Acta Histochemica 113 (2011) 423–427

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Potential role of melatonin in DNA damage caused by nitrosourea-induced mammary carcinogenesis

Janka Vašková [a,*], Monika Kassayová [b], Ladislav Vaško [a]

[a] Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University, Tr. SNP 1, 040 11 Košice, Slovak Republic
[b] Department of Animal Physiology, Institute of Biological and Ecological Sciences, Faculty of Sciences, Pavol Jozef Šafárik University, Košice, Slovak Republic

ARTICLE INFO

Article history:
Received 28 January 2010
Received in revised form 11 March 2010
Accepted 15 March 2010

Keywords:
Alkaline comet assay
Melatonin
Nitrosourea
Immobilization
Mammary carcinogenesis
Rat

ABSTRACT

Mammary carcinogenesis was induced in female Sprague-Dawley rats by exposure to N-methyl-N-nitrosourea (NMU). Animals were kept under constant light conditions to arrest endogenous melatonin synthesis and were fed the same melatonin dosage, since nitrosourea exposure may also induce cellular injury, especially with extensive proliferative activity. The pro-apoptotic effects of the biogenic amine, melatonin, on rat whole blood leukocytes were assessed by alkaline single cell gel electrophoresis (comet) assay. Potential induction of stress due to animal immobilization and its additional effect on DNA damage was studied. The parameters relevant to the degree of DNA damage in groups with chemocarcinogen treatment demonstrated no significant effects as a result of the immobilization. A significant increase in DNA damage after melatonin treatment in NMU-induced carcinogenesis confirms its involvement in the activation of apoptosis.

Introduction

Melatonin (5-methoxy-N-acyltryptamine) is a hormone-like substance secreted by the pineal gland and gastrointestinal tissue which influences circadian physiology and seasonal reproductive events (Tan et al., 2003). In addition to this physiological regulatory effect, melatonin (Mel) also functions as an antioxidant (Tan et al., 2002). It has been postulated that melatonin may be involved in development of mammary cancer. The hypothesis is based on evidence that melatonin down-regulates some of the pituitary and gonadal hormones that control mammary gland development and are also responsible for hormone-dependent mammary carcinogenesis. Melatonin may also act directly on tumor cells, as a naturally occurring anti-estrogen, thereby influencing their proliferative rate (Sánchez-Barceló et al., 2003). Melatonin has been shown to exert important effects by altering the expression of cytokines IL-2 and IL-12 in human neoplasms (Pandi-Perumal et al., 2006) and by modulating synthesis of TNF-α (Perianayagam et al., 2005). The anti-proliferative effects of melatonin are mediated in part by activation of melatonin membrane receptor type I (Shiu et al., 2003) including the expression of cyclin D1, cyclin E, p21 and p53 (Blask et al., 2002).

It has recently been reported that melatonin may play a role in the mediation of apoptotic events (Bejarano et al., 2009). The mechanisms of how melatonin affects apoptosis have not been fully explained, although various modes of action have been proposed (Reiter et al., 2008).

Treatment with N-methyl-N-nitrosourea (NMU) transforms mouse mammary epithelial cells to proneoplastic and neoplastic states. NMU rapidly degrades, so any effect of this pluripotent direct-acting mutagen is observed within 24 h (Budán et al., 2008).

We used single cell gel electrophoresis (SCGE), comet assay, to measure and compare the harmful effects of the chemical compound NMU on DNA and also took into consideration any potential stress responses due to immobilization. In addition to observations on melatonin action on mammary cancer cells, we also tried to assess its effects on floating cells originating from chemocarcinogen-affected cells. Data on light exposure conditions together with data on melatonin supplementation in the rats could provide relevant information on the efficacy of melatonin in afflicted animals.

Materials and methods

Sprague-Dawley female rats were obtained from AnLab (Prague, Czech Republic) when they were aged 35 ± 2 days old, with body weight of 130–150 g. The animals were acclimatized to standard vivarium conditions (temperature 23 ± 2 °C, relative humidity 60–70%, constant artificial light regimen, intensity 150 lx per cage). The rats were fed a standard mixed protein diet (Top-Dovo, Dobrá voda, Slovakia) and supplied tapwater *ad libitum*. After reaching a body weight of 250–300 g they were sacrificed by cervical displace-

* Corresponding author.
E-mail address: janka.kubalkova@upjs.sk (J. Vašková).

0065-1281/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.acthis.2010.03.006

ment and decapitation. The experimental protocols were approved by the State Veterinary and Food Administration of the Slovak Republic (Accreditation No. 2241/06-221).

