Experimental and Molecular Pathology 88 (2010) 96–106

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Prostate carcinogenesis induced by N-methyl-N-nitrosourea (mnu) in gerbils: Histopathological diagnosis and potential invasiveness mediated by extracellular matrix components

Bianca F. Gonçalves [a], Cristiani Zanetoni [b], Wellerson R. Scarano [c], Rejane M. Góes [a], Patricia S.L. Vilamaior [a], Sebastião R. Taboga [a,*], Silvana G.P. Campos [a]

[a] São Paulo State University, Institute of Biosciences, Humanities and Exact Sciences, UNESP/IBILCE, Department of Biology, Laboratory of Microscopy and Microanalysis, 15054-000, São José do Rio Preto, SP, Brazil
[b] State University of Campinas, UNICAMP, Department of Cell Biology, Institute of Biology, Box 6109-13083-864, Campinas, SP, Brazil
[c] São Paulo State University, Department of Morphology, Institute of Biosciences, Box 510-18618-000, Botucatu, SP, Brazil

ARTICLE INFO

Article history:
Received 13 May 2009
and in revised form 28 September 2009
Available online 8 October 2009

Keywords:
MNU
Testosterone
Prostate cancer
α-Methylacyl-CoA racemase
Extracellular matrix
Gerbil

ABSTRACT

In the present study prostate lesions were induced in gerbils (*Meriones unguiculatus*) treated with a single N-methyl-N-nitrosourea (MNU) dose; thus, the incidence, latency and histology of these lesions were evaluated. Fibrillar elements of the extracellular matrix associated with microinvasive sites were also investigated. Animals were divided into 5 groups, including 2 control groups: (1) remained untreated; (2) received the corn oil vehicle (vehicle, 0.1 ml/application) and three different tumor induction regimens: (1) received MNU (30 mg/kg) and weekly testosterone (2 mg/kg) (MNU + testosterone); (2) received only MNU (30 mg/kg); (3) received weekly testosterone doses (2 mg/kg). After 3 and 6 months the animals were dissected and the prostates were evaluated morphologically, immunohistochemically and quantitatively. MNU plus androgen contributed to the development of prostatic intraepithelial neoplasia, microinvasive carcinoma and adenocarcinoma in gerbil prostate. However, these lesions occurred earlier in time in groups that received MNU and androgen compared to control animals as they over time also developed to a high extent microinvasive lesions. Cytochemistry and immunohistochemistry showed that these injuries were commonly associated with inflammatory cells whereas the epithelial cells presented proliferative activity. The α-methylacyl-CoA racemase (AMACR) expression in prostate cancer cells facilitated diagnosis of gerbil lesions. Testosterone, MNU and MNU + testosterone showed an increased epithelial volume, although the secretory activity was significantly suppressed mainly at neoplastic foci. In the prostatic stroma, reticular fibers increased significantly in MNU, MNU + testosterone and among the lesions found in these groups, while collagen fibers decreased at neoplastic sites. The disruption of the basement membrane was proven at malignant sites by ultrastructural analysis and type IV collagen and laminin degradation. The prostate carcinogenesis mediated by MNU and androgen stimulated the emergence of proliferative lesions in gerbils after short periods and showed the importance of a dynamic remodeling of stromal components for cellular invasiveness.

Published by Elsevier Inc.

Introduction

Although prostate cancer is the second most common cancer and third most frequent cause of cancer mortality in the Western world, its pathogenesis is relatively poorly understood and its etiology is believed to be associated with genetic, hormonal, dietary, age-related, racial and environmental causes (Arunkumar et al., 2006; Bosland, 1988; Konishi et al., 1995). Like other cancer types, the prostate cancer progression is a multistep process that begins with the transformation of normal epithelial cells and continues with tumor growth, stromal invasion and metastasis. There is considerable evidence suggesting that the severity of cancer cells co-depends on alterations in the stromal components, such as stromal cells and extracellular matrix (ECM) elements, to create a microenvironment that promotes tumors to a greater degree (Tuxhorn et al., 2001).

