Methotrexate (MTX) and albumin coupled with MTX (MTX-HSA) suppress synovial fibroblast invasion and cartilage degradation in vivo

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Objective: To investigate the effect of methotrexate (MTX) and albumin coupled with methotrexate (MTX-HSA) on cartilage invasion and induction of perichondrocytic degradation by rheumatoid arthritis synovial fibroblasts (RA SF) in vivo.

Methods: Human cartilage and RA SF were co-transplanted in three groups of severe combined immunodeficient mice (SCID), which received 1 mg/kg free MTX (n = 9), 1 mg/kg MTX-HSA (n = 6), or 0.9% NaCl (n = 5), respectively, intraperitoneally twice a week. After 4 weeks’ treatment, the mice were killed and the implants analysed histologically.

Results: The control group had a mean (SEM) score for cartilage invasion of RA SF of 2.0 (0.26) and for perichondrocytic cartilage degradation of 1.5 (0.34). In contrast, mice which received MTX showed a significantly reduced invasion (0.78 (0.14), p < 0.01) and a reduction in perichondrocytic cartilage degradation scores (0.69 (0.2), p < 0.05) in comparison with the control group. Mice treated with MTX-HSA also had significantly reduced scores for RA SF invasion into the cartilage (0.92 (0.41), p < 0.05) and for cartilage degradation (0.83 (0.44), p < 0.05) compared with controls. The effects of MTX and MTX-HSA were not significantly different between these two groups.

Conclusion: Treatment with MTX or MTX-HSA significantly ameliorates cartilage destruction in the SCID mouse model for human RA.

Methotrexate (MTX) and albumin coupled with MTX (MTX-HSA) as a new drug conjugate, which consists of albumin and MTX covalently coupled to each other in a 1:1 molecular ratio. MTX-HSA has a substantially prolonged half life in the circulation owing to increased extravasation of albumin in inflamed tissue and metabolism of albumin by cells with a high protein turnover, such as cells of the rheumatoid synovial tissue. MTX-HSA accumulates also in inflamed joints of mice with collagen induced arthritis (CIA). We have shown that in comparison with equivalent concentrations of MTX, MTX-HSA is significantly more efficient than MTX in the prevention of arthritis in mice with CIA. Moreover, human RA SF metabolised albumin conjugates in vitro and accumulated them intracellularly.

In this study, we provide new data showing that MTX and MTX-HSA can reduce the invasion of RA SF into cartilage, and the RA SF dependent stimulation of perichondrocytic degradation, in an in vivo model for human RA using severe combined immunodeficient (SCID) mice which were co-transplanted with human cartilage and SF from patients with RA.

METHODS

The SCID mice model for human RA was used as described previously. Briefly, synovial tissue was obtained from two patients with RA undergoing joint replacement surgery. The synovial tissue was obtained from the knee joint and from the wrist joint. The tissue was minced and placed in sterile saline solution. The tissue was then transplanted into SCID mice which were co-transplanted with human cartilage and RA SF. Thereafter, they were treated with intraperitoneal injections twice weekly of 0.9% NaCl (control, n = 5), or 1 mg/kg MTX (n = 9), or 1 mg/kg MTX-HSA (n = 6). After 4 weeks of treatment, the implants were analysed histologically (fig 2), and cartilage invasion by SF as well as perichondrocytic cartilage degradation were scored (*p < 0.05; **p < 0.01; error bars represent SEM).

Abbreviations: CIA, collagen induced arthritis; HAS, human serum albumin; IL, interleukin; MTX, methotrexate; RA, rheumatoid arthritis; SCID, severe combined immunodeficient; SF, synovial fibroblasts.
synovial membrane was dissected and RA SF were isolated, cultured, and characterised.24 Twenty female Crl-scidBR 4 week old SCID mice were co-transplanted with RA SF and normal human cartilage using the “inverse wrap” technique.25

MTX-HSA was prepared according to the protocol performed in our laboratory,26 using DCC = N,N'-dicyclohexyl-carbodiimide and HSI = N-hydroxysuccinimide (Aldrich, Steinheim, Germany) for activation and linking MTX (Deisenhofen, Germany) to HSA (Pharma Dessau, Dessau, Germany). For separation of the compounds, ultrafiltration units (exclusion size 30 kDa) from Millipore (Eschborn, Germany) were used.

To examine the effect of MTX and MTX-HSA in vivo, 20 SCID mice were treated randomly into three groups. Six days after implantation, 1 mg/kg free MTX, 1 mg/kg MTX covalently coupled with albumin (MTX-HSA), or 0.9% NaCl, respectively, were administered to the mice of the different groups intraperitoneally twice a week. The dose of MTX and MTX-HSA was chosen in accordance with previous studies, which had evaluated the maximum tolerable doses of MTX in SCID mice.27 Both drugs were given in the same dose of 1 mg/kg in order to examine the effect of the different pharmacokinetic properties of MTX and MTX-HSA in this model. After 4 weeks of treatment (eight injections) the mice were killed and the implants were removed and cut into 5 μm sections. After staining of the sections with haematoxylin and eosin (H&E), the grade of cellular invasion into the cartilage by the co-implanted SF and the grade of perichondrocytic cartilage degradation did not differ significantly between the two treatment groups.

DISCUSSION

The results of the study show that treatment with either MTX or the new conjugate MTX-HSA significantly ameliorates cartilage destruction in the SCID mouse model for human RA. The experiments demonstrate also that these inhibitory effects are not only due to a direct effect on RA SF, which is illustrated by their reduced invasivity, but most likely also due to a reduction of the interleukin (IL)1 mediated effects of RA SF on chondrocytes.28 29 Therefore, indirect effects of MTX by modulation of the IL1/IL1 receptor antagonist ratio as outlined previously,20 31 might additionally be responsible for the observed effects of MTX in this model.

Most interestingly, the data demonstrate that MTX-HSA and MTX both inhibit fibroblast dependent invasion and degradation of cartilage in the SCID mouse model of RA. Therefore, it can be speculated that MTX-HSA may combine the effectiveness of MTX in the treatment of rheumatoid joint destruction with improved pharmacokinetic properties—namely, a prolonged half life and a potential accumulation of the drug in the inflamed joints, as recently demonstrated.30

In summary, MTX and MTX-HSA inhibit both aspects of RA SF mediated destruction of cartilage in vivo. With the use of MTX-HSA, a treatment specifically targeted to the destructive compartments might be feasible, which might also lower the rate of side effects of MTX treatment.

REFERENCE

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