The Effects of Low Dose γ-Irradiation on MIA Induced Joint Inflammation

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1. Introduction

Inflammation of the synovial membrane is associated with the progression of cartilage degeneration and unexpected pain in osteoarthritis (OA). Inflammation produces painful sensations which are largely divided into spontaneous (non-evoked) pain and evoked pain depending on the presence of external stimuli and are characterized by hyperalgesia and allodynia
Nitric oxide (NO) is related to the pathogenesis of OA as inflammatory mediator. Inducible nitric oxide synthase (iNOS) is marker of enhanced NO production in arthritic pain. In previously, low dose irradiation can suppress pro-inflammatory cytokines. But, ray therapeutic effect is unclear.
Thus, present study examined the preemptive effect of low dose irradiation on the development of inflammatory pain in MIA induced OA animal model.

2. Methods and Results

All experimental procedures were conducted in accordance with guidelines set by the Korea University College of Health Science Animals Research Policies Committee. The animals were kept in a 12-h light:12-h dark cycle with light on at 7:00 a.m.

2.1 Experimental Animal Model

Male Sprague–Dawley rats (*N*= 59, 200–250 g; Sam, Korea) were used. To induce OA, the animals were injected with 4 % monosodium iodoacetate (MIA) (40 ul, in saline) in the right knee joint under enflurane anesthesia [1].

2.2 Low dose γ-irradiation

Animals received a whole body irradiation 0.1, 0.5 and 1 Gy [2] with 7 days using an X-ray generator (SFC-31R, Dong A X-ray, Korea). As a control non-irradiated rats were used. These rats were exactly treated as irradiated rats, but no irradiation was applied.

2.2 Weight Bearing

In the inflamed joint, weight loading generates pain perception, which is the major symptom in arthritis. We utilized the weight-bearing device [1] for assessment of arthritic pain in freely walking rats. This allowed for the measurement of weight load on each limb while the animal was walking through a path, the bottom of which was equipped with strain gauge weight sensors (Dana Load Cell, Korea). Preemptive irradiation (0.5 and 1 Gy) significantly reduced the degree of weight load reduction when it compared to the controls

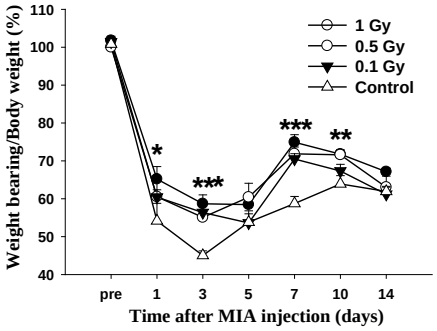

Fig. 1. Effects of preemptive irradiation on weight bearing. Animals were irradiated before 4% MIA injection into the knee joint.

2.3 Mechanical Allodynia

The threshold of brisk paw withdrawal response to a graded mechanical stimulus with a series of eight different von Frey filaments (0.41–15.10 g, Stoeling, Wood Dale, IL, USA) was measured. A von Frey filament was applied for 3–4 s to each hind paw while the filament was bent. The 50 % withdrawal threshold was determined using the up–down method [3] while initiating with the 2.0 g (4.31 mN) strength of filament. After MIA injection, rats showed a significant decrease in paw withdrawal threshold to mechanical stimuli compared to pre-value. Only preemptive irradiation at 1 Gy significantly prevented the MIA-induced decrease paw withdrawal threshold to mechanical stimuli, interpreting as mechanical allodynia.

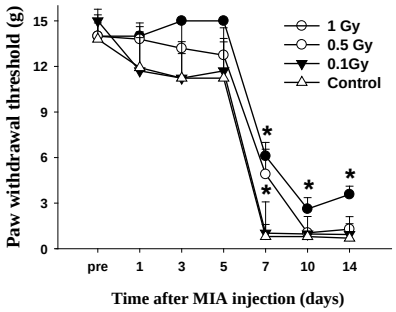

Fig. 2. Effects of preemptive irradiation on mechanical allodynia. Preemptive irradiation showed an antinociceptive effect on secondary mechanical allodynia.

2.4 Thermal Hyperalgesia

To assess hind paw heat sensitivity, Hargreaves' test was conducted using a plantar test device (7371 plantar test; Ugo Basile, Italy) (Hargreaves et al. 1988). A mobile radiant heat source was placed under the glass floor and focused onto the hind paw. Paw withdrawal latencies were measured with a cutoff time of 15 seconds. After MIA injection, rats showed a significant decrease in paw withdrawal latency to theramal stimuli compared to pre-value. Only pre-emtive irradiation at 1 Gy significantly prevented the MIA-induced decrease paw withdrawal latency to thermal stimuli, interpreting as thermal hyperalgesia.

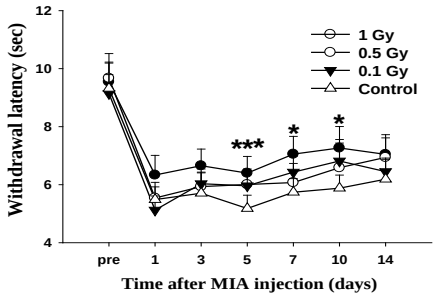

Fig. 3. Effects of preemptive irradiation on thermal hyperalgesia. Animals were irradiated before 4% MIA injection into the knee joint.

2.5 Western blot

To evaluate the possible preemptive effects of low dose irradiation on the development inflammation pain, we assessed the expression of iNOS level in spinal cord at 7th day after MIA induced arthritis, rats are deeply anesthetized. The spinal segments L3-5 are removed. Spinal cord homogenate was diluted by electrophoresis sample buffer and heated for 5 min at 95˚C. Samples containing about 40 ug protein were separated by 8 % SDS-PAGE and then transferred to PVDF membranes.
The membranes were incubated for 1h with 5 % blocking solution at room temperature. After 1h incubation, iNOS and β-tubulin was detected using their antibody (iNOS-1:2000, β-tubulin -1:200,000) with overnight incubation at 4˚C. The membranes were washed with TBST. And then, incubated with 1:6000 dilution of HRP conjugated anti-rabbit IgG secondary antibody for 30 min at room temperature. The membranes were washed again with TBST and then, reacted with ECL detection reagents. Preemptive irradiation (1 Gy) significantly reduced the degree of expression level of iNOS L3-5 spinal segments when it compared to the controls.

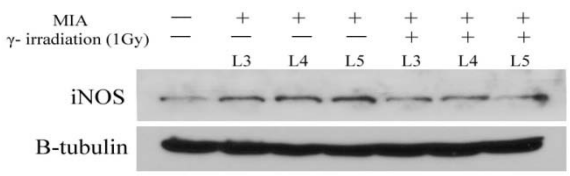

Fig4. Effect of preemptive irradiation induced suppression of spinal iNOS expression on OA rats.

3. Conclusions

Preemptive low dose irradiation on OA model produced anti-inflammatory and analgesic effect which is detectable by behavior tests and western blot. Therefor, low dose irradiation can be useful therapeutic tool in chronic inflammation disorder.

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