Experimental models with well-defined etiological agents, which permit the induction of specific tumor types, provide pathways to studying the important events involved in human prostate cancer (Bosland and Prinsen, 1990; Shirai et al., 1997).The combination of the N-methyl-N-nitrosourea (MNU) carcinogen and testosterone androgen emerged as a valuable tool for inducing higher incidence of prostate carcinogenesis in some rodent models in short-term treatments (Liao et al., 2002; McCormick et al., 1998; Pollard and

* Corresponding author. Departamento de Biologia, IBILCE/UNESP, Rua Cristóvão Colombo, 2265, Jardim Nazareth, São José do Rio Preto, SP, Brazil, 15054-000. Fax: +55 17 32212390.
E-mail address: taboga@ibilce.unesp.br (S.R. Taboga).

0014-4800/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.yexmp.2009.09.017

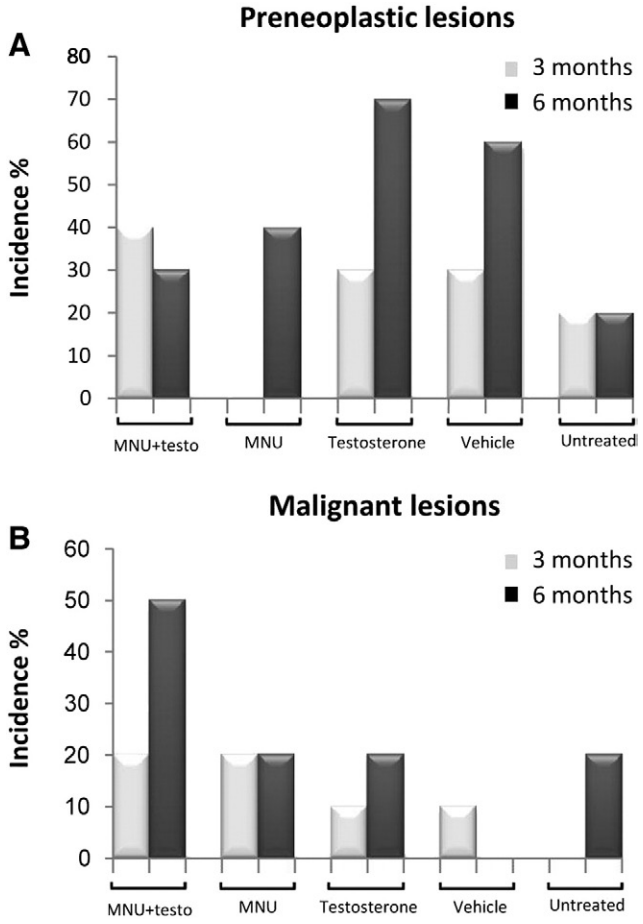

Fig. 1. Graph for incidence of preneoplastic (A) and malignant lesions (B) in gerbil's prostate.

Luckert, 1987). MNU is a well-characterized direct-acting methylating agent that reacts with cellular macromolecules including proteins and DNA, causing relatively high levels of O^6-methylguanine (Beranek, 1990; Jiricny, 2006; Van Zeeland et al., 2008), a DNA adduct with cytotoxic and recombinogenic properties (Kaina et al., 2007); MNU is also related to increased risk for developing cancer (Kyrtopoulos, 1995). In the prostate, rodent models treated with MNU and androgen constitute systems anatomically and physiologically relevant to the preclinical evaluation of substances that are hypothesized to inhibit or enhance human prostate carcinogenesis (Boileau et al., 2003). Thus, it seems common that MNU affects many organs in which neoplastic development seems to be stimulated by promotional agents, for example, breast and colon tumors influenced by diets rich in unsaturated fat (Chang et al., 1977) and bladder tumors associated with methyl sulfonate (Tudor et al., 1984) and possibly by the lineage under investigation (Pollard and Luckert, 1986).

Although few studies have characterized biomarkers in this model, the interrelationship between angiogenesis, proliferation and apoptosis during MNU-induced prostate cancer shows a strong correlation with published human data (Liao et al., 2002).

