



Evaluation of bone and kidney toxicity of BT2-peg2, a potential carrier for the targeted delivery of antibiotics to bone

Zaineb A.F. Albayati^a, Narsimha R. Penthala^a, Shobanbabu Bommagani^a, Ginell R. Post^b, Mark S. Smeltzer^c, Peter A. Crooks^{a,*}

^a Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, United States

^b Department of Clinical Pathology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, United States

^c Department of Microbiology & Immunology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, United States

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ABSTRACT

Previous studies have demonstrated that the bone targeting agent BT2-peg2 (BT2-minipeg2, 9), when conjugated to vancomycin and delivered systemically by intravenous (IV) or intraperitoneal (IP) injection accumulates in bone to a greater degree than vancomycin alone, but that this accumulation is associated with severe nephrotoxicity. To determine whether this nephrotoxicity could be attributed to BT2-peg2 itself, we used a rat model to assess the distribution and toxicity of BT2-peg2 after IP injection of 11 mg/kg twice daily for 21 days. The results demonstrated that BT2-peg2 accumulates in bone but there was no evidence of nephrotoxicity or any histopathological abnormalities in the bone. This suggests the nephrotoxicity observed in previous studies is likely due to the altered pharmacokinetics of vancomycin when conjugated to BT2-peg2 rather than to BT2-peg2 itself. Thus, BT2-peg2 may be a safe carrier for the enhanced delivery of antibiotics other than vancomycin to the bone as a means of combating bone infection. However, the data also emphasizes the need to carefully examine the pharmacokinetic characteristics of any BT2-peg2-antibiotic conjugate utilized for treatment of bone infections.

1. Introduction

Osteomyelitis is a serious inflammatory condition of bone that is most often associated with infection by the bacterial pathogen *Staphylococcus aureus* [1]. Treatment of these infections is extremely challenging owing in part to the increasing prevalence of *S. aureus* strains resistant to methicillin and other beta-lactam antibiotics [2]. Despite the development of a number of newer antibiotics with efficacy against methicillin-resistant *S. aureus* (MRSA), vancomycin remains the antibiotic of choice in the clinical treatment of bone and joint infections [3–5]. Moreover, bone infections are characterized by formation of a biofilm, which confers a therapeutically-relevant level of intrinsic resistance to all conventional antibiotics, including vancomycin [6]. *S. aureus* can also be internalized by osteoblasts, which complicates conventional antibiotic therapy even further [7].

Thus, at a minimum it is necessary to administer high doses of antibiotics for long periods of time, but even then surgical debridement is most often also required [8]. Additionally, such prolonged antibiotic administration, particularly with vancomycin, is associated with

nephrotoxicity and the emergence of *S. aureus* strains that exhibit intermediate but therapeutically relevant levels of resistance to vancomycin (vancomycin intermediate *S. aureus* or VISA) [9]. At present, the primary means of overcoming these limitations is the use of localized, carrier-based antibiotic delivery as part of the surgical debridement protocol [8]. Thus, there is an urgent need for improved methods for the more effective systemic delivery of antimicrobial agents to bone. Indeed, such methods could limit the degree of debridement required to ensure the desired therapeutic effect or perhaps, at least in some cases, eliminate the need for debridement entirely.

BT2-peg2 (9) is derived from the hydroxyapatite-binding moiety of tetracycline and was used successfully to enhance the delivery of estradiol and other bioactive compounds to bone [10–13] Karau et al. [14] demonstrated that, under *in vitro* test conditions, vancomycin conjugated to BT2-peg2 had similar activity to vancomycin itself against MRSA, an observation that we subsequently confirmed [15]. Moreover, treatment of experimental osteomyelitis with vancomycin and the molar equivalent of BT2-peg2-vancomycin using the same dosing regimen confirmed that BT2-peg2-vancomycin exhibits enhanced therapeutic

* Corresponding author.

E-mail address: pacrooks@uams.edu (P.A. Crooks).

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efficacy by comparison to vancomycin alone [14]. It was also confirmed by pharmacokinetic (PK) analysis that BT2-peg2-vancomycin significantly accumulates in bone and plasma after systemic administration by comparison to the systemic administration of vancomycin alone [15].

