CONSTITUTIVE AND IL-1ß INDUCED EXPRESSION OF METALLOPROTEINASES BY SYNOVIAL LINING CELLS MEDIATED BY GAP-JUNCTION INTERCELLULAR COMMUNICATION

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INTRODUCTION:

The ability of cells to communicate directly though gap junctions, which probably occur between most cell types including synovial lining cells (1), has led to the idea that gap junctions transmit metabolic regulatory signals, and there is now a need to define the circumstances in which the intercellular communication actually determines the overall cellular response. If progression of osteoarthritis (OA) were one such case, we would expect that intercellular communication would be critical to a secretory pathway leading to clinical manifestations of the disease. One such pathway is the process by which synovial lining cells control cartilage remodeling. Dysregulation leads to increased production of matrix metalloproteinases (MMPs), resulting in destruction of cartilage or its inadequate repair. The aim of our study was to test the hypothesis that the MMP activity produced by standardized synovial biopsies in response to stimulation by interleukin-1ß (IL-1ß) was mediated by gap-junction intercellular communication.

METHODS:

Synovial biopsies were obtained from patients having total knee replacement for OA, Kellgren-Lawrence Grade IV. All experimental procedures were approved by the Institutional Review Board for Human Research at our institution. The tissues were taken from the suprapatellar pouch and the fat pad using the full bite of an arthroscopic basket punch. Only relatively flat areas were biopsied to minimize variations in the number of synovial lining cells attributable to irregularities in the synovial surface. A second bite was made as close as possible to the same location, but deeper in the tissue, to obtain tissue containing a minimal number of synovial lining cells (fat biopsy). In preliminary studies, we established that the biopsies averaged 20 mg (0.12 cm^2) ±15%. Up to 12 biopsies of each type were obtained from each patient.

Immediately after the biopsies were done, the tissues were washed 4 times in Neuman-Tytell medium (NTM) and then incubated at 37°C for up to 24 hours in 200 µL NTM containing various combinations of IL-1ß and the gap-junction inhibitors octanol (1 mM) or 18□-glycyrrhetinic acid (10 µM). The MMP activity of the supernatant (principally collagenase (MMP-1) and stromelysin (MMP-3)) was then measured using collagen film as a substrate after MMP activation by 1 mM p-amino-phenylmercuric acetate; further details are given elsewhere (1).

RESULTS:

Intercellular communication through gap junctions was critical to the ability of the synovial lining cells to produce MMPs in response to IL-1ß (Figure 1). When gap junction inhibitors were added to the culture media 10 minutes before addition of the IL-1ß, an effect on IL-1ß-induced MMP production occurred beginning about 8 hours after addition of the cytokine, and statistically significant reductions of MMP activity were found after 18 and 24 hours.

The constitutive secretion of MMP activity by OA synovia was also mediated by gap-junction intercellular communication (Figure 2). Each gap-junction inhibitor significantly reduced the amount of MMP activity secreted into the culture medium.

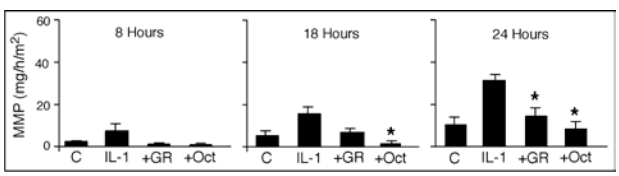

*Figure 1. Gap-junction inhibitors blocked the ability of IL-1ß to induce MMP production by synovial biopsies from patients with OA. Control (C) (no IL-1ß or inhibitors). IL-1ß (100 pg/mL IL-1ß). +GR, addition of IL-1ß and 18alpha-glycyrrhetinic acid (10 µM). +Oct, addition of IL-1ß and octanol (1 mM). Octanol or 18alpha-glycyrrhetinic acid were added to the culture media 10 minutes before addition of IL-1ß. Matrix metalloproteinase activity was measured after the tissues were exposed in culture to the indicated conditions for 8, 18, and 24 hours. Three measurements per condition per patient, N=4 patients. Mean ± SE, *, $p < 0.05$, compared with IL-1ß, paired t test.*

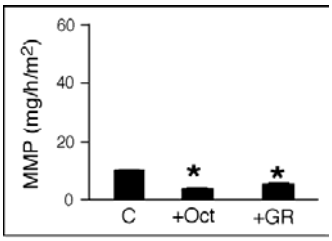

*Figure 2. Gap-junction inhibitors significantly reduced the amount of constitutive secretion of MMPs by synovial biopsies from patients with OA. Control (C) (no gap-junction inhibitors). +GR, addition of 18□-glycyrrhetinic acid (10 µM). +Oct, addition of octanol (1 mM). The tissues were exposed in the presence and absence of the inhibitors for 24 hours, and the enzymatic activity of the supernatant was then measured. Three measurements per condition per patient, N=5 patients. Mean ± SE, *, $p < 0.05$, paired t test.*

Control experiments using the fat biopsies produced only negligible MMP levels, either constitutively or in response to IL-1ß (data not shown). It could be concluded, therefore, that the observed effects (Figures 1 and 2) were due to intercellular communication between synovial cells rather than fat cells.

DISCUSSION:

Synovial lining cells in synovial biopsies expressed MMPs constitutively and in response to stimulation with IL-1ß (Figure 1). Intercellular communication through gap junctions between the synovial lining cells was essential for both stimulated and constitutive secretion of MMPs, as evidenced by the significant reductions of MMP activity when the cells were cultured in the presence of each of two chemically distinct gap-junction inhibitors (Figures 1 and 2). The possibility cannot be excluded that the inhibitory effects were nonspecific and did not evidence a role for intercellular communication in the secretory activity of the cells. However, that possibility seems unlikely because it would require two standard gap-junction inhibitors to have produced a nonspecific effect of approximately the same magnitude.

Overall, the results supported the view that intercellular communication between synovial lining cells was critical to the ability of the cells to secrete MMPs constitutively and in response to stimulation by IL-1ß.

REFERENCES:

1. Kolomytkin OV, Marino AA, Waddell DD, Mathis JM, Wolf RE, Sadasivan KK, Albright JA. Am J Physiol 282:C1254-C1260, 2002.

ACKNOWLEDGEMENT:

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