Topic : Calcium, vitamin D and bone

Roles of membrane estrogen receptor alpha in bone sparing effects of estrogens

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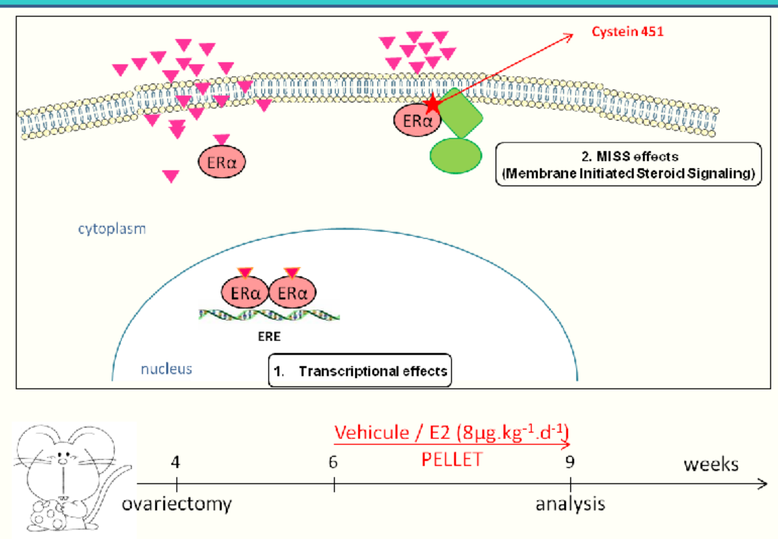
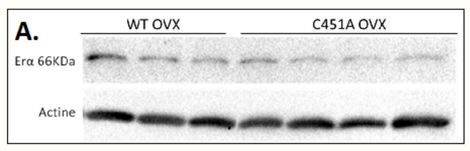
Introduction and objective

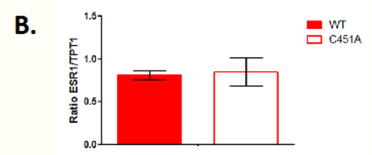
Estrogen bone-sparing effects are mediated *via* Estrogen Receptor alpha (ERα), which stimulates transciptional action through its two transactivating functions (AF1 and AF2) [1, 2]. Whereas ERα AF-1 plays a crucial role in trabecular bone, but not in cortical bone, ERα AF-2 is necessary for the estrogen effects in both types of bone [3]. In addition to these nuclear effects, a fraction of this receptor is targeted to the plasma membrane where it triggers membrane initiated steroid signaling (MISS). A pharmacological approach using an estrogen dendrimer conjugate suggested that the selective activation of membrane ERα is sufficient to elicit a sparing effect in cortical but not trabecular bone [4]. The aim of this study was to define the role of ERαMISS on the beneficial actions of estrogens on bone *in vivo*, using a mouse model in which ERα membrane localization is abrogated due to a point mutation of the palmitoylation of ERα (C451A-ERα).

Methods



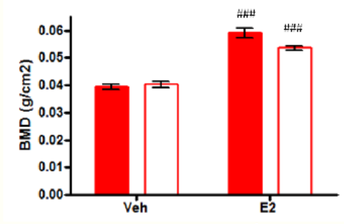
ERα expression is preserved in bone tissue of C451A-ERα mice





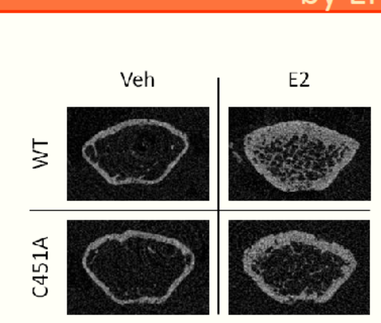
A. ERα protein levels in femoral shatfs of WT and C451A mice by western blot
B. ESR1 (ERα) mRNA levels by quantitative PCR in femoral shafts (n=5 animals per group)

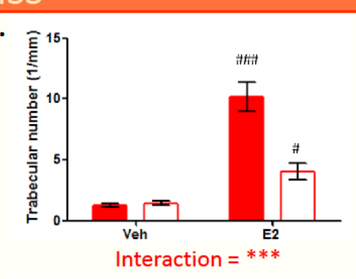
E2 effects on bone mineral density is partially lost in C451A mice