While promising, Karau et al. [14] also found that systemic administration of BT2-peg2-vancomycin in rats was associated with high plasma concentrations of this drug, elevated levels of serum creatinine and blood urea nitrogen (BUN), decreased serum albumin, leukocytosis, and severe nephrotoxicity, as has been reported with exposure to high plasma concentrations of vancomycin [16]. However, these investigators determined that systemic administration of an equi-molar amount of vancomycin under similar conditions did not result in nephrotoxicity. Karau et al. hypothesized from these observations that the adverse consequences after systemic administration of BT2-peg2-vancomycin were likely due to an altered PK of BT2-peg2-vancomycin compared to vancomycin, resulting in prolonged accumulation of the former compound in plasma causing nephrotoxicity. To test this hypothesis, the present study was aimed at determining whether BT2-peg2 (9) alone, administered systemically under the same conditions and concentrations as in the Karau et al. study exhibits significant nephrotoxicity and/or histopathological changes in the bone itself.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were of LC/MS grade or equivalent quality. Acetonitrile, methanol, formic acid, and normal saline were obtained from Fisher Scientific (Pittsburgh, PA, USA). Benzophenone was obtained from Sigma-Aldrich (St. Louis, MO, USA). Heparin sodium injection (10,000 USP units/mL) was purchased from Baxter Healthcare Corporation (Deerfield, IL, USA). The raw materials for the synthesis of BT2-peg2 were purchased from the AK scientific product catalog (Union City, CA, USA).

2.2. Synthesis of BT2-peg2 (9)

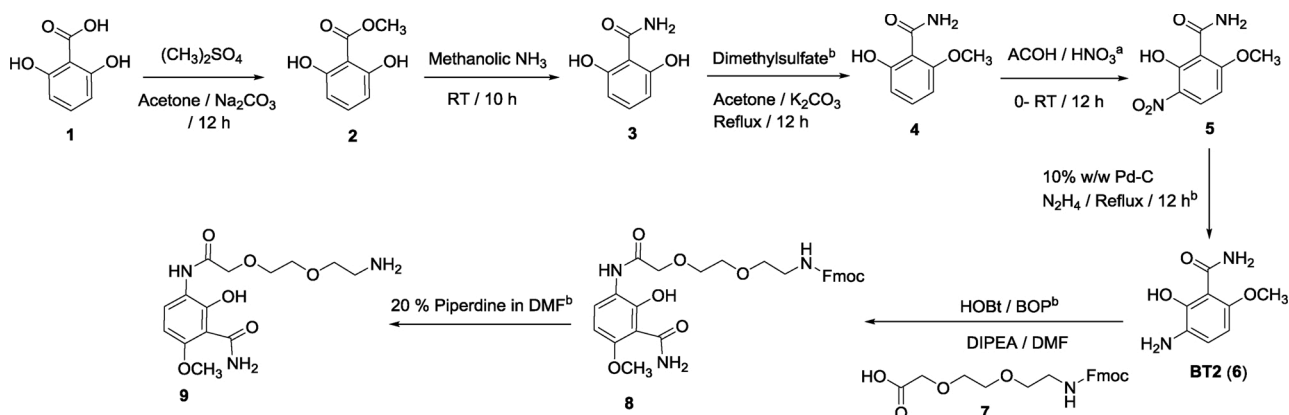
It is noteworthy to mention that BT2-peg2 (9) is stereochemically, structurally, and functionally similar to the BT2-peg2 that has been discussed by Karau et al. and Albayati et al. [14,15]. Synthesis of BT2-peg2 requires the preparation of the key raw material BT2 (6), which can be made in five steps via a modification of the procedures described by Neale et al. [10] and Brooke [17] from the readily available starting material, 2,6-dihydroxy benzoic acid (1) (Scheme 1). Esterification of 1 to methyl 2,6-dihydroxybenzoic acid (2) is the first step in the synthesis of BT2. Neale et al. have reported on the esterification of 1 using methyl iodide, NH_4OH , and AgNO_3 in 89% yield [10]. However,

