

Title

Effect of conditioned medium of peritoneal macrophages treated with all-trans-retinoic acid in experimental model of rheumatoid arthritis

Abstract:

This study aims to evaluate the effectiveness of ATRA on the conditioned medium of macrophage cells in the process of the appearance of anti-inflammatory agents and also to investigate the possibility of the application and therapeutic effects of the substance derived from the impact of ATRA on the conditioned medium of macrophage cells as an alternative strategy in the treatment of rheumatoid arthritis. To isolate macrophages, intraperitoneal injection of cold PBS was used in rats; For this purpose, after excising the spinal cord of the animal, 20 cc of cold PBS (4°C) with 10% FBS was injected into the peritoneum of the rats. The ventricular area was rubbed for 5 minutes and the cells were separated in aseptic conditions and transferred to a sterile falcon using aspiration. After centrifugation and repeated washing, the cells were transferred to a sterile flask. After 2 hours of incubation, the non-adherent cells (mainly B1 lymphocytes) were removed and the adherent cells were treated as macrophages with All-trans-retinoic acid in one micromolar for 24 hours. After this period, the culture medium was removed, and the macrophage cells were cultured for 24 hours in the RPMI culture medium without FBS. The supernatant was removed, and after centrifugation, it was preserved at -80 degrees Celsius as a conditioned medium until the experiments were performed. 25 male Wistar rats were randomly divided into 5 groups. First, rheumatoid arthritis was induced using 200 microliters of Freund's complete adjuvant in the plantar cushion area. In the negative control group, no treatment was administered. In the positive control group, methylprednisolone was given orally at a dosage of 2 mg/kg for the first 5 days after the clinical symptoms appeared. In the treatment groups, injections were performed on days 7, 14, and 21. In one group, peritoneal macrophages were cultured, while in the other group, a substance derived from the conditioned medium of macrophages treated by all-trans-retinoic acid at a dose of 400 microliters was injected intraperitoneally. During the study period, there was a significant difference in the improvement rate of the mentioned complication in the joint when comparing the groups. Before conducting the final tissue tests, we based our evaluations on the use of Collis calipers and typical clinical symptoms such as swelling, redness, and pain. On the 14th day, we noticed that the ATRA treatment group showed the best healing response compared to the positive control group.

Keywords:

Rheumatoid arthritis , Macrophage, Conditioned medium, All-trans-retinoic acid