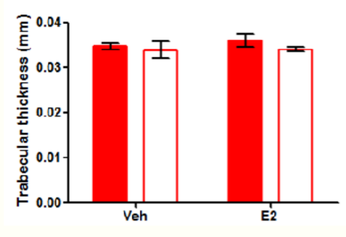


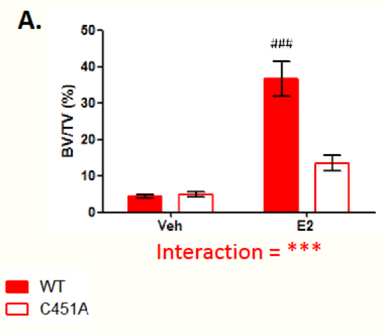
Bone Mineral Density (BMD) measurement in the whole femur of WT and C451A mice.
(n=5 animals per group).
p<0.001 *vs* Veh. (2 ways ANOVA)

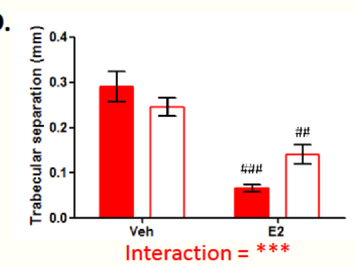
E2 effects on trabecular bone are mediated in part by ERαMISS





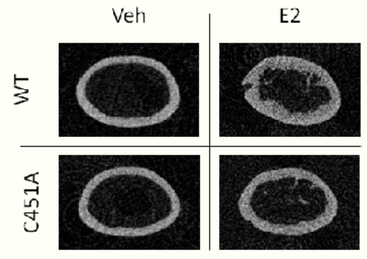


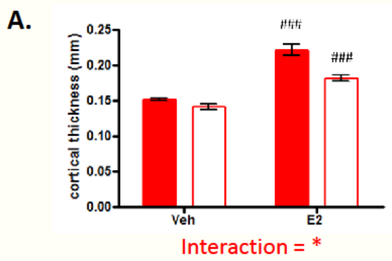


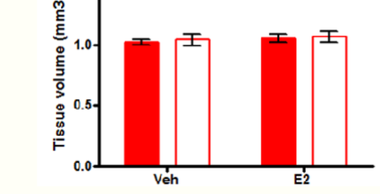


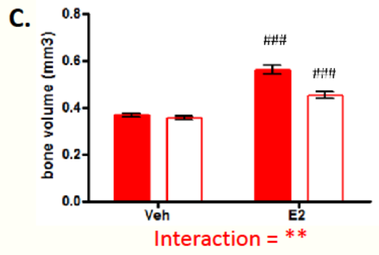
A. Trabecular bone (*i.e.* BV/TV) in femur analyzed by using µCT.
Detailed bone analyses of the E2 effects of in C451A mice on **B.**trabecular number, **C.** trabecular thickness and **D.** trabecular separation (n=5 animals per group)
p<0.001 *vs* Veh ; ## p<0.01 *vs* Veh ; # p<0.05 *vs* Veh ; 2 ways ANOVA

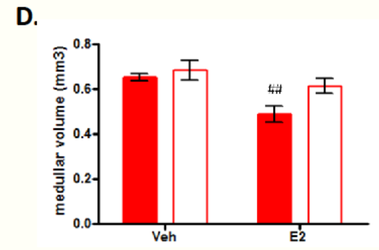
ERαMISS is involved in E2 effects on cortical bone