we were not able to obtain such yields using this methodology. By reacting 1 with dimethyl sulfate/sodium carbonate at room temperature over 12 h we were able to obtain a 95% yield of 2 which could be isolated in greater than 99% purity. The second step in the synthesis of BT2 is aminolysis of 2 with aqueous ammonia to form 2,6-dihydroxybenzamide (3), [10]. We found that by replacing aqueous ammonia with methanolic ammonia we were able to improve the yield of 3 to 84% with a simplified work-up procedure. For the *O*-methylation of 3 with dimethyl sulfate in acetone/potassium carbonate to afford 6-methoxybenzamide (4) we utilized the procedure of Brooke [17]. Nitration of 4 with nitric acid/acetic acid reagent then afforded 3-nitro-2-hydroxy-6-methoxybenzamide (5) [10]. Reduction of 5 is the final step in the synthesis of BT2 (6). Neale et al. has reported using Pd-C hydrogenation in methanol at 46 psig for the reduction of 5 to BT2 [10]. We modified this procedure by carrying out the reaction with 10 mol equivalents of 50–60% hydrazine hydrate and 10% w/w Pd-C in ethanol at reflux temperature and at atmospheric pressure. This procedure improved the yield of BT2 from 67% to 77%. The analytical data for intermediates 2–5 and BT2 (6) are in agreement with the reported literature [10]. The synthesis of BT2-peg2 (9) from BT2 (6) was carried out as per the reported literature procedure (Scheme 1) [17]. The overall yield of 9 from 1 utilizing the above procedures is improved by 43%. The H-1 and C-13 NMR spectra and other analytical data for BT2-peg2 (see Supplementary Information) were consistent with the reported analytical data [17].

2.3. Animal experimental protocol

All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences (UAMS) and the Animal Care and Use Review Office (ACURO) of the U. S. Army Medical Research and Materiel Command (USAMRMC).

BT2-peg2 (9) was administered at a concentration of 11 mg/kg, which is the molar equivalent of the amount of BT2-peg2 present in the BT2-peg2-vancomycin conjugate. BT2-peg2 was formulated in phosphate buffered saline (PBS) and delivered by intraperitoneal (IP) injection into each of four Albino Wistar male rats (200–250 gm, Charles River, Wilmington, MA). Administration was carried out twice daily for 21 days for a total of 42 doses, as employed in the study by Karau et al. [14]. Four Albino Wistar male rats (200–250 gm) were administered PBS IP injections twice daily for 21 days and served as the control group. BT2-peg2-treated and untreated rats were weighed daily throughout this period. Twelve hours after the last dose, rats were humanely euthanized using CO_2 , and blood, kidneys and the right and left tibia harvested from each rat. Blood was collected via cardiac puncture and placed into sodium heparin blood collection tubes for hematology. Plasma was obtained by centrifugation at 10,000 rpm at room temperature (RT) for 5 min. Samples were stored at -80°C prior to analysis.



Scheme 1. Synthesis of BT2-peg2 conjugate (9).

2.4. Histological, biochemical and hematological analysis

Kidneys from untreated and BT2-peg2-treated rats were collected, weighed, observed for abnormalities in size and color, and fixed in neutralized buffered formalin (NBF) for 24 h prior to more detailed histological analysis. Histological analysis of the kidney was performed using Hematoxylin and Eosin (H&E) and Periodic acid–Schiff (PAS) stained sections. The left and right tibia were cleaned of soft tissues, washed with PBS and weighed. Right tibia samples were fixed in NBF for 24 h and decalcified using 10% EDTA (pH 7.0) prior to histological analysis of H&E-stained sections. Plasma and the left tibia were used for quantitative determination of BT2-peg2 (9) concentrations by LC/MS/MS, as detailed below. Hematological analysis of blood was performed to determine white blood cell (WBC) count. UV/visible colorimetric analysis was used to determine plasma creatinine, blood urea nitrogen (BUN) and albumin levels, as previously described [18–20].

