Cyclooxygenase-2 (COX-2) expression determination in prostate cancer and benign prostatic hyperplasia tissue

Floriano-Sánchez E, Torres-Salazar JJ, Cárdenas-Rodríguez N, Castro-Marin M, Zapata-Villalba MA, Flores Terrazas JE.

ABSTRACT

Background: Recent reports have placed prostate cancer in third place worldwide; in Mexico it is the principal cause of death by cancer in men after lung cancer and more than 70% of cases present in advanced stages.

Several genetic markers for prostate cancer are being studied but there are very few studies on cyclooxygenase-2 in the literature. For this reason the present study evaluates whether or not there is any alteration of cyclooxygenase-2 levels in prostate cancer and benign prostatic hyperplasia.

Methods: From a total of 81 samples, 39 corresponded to benign prostatic hyperplasia tissue and 42 corresponded to prostate cancer tissue. Conditions were standardized in order to immunohistochemically detect the presence of cyclooxygenase-2.

Results: The percentages of the area that was immunoreactive to cyclooxygenase-2 in stroma were 7.41% in prostate cancer and 12.11% in benign prostatic hyperplasia. These percentages in the gland were...
INTRODUCTION

Prostate cancer (CaP) is the second cause of death in men after lung cancer. CaP incidence increases three to four times every 1 years after 50 years of age. In Mexico, CaP incidence is second to that of skin cancer and CaP mortality is second to that of cervical cancer.

Although prostate specific antigen (PSA) is the best serum indicator for CaP diagnosis available today, it has great limitations in spite of its sensitivity. PSA use is limited due to its poor specificity and can result in unwarranted prostate biopsy in a solid number of patients clinically evaluated with elevated PSA.

Cyclooxygenase-2 (COX-2) has been associated with carcinogenesis due to angiogenesis stimulation which is a crucial process for tumor growth and expansion. There is COX-2 overexpression in the majority of primary cancers in humans regardless of histological type or grade. In contrast, COX-2 is not expressed in benign pancreatic tumors, suggesting that COX-2 represents a marker for potential malignancy in pancreatic cancer.

In a study carried out on intestinal tumors, the expression of COX-1 levels was normal in intestine whereas COX-2 levels were undetectable in normal intestine and its levels were elevated up to 85% in colorectal adenocarcinoma.

There are studies supporting the idea that there is a COX-2 expression increase in specimens with CaP and a high Gleason score while others show that COX-2 expression is not congreuntly elevated in CaP and is not related with the establishment of clinicopathological risk factors similar to Gleason score and pathological stage.

Immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR) have enabled these studies to be carried out and COX-2 has been shown to play an important role in cancerous cell proliferation in the prostate. The inhibition of COX-2 expression inhibits carcinogenesis. Another study related glucocorticoid therapy to COX-2 transcription reduction.

Endothelin 1 also significantly increases COX-1 and COX-2 expression. COX-2 expression promotes prostaglandin E2 (PGE2) activity and production. These effects were seen to depend on endothelin A (ET-A) receptor activation and mitogen-activated protein kinase (MAPK) in a study carried out on human ovarian carcinoma cells.

CaP is the second cause of death from cancer in men and in some studies a diet rich in animal fat has been described as a triggering factor in the appearance of cancer and COX-2 expression has been associated with tumor grade.

METHODS

Study subjects: Samples were collected towards the end of September 2008 and up to April 2009 from patients seen at the Hospital Central Militar who met the following inclusion criteria: (a) patients diagnosed with CaP who were candidates for radical prostate surgery, (b) patients diagnosed with CaP with prostate tunnelization indication and (c) patients with BPH diagnosis with transurethral resection of the prostate (TURP) indication. Exclusion criteria were: (a) patients who did not give their authorization to participate in the study and (b) patients who were not TURP candidates. Elimination criteria were: (a) inadequate purity and concentration of extracted RNA (b) insufficient amount of tissue and (c) tissue suffering degradation of genetic material during

Conclusions: Cyclooxygenase-2 expression levels in prostate cancer tissue were significantly lower than those obtained in benign prostatic hyperplasia tissue, suggesting that this gene could be used as a marker in early development of prostate cancer.

Key words: prostate cancer, benign prostatic hyperplasia, cyclooxygenase-2, Mexico.
transfer. The amount of tissue necessary for the study was 1-5 g. Samples were obtained immediately after surgery. Maximum transfer time was one hour from the moment of obtaining the samples to their completed transfer the Molecular Biology Laboratory of the Escuela Médico Militar, where they were kept at a temperature of -70°C in a Revco® ultrafreezer (Legaci ULT2186 3-35 Dupont SVVA Refrigerants).

