Changes in cyclooxygenase-2's expression, and PGE2's and 6-keto-PGF1α's levels in the presence of the muscarinic acethylcholine receptor antibody in primary Sjögren Syndrome

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Objective:

to assess the inflammatory process provoked by the M3 muscarinic acetylcholine receptor antibody from pSS patient's sera in rat submandibular gland by measuring the expression of mRNA COX-2 and production of PGE2 and PGI2

Methods:

The levels and the generation of PGE2, 6-keto-PGF1α by Enzyme-linked immunoabsorbent assay (ELISA), cyclic AMP (cAMP) by cAMPc-RIA kit and COX-2 mRNA gene's expression at Real Time PCR in rat submandibular gland acini's preparations were measured in the presence of the autoantibodies alone or after incubation with different inhibitors. PGE2 and 6-keto-PGF1α were also measured in serum from pSS patients.

Statistical analyses:

The Student's "t" test for unpaired values was used to determine the level of significance. Differences between means were considered significant if $P<0.05$.

Results:

To determine the effect of pSS IgG anti M3 peptide on gland acini's we analyzed the time-course of COX-2 mRNA expression by Real Time-PCR. COX-2-mRNA was significantly ($P<0.001$) increased in the presence of pSS IgG anti M3 versus normal serum (control). When the autoantibody was incubated in the presence of DuP697 (1x10-6 M) [a specific COX-2 antagonist] under the same experimental conditions, it abrogated the increment of COX-mRNA's expression in a significant manner ($P<0.01$).

Primary Sjogren Syndrome IgG anti M3 antibody increased the production of both prostanoids PGE2 and 6-keto-PGF1α on submandibular gland acini's in a dose-response concentration curve reaching the maximal when the antibody concentration is 1x10-8 M. The increment in the generation of both prostanoids is abrogated, reaching values similar to basal ones, when the tissue preparations are incubated with prostanoid antagonists PF-04418948 2x10-9 M and RO3244794 5x10-8 M for PGE2 and 6-keto-PGF1α respectively, and in the presence of synthetic M3 peptide 5x10-6 M.

To ascertain if cAMP increment is caused by the generation of PGE2 and 6-keto-PGF1α in our preparation, we studied the action of both prostanoids on the production of this nucleotide. Both prostaglandins were able to increase cAMP production, whereas the selective prostanoids antagonists (PF-04418948 for PGE2 and RO3244794 for 6-keto-PGF1α) blunted the stimulatory action provoked by the prostaglandins.

The levels of PGE2 and 6-keto-PGF1α were studied in serum of 28 pSS patients and in 25 healthy individuals. The concentration of 6-keto-PGF1α and PGE2 in serum of pSS patients was two standard deviations higher than that in normal individuals ($P<0.001$).

Conclusion: The present study suggests a complex interplay between different factors involved in adaptativa autoimmunity in pSS patients at the level of exocrine glands. The presence of anti M3 IgG autoantibody from pSS sera was able to stimulate COX-2 mRNA gene's expression and the increment in the generation of PGE2 and 6-keto-PGF1α abolished by M3 specific cholinergic antagonist. The prostanoids play an important role in the inflammatory process at exocrine gland level.