IN VIVO ^{19}F MRI FOR SENSITIVE ASSESSMENT OF ARTHRITIS: ANTIINFLAMMATORY ACTION OF A2A RECEPTOR ACTIVATION

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Introduction: Since rheumatoid arthritis (RA) is associated with persistent high levels of inflammation, the present study made use of the underlying autoimmune response to sensitively monitor the development of RA. For this purpose, we used emulsified perfluorocarbons (PFCs) which are preferentially phagocytized by monocytes/macrophages and readily detected by ^{19}F MRI [1,2]. This approach was employed to assess the therapeutical feasibility of nucleoside-5'-monophosphates derivatives to serve as prodrugs of adenosine A2A receptor agonists activated by ecto-5'-nucleotidase (CD73) [3]. Because CD73 is upregulated in inflamed tissue, the A2A agonists are expected to be released from their prodrug at the site of inflammation.

Methods: For induction of arthritis, DBA mice were immunized by sc injection of 100 mg collagen type II (CII) dispersed in complete Freund's adjuvant followed by an ip booster injection of CII at day 21 [4]. Collagen-induced arthritis (CIA) was graded by scoring each paw from 0 to 2. Twentyfour hrs prior to MRI, mice received an iv injection of PFCs (10% 15C5) at various times after surgery (n=5 each). ^{1}H/^{19}F MRI was performed at a vertical Bruker DRX Wide Bore NMR spectrometer operating at 400.1 MHz for ^{1}H and 376.5 MHz for ^{19}F measurements using a Bruker Microimaging unit (Mini 0.5) equipped with an actively shielded 57-mm gradient set (200 mT/m maximum gradient strength, 110 µs rise time at 100% gradient switching) and a ^{1}H/^{19}F 30-mm birdcage resonator. Anatomically corresponding ^{1}H and ^{19}F MR images were acquired with the following parameters: FOV 4x4 cm^2, slice thickness 1 mm; ^{1}H: MSME, matrix 256×256, acquisition time 10 min; ^{19}F: RARE (RARE factor 64), matrix 128×128, acquisition time 20 min. For therapy experiments, 14 d after booster injection (day 35) osmotic pumps were implanted to treat mice for one week continuously with cyclohexylethylthio-adenosine (chet-Ado) and the prodrug chet-AMP (n=6 each) at concentrations ≤ 1.5 µg/µl and an infusion rate of 0.5 µl/h. Degree of arthritis was measured 3 weeks after booster injection of CII (day 42) by ^{1}H/^{19}F MRI.

Results: In this RA model the first signs of joint inflammation become visible at day 24 after induction [4]. However, ^{1}H/^{19}F MRI at 9.4 T enabled us to detect the initial immune response not later than day 22 (1 day after the booster injection). The observed ^{19}F signal strongly increased with time and remained restricted to the joint space. Anatomically matching example images obtained at day 28 (one week after induction) are displayed below (Fig 1A). ^{1}H MRI shows the anatomy of the hind legs, while the concurrent ^{19}F image – after merging with the ^{1}H image – clearly reveals PFC accumulation in both synovial joints of the knees and also to a lower degree in the right paw. With developing arthritis we found a good correlation between ^{19}F signal intensity and visual score of the paws (R=0.974, n=11). Without CIA at no time were ^{19}F signals observed within the joints. In separate studies, this approach was applied to evaluate the success of chet-AMP treatment of CIA. Two weeks after booster injection animals showed severe signs of CIA, as indicated by strong ^{19}F MR signals localized within the joint spaces of knees and paws. Treating mice with chet-AMP for one week dose-dependently reduced the degree of arthritis by more than 60% (n=6, P<0.05, Fig. 1B). These results were confirmed by assessing the degree of CIA *via* visual scoring of paw edema formation. Measurement of cytokines/chemokines in plasma of chet-AMP treated and untreated mice revealed that in the prodrug-treated group IFN-γ, IL-6, IL-1β, MIP-1α were decreased. IL-10, which is known to inhibit activation and effector function of T cells, monocytes, and macrophages, was found to be increased. Hemodynamic studies revealed that upon acute iv infusion chet-AMP caused less vasodilatation compared to chet-Ado.

Conclusions: PFCs can serve as MRI contrast agent for the early detection of RA, thereby permitting a more timely therapeutic intervention. Furthermore, this study provides first *in vivo* evidence to establish the prodrug concept as a novel site-specific anti-inflammatory therapy.

References:
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Figure 1: (A) Anatomical matching ^{1}H and ^{19}F MRI of DBA mouse hind legs 1 week after acute induction of CIA. (B) Quantification of ^{19}F signals 3 weeks after acute induction of CIA and 1 week of therapy. DMSO treatment served as negative control.