2.5. Preparation of plasma samples for LC/MS/MS analysis of BT2-peg2 (9) levels

A hundred μL of plasma from untreated and BT2-peg2-treated rats was spiked with benzophenone (10 μL of a 0.1 $\mu\text{g}/\text{mL}$ solution in acetonitrile) as an internal standard. Protein precipitation was performed by adding 400 μL of acetonitrile. After 30 s of vortex mixing, the solution was centrifuged at 10,000 rpm for 10 min at RT. The supernatant was evaporated to dryness under nitrogen and each pellet was reconstituted with 60 μL of a 2:1 mixture of acetonitrile:water. Resuspended pellets were vortexed, sonicated for 1 min, and centrifuged at 10,000 rpm for 10 min at RT prior to analysis by LC/MS/MS, as detailed below.

2.6. Preparation of bone samples and extraction procedure for analysis of BT2-peg2 (9)

Stainless steel balls (3.5 mm, Next Advance Inc, Troy, NY, USA) were placed in a 5 mL tube along with the tibia, 0.5 mL hexane, and 1 mL of water. Bone samples were homogenized for 3 min using a Bullet Blender Storm 5 homogenizer (Next Advance, Inc. Troy, NY, USA). Ten μL (0.1 $\mu\text{g}/\text{mL}$) of benzophenone was added to 0.2 mL of each bone homogenate as an internal standard. Samples were vortexed and extracted with 600 μL acetonitrile, followed by the addition of 400 μL water. Samples were then vortexed for 30 s and centrifuged at RT for 10 min at 10,000 rpm. Supernatants were then processed as described above for analysis by LC/MS/MS.

2.7. LC/MS/MS analysis of BT2-peg2 (9) in plasma and bone samples

Analysis of BT2-peg2 (9) in plasma and bone samples was performed using an Agilent LC/MS/MS Triple Quad 6410 instrument (Santa Clara, CA, USA) utilizing positive electrospray ionization (ESI) in the multiple reaction monitoring (MRM) mode with optimal ion source settings determined by standards of BT2-peg2 (9), and benzophenone as the internal standard. A curtain gas of 20 psi, an ion spray voltage of 4000 V, an ion source gas1/gas2 of 35 psi and a temperature of 300 °C were employed in the collection of chromatographic data. Chromatographic separation was carried out on an Alltech Altima C-18 column (150 mm \times 3.2 mm, 5.0 μm) fitted with an Alltech Altima C-18 guard column (7.5 \times 3.0 mm, 5 μ , Grace Discovery Sciences, IL, USA). A gradient method was used with the mobile phase consisting of water containing 0.1% v/v formic acid as solvent A and ACN as solvent B. The separation was achieved using a gradient of 10–90% solvent B over 3.50 min, which was maintained at 90% B for a further 3.50 min, and then equilibrated back to the initial conditions over 3.20 min. The flow rate was 0.8 mL/min and injection volume through the auto sampler unit for all the samples was 5 μL . BT2-peg2 and the benzophenone internal standard (IS) exhibited retention times of 3.2 and 5.6 min, respectively. MRM

transitions monitored were m/z 328.1/166.0 for BT2-peg2, and m/z 183.1/105.1 and m/z 183.1/77.1 for benzophenone.

2.8. Calibration standards

Calibration standards for BT2-peg2 in plasma and bone samples were constructed by spiking 100 μL of drug free plasma or bone homogenate samples with 10 μL of freshly prepared standard solutions of BT2-peg2 (0, 1, 10, 20, 40, 100, and 200 ng/mL). Calibration curves of BT2-peg2 in plasma and bone were established by plotting the peak area ratios of BT2-peg2 and IS vs. concentrations of BT2-peg2. Linear regression equations were obtained by using the least-squares method. The calibration curves of BT2-peg2 in plasma showed excellent linearity between 10–200 ng/mL with correlation coefficient (R^2) of 0.975 and 10–200 ng/mL with correlation coefficient of 0.982 for bone. The lower limit of detection (LOD) and lower limit of quantitation (LOQ) values were 1 and 10 ng/mL for plasma and 10–20 ng/mL for bone, respectively.

