected adults: ASSERT study. *Antivir Ther*. 2013;18(7):905–913.
16. **Stellbrink H-J**, **Orkin C**, **Arribas JR**, **et al**. Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study. *Clin Infect Dis*. 2010;51(8):963–972.
17. **Hansen AB**, **Obel N**, **Nielsen H**, **Pedersen C**, **Gerstoft J**. Bone mineral density changes in protease inhibitor-sparing vs. nucleoside reverse transcriptase inhibitor-sparing highly active antiretroviral therapy: data from a randomized trial. *HIV Med*. 2011;12(3):157–165.
18. **Cotter AG**, **Vrouenraets SME**, **Brady JJ**, **et al**. Impact of switching from zidovudine to tenofovir disoproxil fumarate on bone mineral density and markers of bone metabolism in virologically suppressed HIV-1 infected patients; a substudy of the PREPARE study. *J Clin Endocrinol Metab*. 2013;98(4):1659–1666.
19. **Tebas P**, **Zhang J**, **Yarasheski K**, **et al**. Switching to a protease inhibitor-containing, nucleoside-sparing regimen (lopinavir/ritonavir plus efavirenz) increases limb fat but raises serum lipid levels: results of a prospective randomized trial (AIDS clinical trial group 5125s). *J Acquir Immune Defic Syndr*. 2007;45(2):193–200.
20. **Rivas P**, **Górgolas M**, **García-Delgado R**, **Díaz-Curiel M**, **Goyenechea A**, **Fernández-Guerrero ML**. Evolution of bone mineral density in AIDS patients on treatment with zidovudine/lamivudine plus abacavir or lopinavir/ritonavir. *HIV Med*. 2008;9(2):89–95.
21. **McComsey GA**, **Kitch D**, **Daar ES**, **et al**. Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. *J Infect Dis*. 2011;203(12):1791–1801.
22. **Kinai E**, **Nishijima T**, **Mizushima D**, **et al**. Long-term use of protease inhibitors is associated with bone mineral density loss. *AIDS Res Hum Retroviruses*. 2014;30(6):553–559.
23. **Crespo M**, **Navarro J**, **Martinez-Rebollar M**, **et al**. Improvement of BMD after switching from lopinavir/R plus two nucleos(T)ide reverse transcriptase inhibitors to lopinavir/R plus lamivudine: OLE-LIP substudy. *HIV Clin Trials*. 2016;17(3):89–95.
24. **Haskelberg H**, **Hoy JF**, **Amin J**, **et al**. Changes in bone turnover and bone loss in HIV-infected patients changing treatment to tenofovir-emtricitabine or abacavir-lamivudine. *PLoS One*. 2012;7(6):e38377.
25. **Rasmussen TA**, **Jensen D**, **Tolstrup M**, **et al**. Comparison of bone and renal effects in HIV-infected adults switching to abacavir or tenofovir based therapy in a randomized trial. *PLoS One*. 2012;7(3):e32445.
26. **Martin A**, **Bloch M**, **Amin J**, **et al**. Simplification of antiretroviral therapy with tenofovir-emtricitabine or abacavir-Lamivudine: a randomized, 96-week trial. *Clin Infect Dis*. 2009;49(10):1591–1601.
27. **Martínez E**, **Arranz JA**, **Podzamczer D**, **et al**. A simplification trial switching from nucleoside reverse transcriptase inhibitors to once-daily fixed-dose abacavir/lamivudine or tenofovir/emtricitabine in HIV-1-infected patients with virological suppression. *J Acquir Immune Defic Syndr*. 2009;51(3):290–297.
28. **Walters SJ**, **Brazier JE**. Comparison of the minimally important difference for two health state utility measures: EQ-5D and SF-6D. *Qual Life Res*. 2005;14(6):1523–1532.
29. **Walter N**, **Rupp M**, **Hierl K**, **et al**. Long-term patient-related quality of life after fracture-related infections of the long bones. *Bone Joint Res*. 2021;10(5):321–327.
30. **Nicholson JA**, **Oliver WM**, **MacGillivray TJ**, **Robinson CM**, **Simpson A**. Sonographic bridging callus at six weeks following displaced midshaft clavicle fracture can accurately predict healing. *Bone Joint Res*. 2021;10(2):113–121.
31. **Nicholson JA**, **Oliver WM**, **MacGillivray TJ**, **Robinson CM**, **Simpson A**. 3D ultrasound reconstruction of sonographic callus: a novel imaging modality for early evaluation of fracture healing. *Bone Joint Res*. 2021;10(12):759–766.
32. **Gaston MS**, **Simpson A**. Inhibition of fracture healing. *J Bone Joint Surg Br*. 2007;89-B(12):1553–1560.
33. **Chang CJ**, **Jou IM**, **Wu TT**, **Su FC**, **Tai TW**. Cigarette smoke inhalation impairs angiogenesis in early bone healing processes and delays fracture union. *Bone Joint Res*. 2020;9(3):99–107.
34. **Miyamura S**, **Lans J**, **He JJ**, **Murase T**, **Jupiter JB**, **Chen NC**. Bone density measurements from CT scans may predict the healing capacity of scaphoid waist fractures. *Bone Joint J*. 2020;102-B(9):1200–1209.

