Qeios

Peer Review

Review of: "Selection of Bone-Targeting Peptides for Therapeutic Intervention: An In Vivo Evaluation and Comparison Study"

Milutin Micic[1]

1. Institute of Anathomy, University of Belgrade, Serbia

In this study, peptides with HA-binding properties, namely D8, (Asp-Asp-Asp-Asp-Asp-Asp-Asp-Asp), E8 (Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu), Y*GNAED8 (YD8: Tyr-Gly-Asn-Ala-Glu-Asp-Asp-Asp-Asp-Asp-Asp-Asp-Asp), and Y*GNAEE8 (YE8: Tyr-Gly-Asn-Ala-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu), were selected, based on previous research results, characterized, and used for *in vitro* testing of binding ability on HA, TCAP, and $CaCO_3$, as well as *in vivo* study of biodistribution in mineralized and soft tissue, including healthy, defective, and pathological (OIM mouse models) bone, in animal models. The research underscores the value of D8 as a binding agent and HA for its binding efficiency. An *ex vivo* fluorescence imaging technique for quantifying peptide biodistribution was refined by the use of the IVIS Spectrum CT system and SPUM algorithms, eliminating tissue autofluorescence, increasing accuracy and sensitivity – thus offering new insights into peptide biodistribution and localization in healthy, defective, and pathological bone. By using this refined visualization technique, the authors were able to precisely determine microlocations of increased peptide binding and to quantify it, which made a substantial contribution to understanding increased peptide affinity in defective and pathological bone models. Adequate statistical tests were used for comparison and data analysis.

This manuscript provides valuable insights for the audience interested in bone pathology and bone-targeting drug development. By addressing the underexplored subfield of *in vivo* biodistribution of bone-binding peptides, refining fluorescence imaging techniques, and providing important recommendations for future research on optimizing peptide design, imaging methodologies, and quantification of binding

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kinetics, this manuscript is well-suited for publication in the journal “Qeios“. Although substantial contribution to the subject is evident, there are minor corrections necessary:

1. “...To apply SPUM algorithms, fluorescence images were captured using nine excitation/emission combinations: excitation at 465 nm (with emission filters at 520, 540, 560, and 580 nm) and excitation at 500 nm (with emission filters at 540, 560, 580, and 600 nm). ... “ It should be clarified what the nine excitation/emission combinations are, because, as stated, two excitations (at 465 and 500 nm) with four filter variations each make eight total combinations.
2. There are numerous typing/writing errors, such as: “...fluorescence intensity was measured using FITC parameters (500/540 nm) to assess peptide binding across tissues and ROIs were defined to encompass the entire tissue area for analysis. ...”, “...Using a tighter ROI confined to the defect area reveals significant differences in the distribution of peptide around the defect when compared to other tissues for both D8 and YD8 peptides. [One-way ANOVA followed by group post hoc comparison was performed on select data, statistical significance is quantified as: ****P < 0.0001; ***P < 0.001; **P < 0.01; *P < 0.05, ns = no significance] ...“, „...Peptide binding and biodistribution in the skeletal tissues of OIM mice were evaluated through fluorescence imaging one hour after intravenous peptide injection, compared with control OIM mice that received a PBS injection...“ and so on.

Declarations

Potential competing interests: No potential competing interests to declare.