2.9. Histopathology

Kidney and bone tissue sections were fixed in 10% NBF. Bone sections were also decalcified in 12% formic acid solution. Tissues were placed in cassettes, embedded in paraffin, processed overnight, sectioned at 5 microns and stained with H&E. The periodic acid-Schiff (PAS) stain was used to highlight the tubular brush border cells. Histologic sections prepared from the kidneys (8 total) and tibia (8 total) were evaluated microscopically by a pathologist in a blinded fashion.

2.10. Statistical analysis

Data are presented as mean \pm standard error of the mean (mean \pm SEM). The data were analyzed for statistical significance using the unpaired Student's *t*-test with Welch's correction factor for unequal variances with $p \leq 0.05$ as the criterion of significance. Statistic for weight vs. time (days) curve was obtained using GraphPad prism software (GraphPad, version 5.0, La Jolla, CA).

3. Results

The change in body weight over the course of 21 days treatment for the untreated and groups were not statistically different ($p \geq 0.05$, Fig. 1). The kidneys from the untreated and the BT2-peg2-treated rats exhibited a deep maroon color and were indistinguishable visually (Fig. 2), and their size and weight indicated no significant difference between the two groups (Table 1). Most importantly, histologic

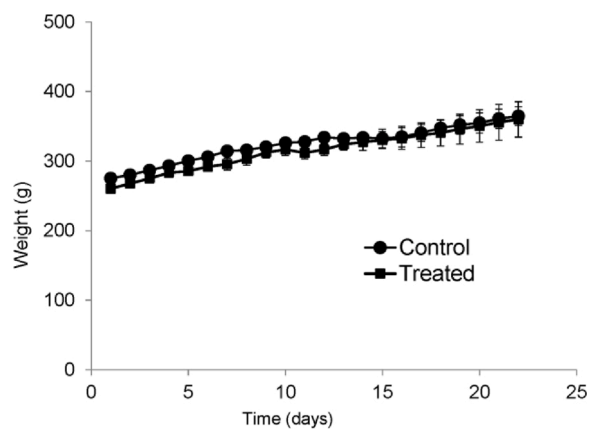


Fig. 1. Body weight change over the 21-day course of treatment with BT2-peg2 (11 mg/kg twice daily) in Albino Wistar male rats. No significant differences were noticed between treated and untreated rats. $P > 0.05$.



Untreated

BT2-peg2

Fig. 2. Representative kidneys from untreated and BT2-peg2-treated rats.

Table 1

Kidney weights, BUN, plasma albumin, creatinine values, and plasma and bone BT2-peg2 levels after treatment with BT2-peg2 at a dose of 11 mg/kg (the molar equivalent of BT2-peg2 used in the BT2-peg2-vancomycin study [14]).

Group	Untreated	BT2-peg2-treated
Kidney weight (g)	2.5 ± 0.22	2.4 ± 0.35
BUN (mg/dl)	22.1 ± 2.5	23.0 ± 0.9
Albumin (gm/dl)	3.4 ± 0.02	3.5 ± 0.01
Creatinine (mg/dl)	0.5 ± 0.01	0.6 ± 0.01
Plasma (ng/mL)	–	< 1
Bone (ng/g)	–	235.0 ± 96.8

evaluation of the renal sections from BT2-miniPEG-2 treated and untreated rats showed unremarkable glomeruli, tubules, vessels and interstitium. There was no evidence of histopathologic features of renal injury, including tubular dilatation, apical budding, brush border or tubular loss. Importantly, there is no evidence of leukocytosis, indicative of tubulointerstitial nephritis, as previously reported by Karau et al. [14].