35. **Yu H**, **Zhang J**, **Liu X**, **Li Y**. microRNA-136-5p from bone marrow mesenchymal stem cell-derived exosomes facilitates fracture healing by targeting LRP4 to activate the Wnt/β-catenin pathway. *Bone Joint Res.* 2021;10(12):744–758.
36. **Wong RMY**, **Choy VMH**, **Li J**, **et al**. Fibrinolysis as a target to enhance osteoporotic fracture healing by vibration therapy in a metaphyseal fracture model. *Bone Joint Res.* 2021;10(1):41–50.
37. **Osagie-Clouard L**, **Meeson R**, **Sanghani-Kerai A**, **Bostrom M**, **Briggs T**, **Blunn G**. The role of intermittent PTH administration in conjunction with allogenic stem cell treatment to stimulate fracture healing. *Bone Joint Res.* 2021;10(10):659–667.
38. **No authors listed**. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. World Health Organisation. https://www.who.int/publications-detail-redirect/9789241549684 (date last accessed 5 August 2022).
39. **Litrenta J**, **Tornetta P**, **Mehta S**, **et al**. Determination of radiographic healing: an assessment of consistency using RUST and Modified RUST in metadiaphyseal fractures. *J Orthop Trauma.* 2015;29(11):516–520.
40. **Keating JF**, **O'Brien PJ**, **Blachut PA**, **Meek RN**, **Broekhuyse HM**. Locking intramedullary nailing with and without reaming for open fractures of the tibial shaft. A prospective, randomized study. *J Bone Joint Surg Am.* 1997;79-A(3):334–341.
41. **Fowler J**, **Dubina AG**, **Castillo RC**, **et al**. Prediction of Tibial Nonunions at 3 Months After Intramedullary Nailing. Orthopaedic Trauma Association Congress; 2014. https://ota.org/sites/files/legacy_abstracts/ota14/OTA%20AM14%20Paper%20110.pdf (date last accessed 8 July 2022).
42. **McClelland D**, **Thomas PBM**, **Bancroft G**, **Moorcraft CI**. Fracture healing assessment comparing stiffness measurements using radiographs. *Clin Orthop Relat Res.* 2007;457:214–219.
43. **Graham SM**, **Maqungo S**, **Laubscher M**, **et al**. Fracture healing in patients with HIV in South Africa: a prospective cohort study. *J Acquir Immune Defic Syndr.* 2021;87(5):1214–1220.
44. **Wijesekera MPC**, **Graham SM**, **Lalloo DG**, **Simpson H**, **Harrison WJ**. Fracture management in HIV positive individuals: a systematic review. *Int Orthop.* 2016;40(12):2429–2445.

Author information:

- S. M. Graham, MBChB, MRCS, MSc (Res), FRCS (Tr&Orth), PhD, Associate Professor of Orthopaedic Trauma, Oxford Trauma and Emergency Care, Nuffield Department of Orthopaedics, Rheumatology & Musculoskeletal Sciences, University of Oxford, Oxford, UK; Liverpool Orthopaedic and Trauma Service, Department of Orthopaedic and Trauma Surgery, Liverpool University Hospital Foundation Trust, Liverpool, UK.
- M. M. K. Jalal, MBChB, MRCS, FRCS, PhD, MD (Clinical), MFSTEd, Specialty Doctor Orthopaedics and Trauma Surgery, Department of Orthopaedic and Trauma Surgery, Royal Infirmary of Edinburgh, The University of Edinburgh, Edinburgh, UK; The Scottish Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh, UK; Basra Health Directorate, Univeristy of Basra, Basra, Iraq.
- D. G. Lalloo, MBBS, FRCP, MD, FFTM, RCPS(Glas), Director, Professor of Tropical Medicine, Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK.
- A. Hamish R. W. Simpson, MA(Cantab), BM, BCh (Oxon), DM(Oxon), FRCS(England & Edinburgh), FIORS, Editor-in-Chief, Professor of Orthopaedics and Trauma, Department of Orthopaedic and Trauma Surgery, Royal Infirmary of Edinburgh, The University of Edinburgh, Edinburgh, UK; The Scottish Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh, UK; *Bone & Joint Research*, London, UK.

Author contributions:

- S. M. Graham: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing.
- M. M. K. Jalal: Formal analysis, Investigation, Methodology, Project administration, Validation, Writing – original draft.
- D. G. Lalloo: Supervision, Funding acquisition, Writing – review & editing.
- A. H. R. W. Simpson: Supervision, Writing – review & editing.

- S. M. Graham and M. M. K. Jalal contributed equally to this work.

- S. M. Graham and M. M. K. Jalal are joint first authors.

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