A ROLE FOR FIBROBLAST GROWTH FACTOR-2 (FGF-2) IN THE ANTI-INFLAMMATORY EFFECT MEDIATED BY A2A RECEPTOR ACTIVATION, IN VIVO.

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It is well known that adenosine acts as a protective endogenous anti-inflammatory agent by engaging its high-affinity receptor A2A (A2AR) (Antonioli et al., 2014).

A2AR has also been shown to mediate adenosine-induced matrix deposition and wound healing in a damaged tissue, contributing to repairing processes and fibrotic disorders (Cronstein and Sitkovsky., 2017). Fibroblast growth factor-2 (FGF-2) is a growth factor that has been implicated in tissue proliferation, wound healing but also in inflammation; however, its role in an inflamed tissue environment is still to be clarified (Redington et al., 2001; Seong et al., 2007).

Here, we evaluated changes in A2AR and FGF-2 in inflamed tissues following systemic administration of the A2A agonist, CGS21680. As model we used the rat paw oedema induced by carrageenan, that is an inflammation model characterized by local changes of epidermis and dermis, already demonstrated to respond to the anti-inflammatory effect of CGS21680 (Caiazzo et al., 2016).

Male Wistar rats (Charles River; 120-150) were slightly anaesthetized with enflurane and treated with the selective A2A agonist, CGS21680 (0.02-2 mg/kg ip.) alone or in co-administration with the A2A antagonist, ZM241385 (3 mg/kg ip.), or with an equal volume of vehicle (DMSO) just before carrageenan (100 µl, 1 % w/v) injection in the left hind paw. Oedema was measured by the means of a plethysmometer at time zero and each hour over a period of 6 hours.

Myeloperoxidase (MPO) assay, morphological analysis, Picro Sirius red staining for collagen, Western blot and immunofluorescence analysis for A2AR and FGF-2 expression and localization were performed on inflamed tissues excised at different time following oedema induction. Contralateral, not injected paws, were also excised as control tissues.

We found that rat treatment with CGS21680 inhibited in a dose related manner carrageenan-induced paw oedema and its effect was reversed by co-administration of A2AR antagonist, ZM241385 (3 mg/kg ip.). MPO activity measured in inflamed paws excised 3 hours after carrageenan injection was reduced after treatment with CGS21680 (2 mg/kg ip), compared with control values. Western blot analysis performed on paw excised at different time after oedema induction showed an increased A2A protein expression starting 1 hour following oedema induction, and peaking between 3 and 4 hours, that was reduced to control values by CGS21680 treatment (2 mg/kg ip). Picro Sirius red staining for collagen detection showed that in tissue section from inflamed animals dermal collagen resulted to be loose; conversely, in control paws (not inflamed) and CGS21680 treated animals dermal elastic fibers were well organized. Furthermore, when sections were analysed under polarized light an increased number of yellow, green and orange collagen fibers were evident in tissue from CGS21680 treated animals,

compared to control tissues. Interestingly, the expression of FGF-2 in rat paws, evaluated at each hour following carrageenan injection, was increased following rat treatment with CGS21680. Immunofluorescence analysis showed increased immunostaining for FGF-2 localized in the derma of paws from CGS21680 treated animals and also showed spots of co-localization between A2AR and FGF-2.

Our study suggest that the anti-inflammatory effect of A2AR activation is paralleled by an increased expression of FGF-2 that, in turn, might participate in matrix deposition.

1 Antonioli et al. (2014). Drug Discov Today. 19:1051-68.

2 Cronstein and Sitkovsky. (2017). Nat Rev Rheumatol. 13:41-51

3 Redington et al. (2001). J Allergy ClinImmunol. 107:384-7.

4 Seong et al. (2007). J AllergyClinImmunol. 119:831-837.

5 Caiazzo et al. (2016). Biochem Pharmacol. 112:72-81