Radiations as source of treatment for Rheumatoid Arthritis: X-rays reverse the effect of TNF-alpha in mouse micromass cultures *in vitro* *

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Introduction

Rheumatoid Arthritis (RA) is an autoimmune disorder which is associated with excess pain due to the accumulation of synovial fluid in the joints and severe cartilage destruction. It has been a huge challenge to find a treatment for this disease. Radon, an inert gas is believed to suppress the severity of the disorder. The mechanism underlying the suppression of pain after Radon exposure is not yet clear. Cholinergic anti-inflammatory pathway (CAIP) is believed to be one of the pathways involved in the suppression of inflammation, though its mechanism of action is yet to be proven. In the present study, we used ionizing radiations (X-rays) as a source of treatment. The results using in vitro cultures showed that treatment with TNF-alpha hindered cartilage and bone formation but X-rays reversed the effects of TNF-alpha. A rise in cholinesterase activity was seen after X-ray treatment, suggesting a possible the involvement of cholinergic system during treatment.

Material and Methods

11/11.5 day-old embryos from pregnant C57BLC wild type mice were collected. Mesenchymal cells were isolated from limb buds, plated as high density micro-mass cultures and incubated for 2 weeks at 37°C. The cultures were then treated with 5 or 10ng/ml human TNF-alpha to mimic a diseased condition in vitro. The cultures were exposed to 0.5, 1 and 2Gy X-rays and were fixed after 3, 5 and 7 days. Alcian blue staining marks cartilage development, while alkaline phosphatase stainings indicates differentiation of osteoblasts. Cholinesterase enzyme activity was visualized by Karnovsky-Roots staining.

Results

TNF-alpha affects mineralization in vivo. hTNF over expressing mice showed reduced Alp activity in vivo compared to wildtype mouse. Transgenic mice were treated with single dose of 0.5Gy X-rays in order to see if X-rays can effectively be used for treatment but at this dosage, no clear effects were noticed.

X-rays reverse the actions of TNF-alpha in vitro. Cultures that were exposed to 5ng/ml TNF-alpha showed severe destruction in cartilage differentiation and also mineralization. 2Gy X-rays reduced the effect of TNF-alpha to a larger extent supporting nodule formation and increased enzymatic activity for alkaline phosphatase suggesting that

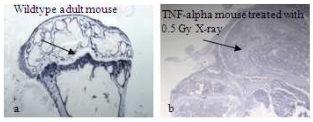

Figure 1: Alp activity in wildtype (a) and 0.5Gy x-ray treated hTNF-alpha over expressing mouse (b). Note Alp activity is severely disturbed in transgenic mouse even after X-ray treatment (arrow) in vivo.

X-rays reverse the action of TNF but these effects were not observed at 0.5Gy in vitro.

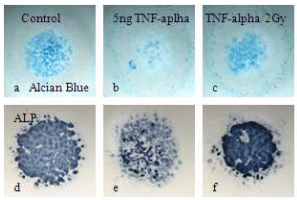

Figure 2: Note that TNF-alpha strongly disturbs cartilage formation (b, while a is control) but X-rays reversed the effects (c). A similar effect is observed in alkaline phosphatase activity as well (d-f).

X-rays increase cholinesterase activity suggesting a role of the cholinergic system during treatment. We provided a direct evidence of the involvement of cholinergic system during cartilage differentiation in our previous report. 5ng TNF-alpha suppressed acetylcholinesterase activity but nterestingly 2Gy X-rays rescued the activity.

Figure 3: Karnovsky and Roots staining for AChE activity revealed that 5ng TNF-alpha down regulated the activity (b, arrow; a, control) while 2Gy X-rays restored it (c, arrow).

Conclusion

– TNF-alpha disrupts cartilage and bone formation in vitro
– X-rays can reverse the effects of TNF-alpha
– A cholinergic mechanism might me involved during treatment

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