**Immunohistochemistry:** Under a light microscope CaP and BPH tissue were fixed by formalin immersion (pH=7.4) and embedded in paraffin. For histological analysis tissue sections (3 mm) were stained with hematoxylin and eosin (H&E) stain. Tissue sections were stained with periodic acid-Schiff stain (PAS) to show polysaccharides, mucopolysaccharides and glycoproteins of the cell membrane.

Slices were incubated with periodic acid for 5 minutes and washed with distilled water. Slices were incubated with Schiff’s reagent for 5 minutes and counterstained with hematoxylin for 30 seconds. Histological profile of 5 randomly selected fields was registered using KS-300 software (Carl Zeiss, Jena, Germany). The percentage of damaged area with histopathological alterations was obtained (400x magnification). For immunohistochemistry tissue sections (3 mm) were deparaffinized and heated to unmask antigen sites. Peroxidase endogenous activity was blocked with 0.03% H2O2 in absolute methanol. Tissue sections were incubated overnight at 4°C at a 1:200 dilution of monoclonal antibody against COX-2 in TRIS solution. Primary antibody was removed and tissue was washed twice with TRIS. Slices were incubated with a 1:500 dilution of rabbit polyclonal antibody as secondary antibody and washed twice with TRIS. Bonded antibodies were detected using avidin-biotin complex (Vectastain ABC kit) and dianimobenzidine as substrate. After repeated washes with TRIS, slices were counterstained with hematoxylin stain. All slices were incubated under the same conditions at the same antibody concentration and in the same run so that immunostain was comparable. All specimens were examined under Axiowert 200M light microscope (Carl Zeiss, Jena, Germany). For automated morphometric analysis, positive cell (brown) percentage was determined using the KS-300 3.0 computerized image analyzer (Carl Zeiss, Jena, Germany). This equipment automatically detects positive cells, determining their percentage by field. Five random fields were studied at 100x magnification (total area 1584 000 μ2). Results were expressed as percentage.

**Data analysis:** COX-2 immunoreactivity was analyzed using the Student t test with mean SD. Both groups (CaP and BPH) were compared and statistically significant difference was considered to exist when \( P < 0.05 \).

**RESULTS**

Biological samples were collected from a total of 81 patients: 42 (51.85%) with established CaP diagnosis and 39 (48.14%) with BPH diagnosis.

Mean age of CaP patients was 67.77 ± 8.65 and mean age of BPH patients was 64.37 ± 11.07. Comparison of both groups with t-test for independent samples was not statistically significant and so age of both groups was accepted as equivalent two-tailed \( t = 1.326, P = 0.190 \). Gleason score in the CaP group was 4.47 ± 3.1; median 5 and maximum value 9.

PSA value was 35.29 in the CaP group and 26.32 in the BPH group.

Quantitative data of COX-2 expression were 12.11% and 14.76% in BPH stroma and gland and 7.41 and 5.12% in CaP stroma and gland, respectively.

By means of t-test for independent variables, statistically significant differences were found: \( P = 0.0314 \) for immunoreactivity percentage in areas per field (400x) in stroma; \( P = 0.0001 \) for immunoreactivity percentage in areas per field (400x) in gland. Gland immunoreactivity was much higher in BPH tissue, and so the study hypothesis was accepted, assuming there was a statistically significant difference between both groups in regard to immunoreactivity percentage of areas per field (400x) in stroma and immunoreactivity percentage of areas per field (400x) in gland (Images 1, 2 and 3).

**DISCUSSION**

COX-2 is an inducible enzyme that is observed in sites where there is inflammation and pain. Recent studies have evaluated COX-2 expression in CaP where Cox-2 over-regulation has been related to inhibition of this enzyme’s activity in cancerous cells. An increase of COX-2 has been observed in both CaP and BPH, but there are discrepancies in the published reports as to COX-2 expression levels in both groups and the type of cells in which the enzyme is expressed. In this regard, it has been seen that there is a difference in COX-2 intracellular localization in BPH and CaP epithelial cells. While COX-2 located in the basal and basolateral cell membrane in BPH is significant, its location in the cytoplasm in CaP is predominant. Bibliographic evidence points out that COX-2 protein is expressed at very low and non-detectable levels in normal prostate tissue. In addition when COX-2 staining is observed in CaP, the degree
Floriano-Sánchez E, et al. Cyclooxygenase-2 (COX-2) expression determination in prostate cancer and benign prostatic hyperplasia tissue of positive stain does not correlate with established clinicopathological risk factors. Nevertheless, other studies show a relation between COX-2 and tumor differentiation grade.

COX-2 expression in the present study showed that this enzyme is significantly expressed in BPH when compared with CaP.

**CONCLUSIONS**

The results of the present study appear to suggest that COX-2 plays an important role in tumor differentiation grade.

**BIBLIOGRAPHY**