Microscopic examination of the right tibial bones from BT2-miniPEG-2 treated and untreated rats showed similar feature. Histologic features in the tibia show cartilage with chondrocytes, trabecular bone and intertrabecular spaces showing morphologically unremarkable trilineage hematopoiesis. There was no evidence of cellular or stromal injury, bone remodeling/repair in the sections examined. Histologic evaluation of the renal sections from BT2-peg2-treated and untreated rats showed unremarkable glomeruli, tubules, vessels and interstitium. There were also no histopathologic features of renal injury, including tubular dilatation, apical budding, brush border or tubular loss (Fig. 3). In addition, biochemical results indicated normal values for blood urea nitrogen (BUN), plasma albumin and creatinine in both the untreated and BT2-peg2-treated animals (Table 1).

There was also no statistical difference in total white blood cell counts for the untreated and BT2-peg2 treated groups, which exhibited values of $8.9 \pm 0.9 \times 10^3$ and $9.3 \pm 0.8 \times 10^3/\mu\text{L}$, respectively. Significant amounts of BT2-peg2 were detected in the left tibia, while BT2-peg2 was undetectable in plasma (LOD < 1 ng/mL, Table 1). Microscopic examination of the right tibia from BT2-peg2-treated and untreated rats showed similar features, including intact cortical and

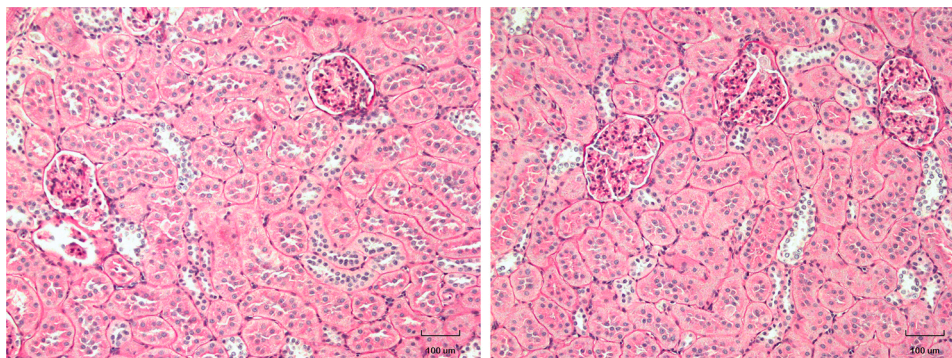


Fig. 3. Histological analysis of kidneys as a function of BT2-peg2 treatment. Periodic acid–Schiff (PAS) stained histopathologic sections from untreated (left) and BT2-peg2-treated kidneys (right). There was no discernible evidence of microscopic glomerular or renal tubular damage as evidenced by tubular dilatation, apical budding, or brush border and tubular loss. The absence of demonstrable histopathology was also confirmed by H&E staining (data not shown).

paratrabeular bone with morphologically unremarkable trilineage hematopoiesis. There was no evidence of cellular or stromal injury in either experimental group (Fig. 4).

4. Discussion

Previous studies employing a rat model of experimental osteomyelitis provided evidence that BT2-peg2-vancomycin delivered systemically by intravenous (IV) or intraperitoneal (IP) injection exhibits greater therapeutic efficacy in the context of bone than an equivalent dose of vancomycin [14]. However, the use of BT2-peg2-vancomycin was also associated with a profound change in pharmacokinetic profile characterized by high plasma levels of BT2-peg2-vancomycin, decreased animal weight, increased kidney size, and severe tubulointerstitial nephritis. Subsequent chemical analysis also confirmed that administration of BT2-peg2-vancomycin resulted in elevated levels of serum creatinine and blood urea nitrogen (BUN), and decreased serum albumin.

It is noteworthy that vancomycin administered via conservative dosing and/or via more intense dosing (twice/day for 21 days) was not nephrotoxic in any of the doses and protocols used. In this respect it should be noted that nephrotoxicity associated with BT2-peg2-vancomycin was minimized if not eliminated using more conservative dosing regimens (i.e. every 12 h for 3.5 days followed by once daily every fourth day or once per week), but these more conservative regimens were not demonstrably associated with an enhanced therapeutic effect [14]. It should also be noted that plasma levels of BT2-peg2-vancomycin were dramatically elevated using the more intensive dosing regimen and this was not the case with either of the more conservative dosing regimens [14]. This suggests a direct correlation between high plasma levels of BT2-peg2-vancomycin and nephrotoxicity.

