Manganese superoxide dismutase (Mn-SOD) expression levels in prostate cancer and benign prostatic hyperplasia tissue

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ABSTRACT

Background: Recent reports place prostate cancer as the third most common cause of death from cancer in the worldwide male population. In Mexico it is the principal cause of death from cancer in men and more than 70% of those cases are in advanced stages. Several genetic markers involved in prostate cancer are currently being studied. Manganese superoxide dismutase (Mn-SOD) is associated with cancer, but there are very few studies in the literature analyzing this relation. Therefore, the objective of the present study was to evaluate the existence of alterations in Mn-SOD levels in prostate cancer and benign prostatic hyperplasia.

Materials and methods: Eighty samples, 50% of which corresponded to benign prostatic hyperplasia and 50% to prostate cancer, were obtained. Immunohistochemical diagnostic method was standardized for the detection of Mn-SOD in tissue.

Results: The percentage of Mn-SOD immunoreactive area was 24.83 ± 10.5% in prostate cancer and 14.73 ± 8.7% in benign prostatic hyperplasia.

Conclusions: Mn-SOD expression levels in prostate cancer were significantly higher than those in benign prostatic hyperplasia.

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RESUMEN

Antecedentes: Publicaciones recientes ubican al cáncer de próstata en el tercer lugar de mortalidad en hombres a nivel mundial, en México es la principal causa de muerte masculina y más del 70% se presenta en estadios avanzados. En la actualidad están en estudio varios marcadores genéticos en cáncer de próstata; la Mn-superóxido dismutasa está asociada con el cáncer pero no existen suficientes estudios al respecto, por ello el objetivo del presente estudio fue evaluar la existencia de alteraciones en los niveles de la Mn-superóxido dismutasa, en cáncer de próstata e hiperplasia prostática benigna.

Material y métodos: Se obtuvieron 80 muestras de las cuales 50% correspondieron a hiperplasia prostática benigna y el resto a cáncer de próstata. Se estandarizó el método diagnóstico por inmunohistoquímica para detectar la presencia de Mn-superóxido dismutasa en los tejidos.

Resultados: El porcentaje de área inmunorreactiva a Mn-superóxido dismutasa fue de 24.83 ± 10.5% y de 14.73 ± 8.7% en cáncer de próstata y en hiperplasia prostática benigna, respectivamente.

Conclusiones: Los niveles de expresión de Mn-superóxido dismutasa en cáncer de próstata fueron significativamente mayores que en la hiperplasia prostática...
prostatic hyperplasia, suggesting that this gene could be used as a tumor marker in biopsies in patients suspected of presenting with prostate cancer.

**Key words:** Prostate cancer, benign prostatic hyperplasia, Mn-superoxide dismutase, Mexico.

**INTRODUCTION**

The male organ that most often suffers benign or malignant processes is the prostate. Benign prostatic hyperplasia (BPH) is the most common benign tumor in men and its incidence is age-related. The number of men presenting with prostate cancer has increased in the last two decades as a result of increased population lifespan. In Mexico, prostate cancer (CaP) in 2000 held second place in relation to neoplasm incidence in men, after skin cancer. The specific causes of CaP onset and progression are not known, however, genetic and environmental factors have been attributed to the progression of this disease. Ninety-five percent of malignant neoplasms of the prostate correspond to acinar adenocarcinomas, 70% of which occur in the peripheral zone.2,3

The Gleason classification system defines tumor grade for prognostic purposes.4 The patient presenting with localized CaP is usually asymptomatic, although digital rectal examination (DRE) may reveal induration, irregularity and loss of symmetry in the gland. If CaP is diagnosed in time, treatment is effective and morbidity is reduced. Diagnosis is made by performing DRE and obtaining prostate-specific antigen (PSA) and it is confirmed by carrying out transrectal ultrasound and biopsy.

BPH prevalence is age-associated and onset is after 40 years of age. There is a 50% prevalence at 60 years of age and a 90% prevalence at 85 years of age. Fifty percent of patients with a histological diagnosis of BPH present with moderate to severe lower urinary tract symptoms.4

BPH has a multifactorial and endocrine etiology. Histologically, the prostate is made up of stroma and epithelium and each one, individually or combined, can give rise to hyperplastic nodules and to characteristic symptoms.5 Factors associated with BPH development are elevated levels of dihydrotestosterone (DHT) and aging. Clinical observation and studies in men have demonstrated the endocrine control involved in BPH.

It is known that oxidative stress plays a part in CaP development. Oxidative stress is a result of an imbalance between reactive oxygen species (ROS) production and antioxidant defenses which can present as excessive ROS production or defense antioxidant reduction or a combination of both. Within enzyme antioxidant systems, manganese-superoxide dismutase (Mn-SOD) plays an important role in maintaining ROS cellular balance. It is in charge of catalyzing the conversion of superoxide to hydrogen peroxide, due to the fact that mitochondrial respiration creates a larger quantity of free superoxide in the cell.6

Reduced Mn-SOD activity has been shown to be associated with different types of tumors. Mn-SOD over-expression suppresses the tumorigenicity of human melanoma, breast cancer cells and glioma cells, suggesting that Mn-SOD acts as a tumor suppressing gene in a wide variety of cancers. Many studies suggest that Mn-SOD may function as a general tumor-suppressing gene, leading to therapeutic applications in the near future.7

The suppressing function of Mn-SOD has been related to ovarian cancer, breast cancer, lung cancer and CaP.8

Taking the above information into account, the decision was made to analyze Mn-SOD expression levels in CaP and BPH.

**MATERIALS AND METHODS**

**Object of study:** Samples were collected from December 2005 to April 2007. They were selected from patients annotated in the surgery registrar of the urology
service at the Hospital Central Militar who met with inclusion, exclusion and elimination criteria.

