Role of Low Frequency Low Energy Pulsed Electromagnetic Fields and Adenosine Receptors in Regulating Inflammatory Responses on Human Synoviocytes

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The present study describes the effect of low frequency low energy pulsed electromagnetic fields on A\textsubscript{3}, A\textsubscript{2A}, A\textsubscript{2B} and A\textsubscript{3} adenosine receptor expression and functionality in human synoviocytes by using in vitro experiments on different inflammatory responses.

INTRODUCTION

Physical stimulation with low frequency low energy pulsed electromagnetic fields (PEMFs) is a viable therapeutic approach to limit cartilage degradation and control inflammation associated to joint diseases [1,2]. In particular, in vitro cultured chondrocytes increase their proliferation as a function of PEMF exposure length [3]. In cartilage explants PEMFs increase proteoglycan synthesis preventing the catabolic effect of the pro-inflammatory cytokine [4]. Further, clinical studies in humans show that PEMF stimulation has beneficial effects in patients after arthroscopic surgery for cartilage lesions [5]. Adenosine is an endogenous nucleoside which mediates a number of physiological functions through the interaction with four cell surface subtypes, coupled to G proteins, classified as A\textsubscript{1}, A\textsubscript{2A}, A\textsubscript{2B} and A\textsubscript{3} receptors (ARs). Adenosine has been reported to reduce inflammation in several in vivo models, suggesting a potential value of adenosine as therapeutic mediator of inflammatory joint disease [6,7]. A role of adenosine in modulating chondrocytes and synoviocytes is well documented [7,8]. Previous studies have demonstrated that PEMFs evoke an up-regulation of the A\textsubscript{2A} and A\textsubscript{3}ARs and increase the anti-inflammatory response of these receptors in human neutrophils [9,10]. It has been also observed that the chondroprotective effects of PEMFs are based on anti-inflammatory mechanisms involving the stimulation of A\textsubscript{2A} or A\textsubscript{3} ARs [11-13].

METHODS

Saturation, competition binding experiments, mRNA and Western blotting assays in the absence and in the presence of PEMFs on ARs in human synoviocytes were performed. In adenylyl cyclase and proliferation assays the potency of high-affinity A\textsubscript{2A} or A\textsubscript{3} AR agonists in the absence and in the presence of PEMFs was evaluated. Adenosine analogs such as cyclohexyladenosine (CHA), 5'-N-ethyl-carboxamido-adenosine (NECA), (2-(p-carboxyethyl)-phenethylamino) -5'-N-ethyl-carbamido adenosine (CGS 21680) or (2-chloro- N\textsuperscript{6}- (3-iodo-benzyl) adenosine-5'-N-methyl-carboxamide (CI-IB-MECA) were added in untreated or exposed to PEMFs cell cultures. The effect of these agonists on p38 mitogen-activated protein kinases (MAPKs) and nuclear factor kB (NFkB) activation, TNF-\alpha, IL-6, IL-8 and IL-10 release were assessed. The release of PGE\textsubscript{2} and COX-2 expression was evaluated in TNF-\alpha or LPS treated synoviocytes in the absence and in the presence of PEMFs. The release of pro-inflammatory cytokines was also tested in the presence of selective AR antagonists to demonstrate the involvement of specific AR subtypes.

RESULTS

The presence of PEMFs evoked an up-regulation of A\textsubscript{2A} and A\textsubscript{3}ARs (Figure 1). In PEMF-treated cells the potency of the A\textsubscript{2A} or A\textsubscript{3} AR agonists on cyclic AMP assays was significantly increased when compared with the untreated cells (Figure 2). Activation of A\textsubscript{2A} or A\textsubscript{3} ARs inhibited p38 MAPK and NF-\kappaB activation, TNF-\alpha, IL-6, IL-8 and IL-10 release were assessed. The release of PGE\textsubscript{2} and COX-2 expression was evaluated in TNF-\alpha or LPS treated synoviocytes in the absence and in the presence of PEMFs. The release of pro-inflammatory cytokines was also tested in the presence of selective AR antagonists to demonstrate the involvement of specific AR subtypes.

![Fig. 1 Effect PEMFs on A\textsubscript{2A}ARs in human synoviocytes.](image-url)
CONCLUSIONS

The major finding of this study is that PEMFs mediate anti-inflammatory effects on human synoviocytes through the A3A or A2AR up-regulation. The pharmacological results revealed the direct involvement of the A3A and A2AR agonists in the inhibition of the inflammatory cascade. Functional data clearly indicate that the PEMFs via an indirect mechanism linked to the increase of A3A and/or A2AR density determine a potentiated anti-inflammatory response. Taken together these results suggest that PEMFs might represent a very innovative therapeutic approach in the control of inflammation associated to joint diseases.

REFERENCES


Functional data obtained in the presence of selective A3A and A2AR agonists or antagonists showed that PEMFs inhibit PGE2, IL-6, IL-8 while stimulate the release of the anti-inflammatory cytokine IL-10. These effects seem to be mediated by the PEMFs-induced up-regulation of A3A and A2 AR stimulation (Figure 3).