Exemestane, a steroidal aromatase inhibitor, activates the TRPA1 channel on sensory neurons causing pain

C. Fusi[1], R. Nassini[1], S. Benemei[1], D. Minocci[1], P. Geppetti[1], S. Materazzi[1]

[1]Dept. of Health Sciences, University of Florence, Florence.

Exemestane (6-methylenandrosta-1,4-diene-3,17-dione) is a new irreversible, steroidal aromatase inhibitor (AI), structurally related to the natural substrate androstenedione, largely used for the treatment of advanced breast cancer in postmenopausal women. It acts as a false substrate for the aromatase enzyme, and is processed to an intermediate that binds irreversibly to the active site of the enzyme causing its inactivation and reducing circulating estrogen levels. Inhibition of aromatase by exemestane has been reported to be a safer and more effective treatment compared to antiestrogen therapy with tamoxifen or raloxifen (Goss et al., NEJM 2011) in postmenopausal patients with hormone-dependent breast cancer. Among the reported side effects associated with exemestane use, musculoskeletal and joint inflammatory pain represent severe and debilitating reactions which can limit compliance and cause therapy discontinuation (Winters L. et al., Clin J Oncol Nurs. 2007).

The Transient Receptor Potential Ankyrin 1 (TRPA1) channel, co-localized on primary sensory nerves with the 'capsaicin receptor' (TRPV1), is activated by a wide variety of noxious and irritant stimuli. These include a series of exogenously or endogenously produced highly reactive molecules that covalently modify cysteine or lysine residues of the protein (Trevisani et al. PNAS 2007). Based on the reactive chemical structure of exemestane, we hypothesized that it could stimulate nociceptive sensory neurons, *via* TRPA1 activation, producing, through this mechanism, a painful response.

By using calcium imaging assay we demonstrated that exemestane is able to activate, in a concentration-dependent manner, the human or rat recombinant TRPA1 channel, expressed in HEK293 cells. In addition, experiments performed in primary cultures of rodent (both rat and mouse) sensory neurons confirmed exemestane's ability to evoke a calcium response in capsaicin-sensitive neurons *via* a selective TRPA1 activation. Calcium response to examestane is, in fact, abated by a selective TRPA1 antagonist, HC-030031, and absent in sensory neurons isolated from TRPA1-deficient mice. In addition, intraplantar injection of exemestane elicits a concentration-dependent acute nociceptive response (licking/lifting) in wild-type mice, an effect that was absent in TRPA1-deficient mice or wild-type mice pretreated with HC-030031. Finally, exemestane injection evokes a mechanical and cold hypersensitivity through TRPA1 activation/sensitization.

These findings indicate that exemestane selectively activates both human and rodent TRPA1 channels. In addition, the ability of exemestane to activate sensory nerve terminals, *via* the TRPA1 channel, may be responsible for the inflammatory and painful side effects related to AI therapy. Present findings suggest that a TRPA1 antagonist could be useful in the treatment of patients with painful states evoked by AIs, such as exemestane.

Evans TR. et al., Cancer Res. 1992;52(21):5933-9.
Goss PE. et al., N Engl J Med. 2011;364(25):2381-91.
Trevisani M. et al., PNAS 2007; 104(33):13519 –24.
Winters L. et al., Clin J Oncol Nurs. 2007;11(3):433-9.

Acknowledgements: The study has been supported by grants from Regione Toscana: FABER – POR CREO, FESR 2007-2013 1.1.C and Associazione Italiana per la Ricerca sul Cancro (AIRC MFAG, 13336) and PRIN 2010-2011.