**Inclusion criteria:** Samples from patients diagnosed with CaP with obstructive lower urinary tract syndrome and samples from patients diagnosed with BPH who were to undergo transurethral resection of the prostate (TURP) and radical prostatectomy were included in the study.

**Exclusion criteria:** Patients unwilling to participate in the study and patients who were not TURP candidates.

**Elimination criteria:** Tissue with CaP and BPH foci under 1 cm² and tissue that suffered genetic material alteration in the transfer process were eliminated from the study.

The amount of tissue necessary for the study was 1-5 grams. Samples were obtained immediately following surgical intervention. Maximum transfer time was 1 hour from the time the samples were taken to their arrival at the Molecular Biology Laboratory of the Escuela Médico Militar, where they were kept at a temperature of -70°C in a Revco® freezer (Legacy ULT2186 3-35 Dupont SVVA Refrigerants).

**Immunohistochemistry:** Using light microscopy, CaP and BPH tissue samples were fixed in formalin (pH=7.4) and embedded in paraffin. Tissue sections (3 μm) were stained with hematoxylin and eosin (H&E) and with periodic acid-Shiff (PAS) to show the presence of polysaccharides, mucopolysaccharides and glycoproteins in the cell membrane. Slices were incubated with periodic acid for 5 minutes and washed with distilled water and then incubated with the Shiff reactive for 5 minutes and counter-stained with hematoxylin for 30 seconds. The histological profile of the 5 randomly selected fields were registered using KS-300 software (Carl Zeiss, Jena, Germany). The percentage of damaged area with histopathological alterations was obtained (400X magnification). To carry out immunohistochemistry, paraffin was removed from the tissue sections (3 μm) and they were heated to unmask antigen sites. Endogenous peroxidase activity was blocked with 0.03% H₂O₂ in absolute methanol. Tissue sections were incubated overnight in Tris solution at 4ºC at a 1:200 dilution of monoclonal antibody to Mn-SOD. Primary antibody was removed and 2 repetitive washouts with Tris were done. Slices were incubated with a 1:500 dilution of rabbit polyclonal antibody as secondary antibody and 2 repetitive washouts with Tris were done. Bound antibodies were detected with avidin-biotin complex (Vectastain ABC-kit) and diaminobenzidine as substrate. After repeated washout with Tris, slices were counter-stained with hematoxylin. All slices were incubated at the same time, under the same conditions and at the same antibody concentration, so immunostaining was comparable. All specimens were examined using an Axiovert 200M inverted microscope (Carl Zeiss, Jena, Germany). To make the automatized morphometric analysis, the percentage of positive cells (brown color) was determined with a KS-300 3.0 computerized image.

**Image 1.** Immunohistochemical localization of Mn-SOD is shown in BPH and CaP glandular tissue. The images are histological slices showing inked Mn-SOD, in A, BPH glandular tissue is observed and in B, CaP glandular tissue is observed. Arrows indicate positive immunoreactivity in both tissue types. An obvious reduction in inked intensity in A, in diffused as well as densitometric form (P=0.0001) is seen. Images are representative (n =40), (400x field magnification).
analyzer (Carl Zeiss, Jena, Germany). This equipment automatically detects positive cells, determining their percentage per field. Five random fields were studied at a magnification of 100x (total area 1, 584,000 µ²). Results were expressed as percentages.

**Data analysis:** Mn-SOD immunoreactivity data were analyzed with the Student *t* test and data were expressed as mean ± standard deviation. Both groups (CaP and BPH) were compared and significant statistical difference was considered when *P*<0.05. Graph Prisma version 3.32 software was used to apply the statistical tests.

**RESULTS**

Biological samples were obtained from a total of 80 patients. CaP diagnosis was established in 40 patients (50%) and BPH diagnosis was established in 40 patients (50%).

The Mn-SOD-immunoreactive area percentage was 24.83±10.5% in CaP and 14.73±8.7% in BPH (Image 1).

**DISCUSSION**

Oxidative damage caused by ROS and other free radicals is involved in prostate carcinogenesis. Evidence in the literature indicates that Mn-SOD functions as a tumor suppressor, possibly through apoptotic modulation, cell growth and cell proliferation. A significant increase in this enzyme was observed in the present study along with a significant increase in the percentage of inked area in tumor tissue. However, several studies have reported a greater Mn-SOD expression in advanced stage cancer tissue.

It is known that oxidative stress can contribute to prostate carcinogenesis. Mn-SOD, the primary antioxidant enzyme of the mitochondria, has recently been associated with CaP pathogenesis. Studies have been carried out which show a relation between CaP and certain polymorphisms, but the association between this type of genetic change and CaP risk is weak. However, it is evident that endogenous and exogenous antioxidants play an important role in clinical CaP prevention.

Mn-SOD has regulating effects on potential cell redox. It influences cell growth and genetic expression. The results of the present study indicate an increase in the marked area percentage in glandular tissue, suggesting that Mn-SOD may be over-expressed in a more diffused form in different tissue – in stroma as well as in CaP glandular tissue areas.

In addition, damage caused by ROS has only been found in the epithelium and not in CaP connective tissue.

Previous studies on different types of cancer concur with the present study in regard to the increase in elevated Mn-SOD enzyme levels.

The tissues analyzed in this study displayed immunoreactivity in epithelial cells, connective tissue, inflammatory cells and even in endothelial cells. The number of immunoreactive cells was not quantified but it was obvious that they exhibited greater immunoreactivity in CaP in relation to densitometric data.

**CONCLUSIONS**

Mn-SOD immunoreactivity densitometric data in CaP glandular tissue were significantly higher than the same data obtained in BPH tissue.

**BIBLIOGRAPHY**