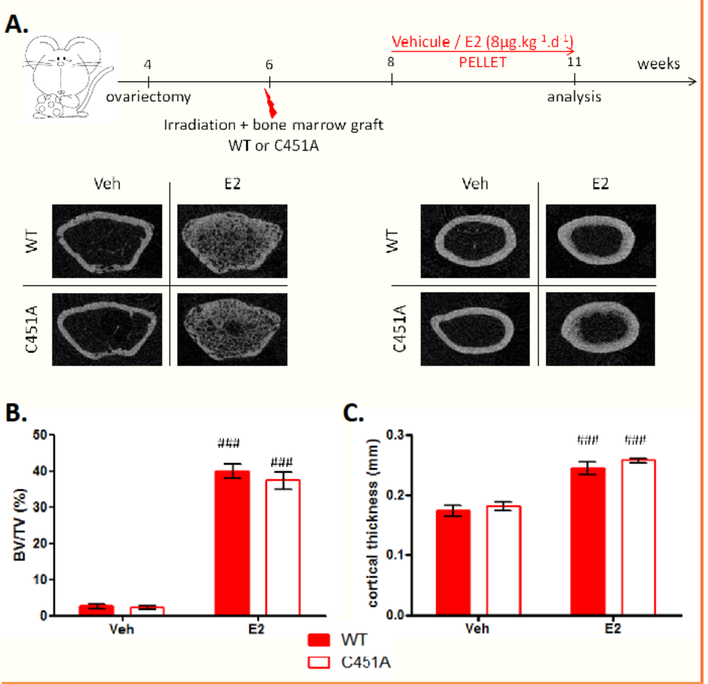






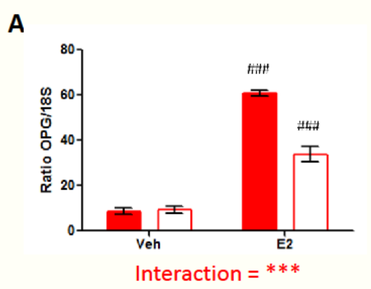
A. Cortical thickness in femur analyzed by µCT. Detailed analyzed of E2 effects in C451A mice on **B.** tissue volume, **C.** bone volume and **D.** medular volume. (n=5 animals per group)
p<0.001 *vs* Veh ; ## p<0.01 *vs* Veh ; # p<0.05 *vs* Veh ; 2 ways ANOVA

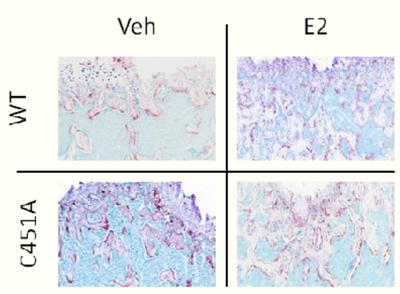
ERαMISS of medullar compartment is not involved in E2 effects on cortical and trabecular bone

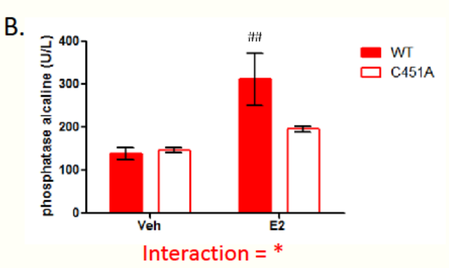


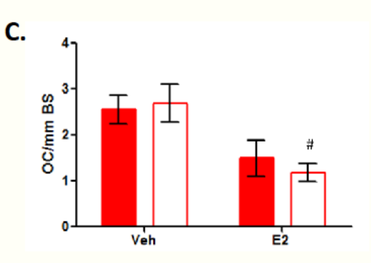
A. Schematic protocol used in order ot evaluate the role or ERαMISS in the hematopoietic compartment. **B.** Trabecular bone (BV/TV) in femur analyzed by µCT in mice grafted with bone marrow of WT or C451A mice. **C.** cortical thickness in femur of the same mice analyzed using µCT. (n=5 animals per group).
p<0.001 *vs* Veh ; 2 ways ANOVA

E2 effects on osteoclasts are preserved in C451A mice whereas they are altered in osteoblasts









A. Osteoprotegerin mRNA levels analyzed by quantitative PCR in femoral shafts. **B.** Alkaline phosphatase levels in serum collected immediately before euthanasia. **C.** histologic identification of osteoclasts (TRAP multinucleated cells) ; the mean number of osteoclasts per mm (±SD) present on the bone surface is shown.
p<0.001 *vs* Veh ; ## p<0.01 *vs* Veh ; # p<0.05 *vs* Veh. 2 ways ANOVA

Conclusion

Using a genomic approach, we demonstrate here that ERαMISS is necessary to elicit a full beneficial effect of estrogens on both trabecular and cortical bone. The use of bone marrow grafts showed that these MISS effects are not mediated by ERα of bone marrow compartment, including osteoclasts and lymphocytes, known to play a role in bone metabolism [5,6]. Accordingly, similar numbers of osteoclasts were found between WT and C451A mice treated by E2 but osteoblasts activity is significantly reduced in C451A mice as revealed by less osteoprotegerin level and alkaline phosphatase activity. In accordance with Bartell et al. [4], our results demonstrate that ERαMISS is not only sufficient but also necessary to induce an optimal beneficial effect on trabecular bone. In addition, althought selective activation of ERαMISS is not sufficient to induce E2 effect on cortical bone, we show here that ERαMISS is involved in this beneficial action, probably through a crosstalk of nuclear and membrane ERα activities.

References

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Calcium, vitamin D and bone
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DOI: 10.3252/pso.eu.17ece.2015
Poster presented at: ECE2015 16-20 May 2015 DUBLIN, IRELAND 17th EUROPEAN CONGRESS OF ENDOCRINOLOGY
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