Since this study was aimed at determining whether BT2-peg2 itself contributed to the above adverse effects, in order to test this hypothesis as stringently as possible, we employed the maximum dosing regimen used by Karau et al. [14], which was twice daily administration of drug for 21 days. To this end, we administered 11 mg/kg of BT2-peg2 (which is the molar equivalent of the BT2-peg2 component of BT2-peg2-vancomycin) to rats using the same dosing regimen (i.e. IP injection twice daily for 21 days) shown to enhance therapeutic efficacy in the study by Karau et al. [14].

As presented in Table 1, we found that plasma levels of BT2-peg2 were below the limit of detection (< 1 ng/mL) despite the clear accumulation of BT2-peg2 in the bone, indicating that BT2-peg2 is targeting bone tissue. The latter finding is consistent with the high affinity of BT2-peg2 for hydroxyapatite and an enhanced tendency to accumulate in bone [17]. Despite vancomycin antibacterial efficacy against both methicillin-resistant and methicillin-susceptible *S. aureus* strains, vancomycin ability to penetrate bone tissue is limited. Also, vancomycin

exhibits poor pharmacokinetics that makes it insufficiently bioavailable in bone tissue, thus limiting its *in vivo* use for bone infections. Our previous studies have sought to optimize vancomycin bioavailability by conjugating the bone targeting agent BT2-peg2 to deliver vancomycin to the bone as BT2-peg2-vancomycin, a strategy of potential clinical use in the treatment of bone infection. We demonstrated that BT2-peg2 can be chemically conjugated to vancomycin via a modified polyethylene glycol (PEG) linker to form BT2-peg2-vancomycin, which retains the antibacterial activity of vancomycin [10,12,14]. Previous *in vitro* studies have confirmed that the MICs of BT2-peg2-vancomycin against methicillin-resistant and methicillin-susceptible *S. aureus* are comparable to those of vancomycin, and that BT2-peg2-vancomycin binds to hydroxyapatite to a greater extent than vancomycin [14].

In summary, the results from the current study demonstrate that systemic administration of BT2-peg2 via the IP route is not associated with any of the nephrotoxic side effects observed in previous studies employing BT2-peg2-vancomycin [14]. Specifically, there was no statistically significant difference between the untreated and BT2-peg2-treated experimental groups, as assessed by weight loss, kidney size and overall morphology, histopathological changes in the kidney, or changes in blood urea nitrogen (BUN), albumin or creatinine levels. All of these results are consistent with the hypothesis that BT2-peg2 itself was not responsible for the nephrotoxicity observed in the earlier studies of Karau et al. [14].

In addition, a hypothetical assumption, if vancomycin is released, *in vivo* from the BT2-peg2-vancomycin conjugate, an equivalent molar amount of BT2-peg2 would have been also released. Neither vancomycin nor free BT2-peg2 was released, since the levels of free vancomycin and/or BT2-peg2 in plasma and bone tissues from BT2-peg2-vancomycin-treated (molar equivalent dose of 50 mg/kg of vancomycin) rats were undetectable after IV or IP treatment with BT2-peg2-vancomycin, indicating either no hydrolysis of BT2-peg2-vancomycin had occurred or their levels were not significant or unquantifiable [15].

It was suggested by Karau et al. that the high plasma levels of BT2-peg2-vancomycin compared to vancomycin observed with the high dosing regimen were likely due to the pegylation component of the BT2-peg2-vancomycin formulation in that pegylation is known to increase drug half-life, although the possibility that release of BT2-peg2-vancomycin from the bone into the systemic circulation also contributed to the high levels observed in plasma could not be ruled out [14].