Melatonin (Sigma–Aldrich, Diesenhofen, Germany) was dissolved in tapwater to a final concentration of 0.086 mmol/l per day and made available in the drinking water from 15.00 to 20.00 h (from 8.00 to 15.00 h animals drank tapwater only). Melatonin administration was initiated 6 days prior to the first chemocarcinogen dose. Three groups of animals were treated with methyl-N-nitrosurea (NMU) (Sigma–Aldrich, Diesenhofen, Germany). The NMU was administered intraperitoneally in two doses (50 mg/kg/body weight each), between postnatal days 40 and 50. Immobilization as a psychoemotional stress model was initiated 6 days after the second chemocarcinogen administration. The animals were repeatedly immobilized in special boxes for 2 h three times a week (Monday, Wednesday, and Friday) for a period of 19 weeks, as described by Kassayová et al. (2007). For evaluation the animals were divided into four groups: *Group 1*: CTRL – intact control group; *Group 2*: NMU – group with chemocarcinogen administration only, *Group 3*: NMU-Im – immobilized group, *Group 4*: NMU-Im-Mel – immobilized group that drank Mel until the end of the experiment. The experiment was carried out for 22 weeks (from September to February) with 20 animals in each group at the beginning of the experiment.

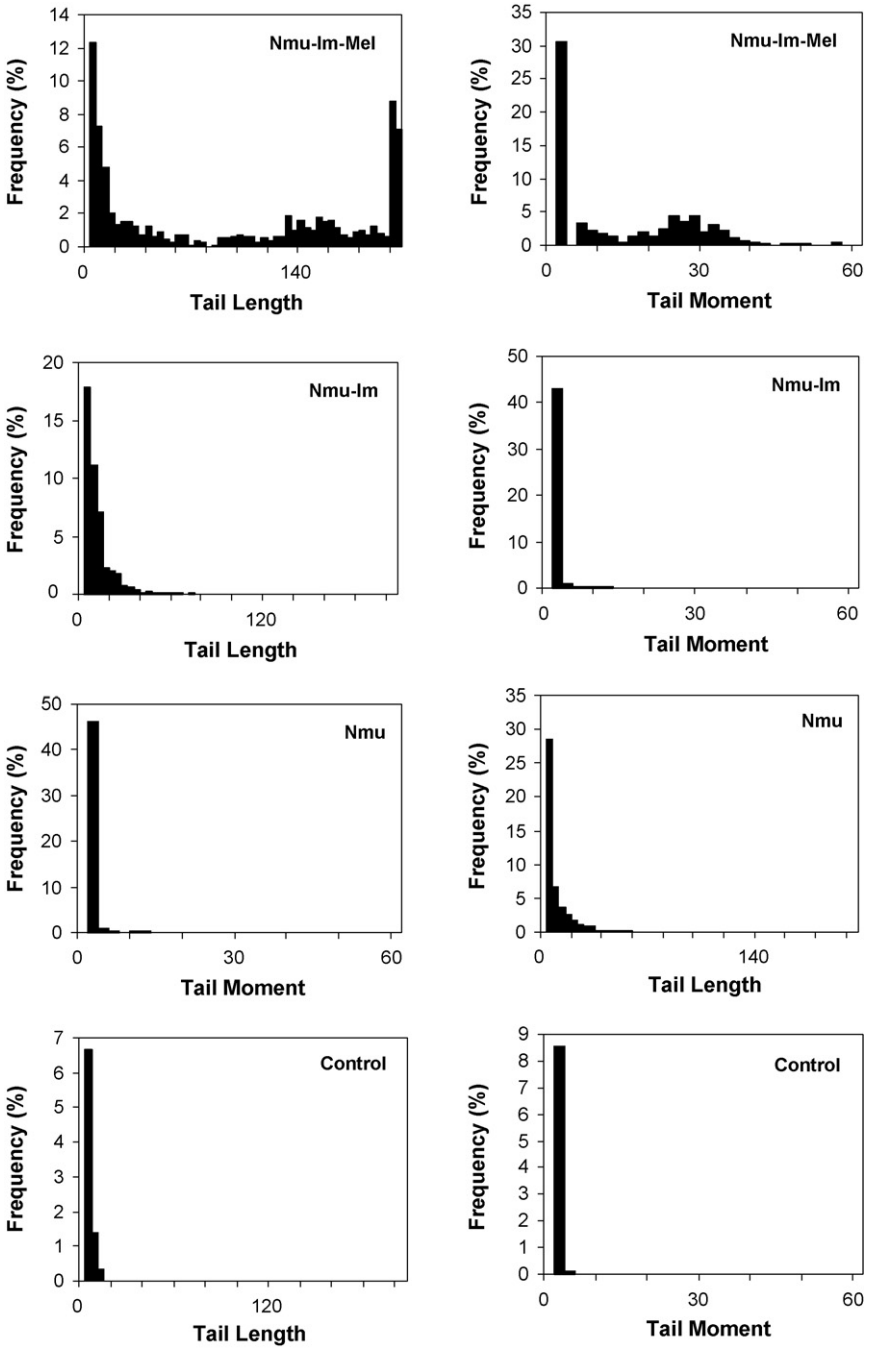

Fig. 1. Comparison of the effects of NMU, NMU-Im, NMU-Im-Mel treatment on rat leukocytes. Blood cells were directly cast into agarose and immersed in lysing solution pH 10. After electrophoresis under alkaline conditions, fluorescent visualization of DNA damage on comet tail lengths and comet tail moments was assessed.