Our research group adopted the gerbil *Meriones unguiculatus* as an alternative experimental model for prostate study. The prostate of this animal presents lobes very close to each other such that, anatomically, the gland is more compact in relation to other rodent species (Pegorin de Campos et al., 2006). Additionally, the model has presented significant responses to hormonal treatments and to development of spontaneous neoplasias associated with aging (Campos et al,. 2008; Santos et al., 2006; Scarano et al., 2006; Scarano et al., 2008).

The goal of the present study was to induce prostate lesions in adult gerbils treated with a single MNU dose and then to evaluate the incidence, latency and histology of these lesions among groups. Additionally, since most histopathological studies on chemical induction of prostatic carcinogenesis in rodents have not focused on the stromal elements, we investigated the behavior of fibrillar ECM elements associated with microinvasive sites by histochemical, immunohistochemical, ultrastructural and quantitative methods.

Materials and methods

Animals, experimental procedures and carcinogenesis induction

90 adult male gerbils (90 days) were conducted in accordance with institutional guidelines for animal treatment and the experiment was approved by Ethic Committee of Experimental Animals of Sao Paulo State University (protocol number: 1306-1), housed in plastic cages, under conventional conditions (25 °C, 40–70% relative humidity, 12 light/12 dark), in pathogen-free conditions, with supply of water and balanced chow *ad libitum*.

N-methyl-N-nitrosurea (MNU) (Sigma, St. Louis, MO) was stored at −20 °C in the dark and the MNU solution was freshly prepared and dissolved in physiological saline just before use.

The animals were randomly divided into 5 groups: controls (2 groups) and experimental (3 groups). Control 1: ($n=10$) untreated group; Control 2: ($n=20$) received once a week only the corn oil vehicle (vehicle, 0.1 ml/application; subcutaneously injected); Experimental 1: ($n=20$) received a single dose of MNU (30 mg/kg, 0.1 ml/application; subcutaneously injected) and weekly doses of the testosterone propionate (2 mg/kg, 0.1 ml/application; subcutaneously injected). Experimental 2: ($n=20$) received a single dose of the MNU solution (MNU, 30 mg/kg, 0.1 ml/application; subcutaneously injected) and weekly doses of the corn oil vehicle (0.1 ml/application; subcutaneously injected); *Experimental 3:* ($n=20$) received once a week testosterone propionate dose (2 mg/kg, 0.1 ml/application; subcutaneously injected; Sigma, St. Louis, MO) dissolved in corn oil vehicle. The animals were killed 3 and 6 months after the beginning of the experiment. Before the necropsy, they were placed in a chamber containing CO_2 and, immediately after, sacrificed and dissected and each animal was subjected to a complete autopsy and the entire prostatic complex (prostate lobes and seminal vesicles) was removed and excised. The lesion-type classification of the groups were executed only after necropsy, histological processing of the samples and light microscopy analysis.

Histochemistry

For light microscopy, some prostatic fragments were fixed for 24 h in 4% paraformaldehyde in phosphate buffer saline and embedded in paraffin (Histosec™, Merck, Darmstadt, Germany), and others in Karnovsky fixative (0.1 M Sörensen phosphate buffer, pH 7.2, containing 5% paraformaldehyde and 2.5% glutaraldehyde) and embedded in glycol methacrylate resin (Leica™ Historesin Embedding Kit, Nussloch, Germany).

Historesin sections were cut at 2 μm thickness and submitted to cytochemical staining of hematoxylin–eosin (general tissue analysis) and the paraffin sections (5 μm) were stained with Gömori reticulin (selective for collagen and reticular fibers), Gömöri trichrome (selective for collagen fibers). The tissue sections were analyzed in an Olympus photomicroscope and the microscopic fields were digitalized using the Image-Pro-Plus software version 4.5 for Windows.

The prostates were serially sectioned and the histopathological classification of prostate lesions was accomplished according to previously described criteria (Shappell et al., 2004). The entire ventral prostate from each animal was examined in order to quantify the