While certainly not definitive, this suggests that the 183-fold elevated plasma levels of BT2-peg2-vancomycin compared to vancomycin observed by Karau et al. [14] were due to altered pharmacokinetic parameters and reduced clearance of BT2-peg2-vancomycin rather than sustained release from the bone. This is consistent with our previous PK studies demonstrating that vancomycin was first detectable in blood 1 h after administration and was cleared within 12 h [15]. In contrast, plasma BT2-peg2-vancomycin was also first detected at 1 h, but remained detectable for at least 168 h, demonstrating a significant

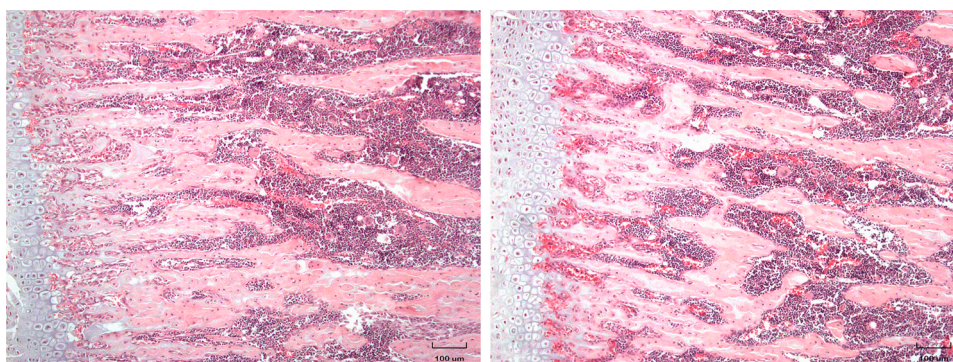


Fig. 4. Bone histology as a function of BT2-peg2 treatment. Representative images of H&E stained sections of the right tibia are shown from untreated (left) and BT2-peg2-treated rats.

decrease in total clearance (Cl_{tot}) from the body [15]. This significant change in the PK properties of vancomycin when conjugated to BT2-peg2 resulted in a higher plasma concentration and a longer *in vivo* exposure to BT2-peg2-vancomycin. In fact, a decrease of 13.5-fold in Cl_{tot} and a 14.7-fold increase in half-life ($t_{1/2}$) was exhibited by BT2-peg2-vancomycin compared to vancomycin with for the former compound producing a 10.8-fold enhancement in the area-under-the-curve (AUC) for BT2-peg2-vancomycin when compared to vancomycin [15].

Finally, in this current study we not only confirmed the targeting efficiency of BT2-peg2 in the context of bone, but also that its accumulation in bone is not associated with adverse bone pathology or leukocytosis during the above 21-day study. Both histological and hematological results indicate normal bone marrow function. However, it is important to note that potential longer term deleterious effects of BT2-peg2 on bone, such as effects on bone mineral and induction of bone resorption were not addressed in this current study. The detection of such longer term bone toxicities may also require the use of additional techniques that include micro-CT and micro-indentation studies to identify possible microstructural damage and effects on bone mechanics.

Overall, we conclude that BT2-peg2 has tremendous potential as a safe and effective bone targeting agent and that the nephrotoxicity observed in earlier experiments is in fact a function of its conjugation to vancomycin, likely owing to prolonged persistence in the blood. This suggests that BT2-peg2 could be used to enhance the systemic delivery of antibiotics other than vancomycin to bone. However, the results we report also emphasize the need to carefully evaluate dose, PK parameters and potential toxicity of alternative BT2-peg2 conjugates in addition to their therapeutic efficacy in the context of bone infection, as well as identifying longer term effects on bone. In this respect we also conclude that BT2-peg2 may have advantages over other approaches in the general area of targeted drug delivery to bone, especially with regard to anticancer drugs that target bone, anti-leukemia drugs that target the bone marrow, bone regeneration drugs, and anti-resorptive drugs.

Author statement

Zaineb A.F. Albayati: Pharmacokinetic studies on animals, Manuscript original draft and editing.

Narsimha R. Penthal: Design and synthesis of BT2- Peg2. Writing and editing the manuscript.

Shobanbabu Bommagani: Synthesis and characterization of BT2-peg2.

Ginell R. Post: Bone and Kidney histopathology study.

Mark S. Smeltzer: Design of bone and kidney histopathology studies.

Peter A. Crooks: Provided funding and resources, supervision of this overall research, Correcting and editing the manuscript.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.toxrep.2021.02.002>.

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