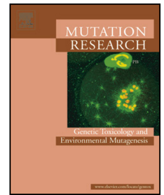
Mutation Research 753 (2013) 48–53

Contents lists available at SciVerse ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres



Role of recombinant human erythropoietin in mitomycin C-induced genotoxicity: Analysis of DNA fragmentation, chromosome aberrations and micronuclei in rat bone-marrow cells

Karima Rjiba-Touati [a], Imen Ayed-Boussema [a], Yosra Guedri [b], Abdelatif Achour [b], Hassen Bacha [a,*], Salwa Abid [a]

[a] *Laboratory of Research on Biologically Compatible Compounds, Faculty of Dentistry, Monastir, Tunisia*
[b] *Department of Nephrology, Dialysis and Transplant, University Hospital of Sahloul. Sousse, Tunisia*

ARTICLE INFO

Article history:
Received 5 July 2012
Received in revised form 11 October 2012
Accepted 22 December 2012
Available online 30 January 2013

Keywords:
Mitomycin C
Rhepo
DNA fragmentation
Micronucleus
Chromosome aberrations

ABSTRACT

Mitomycin C (MMC) is one of the most effective chemotherapeutic agents. However, during clinical use several side effects may occur. Recombinant human erythropoietin (rhEPO), a glycoprotein that regulates haematopoiesis, has been shown to exert an important cyto-protective effect in many tissues. The aim of this study was to explore whether rhEPO protects against MMC-induced genotoxicity in rat bone-marrow cells. Adult male Wistar rats were divided into six groups of 18 animals each: a control group, a 'rhEPO alone' group, an 'MMC alone' group and three 'rhEPO + MMC' groups (pre-, co- and post-treatment conditions). Our results show that MMC induced a noticeable genotoxic effect in rat bone-marrow cells. rhEPO reduced the effects of MMC significantly in every type of experiment conducted, such as the frequency of micronuclei, the percentage of chromosome aberrations and the level of DNA damage measured with the comet assay. The protective effect of rhEPO was more efficient when it was given 24 h prior to MMC treatment.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Mitomycin C (MMC) is a powerful anti-bacterial and anti-tumour fungal antibiotic isolated from *Streptomyces caespitosus*. In cancer therapy, MMC is a commonly used drug to fight several human malignancies including cancers of the stomach, colon, rectum, pancreas, breast, lung, uterus, cervix and bladder [1,2]. MMC is a direct-acting clastogen requiring only intracellular reductive activation to initiate its potent DNA cross-linking action [3]. Therefore, MMC has shown a range of genotoxic effects including mutation induction and inhibition of DNA synthesis in undifferentiated cell lines and in *Drosophila melanogaster* [4–6]. The genotoxic effects of MMC have been proven in chromosome aberration tests and in micronucleus assays in mouse spermatocytes, bone-marrow cells and in *D. melanogaster* [6–8]. In this context, strategies to protect against MMC-induced genotoxicity are of clinical interest and cyto-protective agents are essential to provide this protection [7–9]. The anti-genotoxic effect of recombinant human erythropoietin (rhEPO) against MMC-induced DNA damage has been investigated in human lymphocytes *in vitro* and in P388 ascites tumour cells inoculated in BDF1 mice. Digkas et al. [10] demonstrated that rhEPO used in combination with MMC decreased significantly the sister chromatid exchange levels and increased the proliferation rate indices and the mitotic indices induced by MMC, both *in vitro* and *in vivo*. However, the use of rhEPO as a cyto-protective agent in bone-marrow cells has not been investigated. Our aim was to study the action of rhEPO in two cell types, in the presence and absence of the genotoxic agent mitomycin C (MMC).

Erythropoietin (EPO) is a glycoprotein hormone primarily, but not exclusively, synthesized by renal cortical interstitial fibroblasts in response to tissue hypoxia [11]. EPO, which is used clinically in the form of recombinant human EPO (rhEPO), has been successfully used in treatment of human anaemia associated with end-stage renal failure and cancer chemotherapy [12–14]. More recently, it has been established that the biological effects of rhEPO are not only limited to the haematopoietic system; many studies have shown that rhEPO is a pleiotropic cytokine that exerts broad tissue-protective effects in diverse non-hematopoietic organs [15–17]. Several investigations have shown that rhEPO can reduce genotoxic damage in brain and kidney of rats [18,19]. Genc et al. [20] showed that rhEPO protects against inflammatory effects induced in primary rat oligodendrocyte cultures and may play a protective role in neurological disorders characterized by oligodendrocyte death.

* Corresponding author at: Laboratoire de Recherche sur les Substances Biologiquement Compatibles, (LRSBC), Faculté de Médecine Dentaire, Rue Avicenne, 5019 Monastir, Tunisia. Tel.: +216 73 42 55 50; fax: +216 73 46 11 50.
E-mail address: hassen.bacha@fmdm.rnu.tn (H. Bacha).

1383-5718/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.mrgentox.2012.12.011

In the same context, Liao et al. [21] demonstrated that administration of rhEPO protects rat neurons by enhancing Bcl-2 expression, thereby inhibiting neuronal apoptosis induced by traumatic brain injury. Little is known about the protective effects of rhEPO against MMC-induced genotoxicity in bone-marrow cells. Using the nude mouse as a novel test system for the evaluation of genotoxicity of rhEPO, Yajima et al. [22] demonstrated that errors in the processes of enucleation or differentiation of erythrocytes should be equally considered as possible mechanisms, alongside errors in DNA-repair processes, for the increased frequencies of micronucleated polychromatic erythrocytes (MNPCE) and micronucleated reticulocytes (MNRET). In comparative studies on induction of micronuclei by three genetically recombinant erythropoietins and one native form directly isolated from human urine, the same authors showed that the induction of MNPCE and MNRET was a common characteristic of both native and genetically recombinant EPO [23].

The aim of the present work was to investigate the protective effect of rhEPO against MMC-induced genotoxicity in bone-marrow cells of Wistar rats. For this purpose, we quantified the frequency of micronuclei, the percentage of chromosome aberrations, and the DNA-damage level – measured with the comet assay – in rats treated with MMC and rhEPO under different conditions of treatment. These tests are widely used in industrial genotoxicity testing *in vitro* [24–27] and are also becoming an important tool for evaluating the genotoxic potential of compounds *in vivo* [28–30]. They have been recommended by regulatory authorities for the assessment of genotoxicity and mutagenicity of many chemicals and natural compounds [31,32].

2. Material and methods

2.1. Chemicals

Mitomycin C (CAS: 50-07-7; batch number: 060401) was purchased from Sigma–Aldrich, France. Experiments were performed with a commercially available preparation of rhEPO (Hemax®, Bio SIDUS S.A., Argentina). All other chemicals used were of analytical grade.

2.2. Treatment of animals

Experiments were performed on male Wistar rats according to the guidelines and principles of American College of Toxicology Statement on the Use of Animals in Toxicology.

Wistar rats (weight range, 120–140 g) were kept under controlled environmental conditions at room temperature (22 ± 2 °C) and in 12-h light/dark cycles. They were allowed free access to food and water but were fasted overnight before treatment. For the time-course experiment, rats were divided at random into six groups with 18 animals each. The control group received a single injection of saline solution 0.9%. The rhEPO-group was given only rhEPO and the MMC-group was given only a single injection of MMC. To test the effect of rhEPO on MMC-induced genotoxicity, three treatment conditions were evaluated: in the co-treatment group, a single dose of rhEPO was administered simultaneously with MMC; in the pre-treatment group, a single dose of rhEPO was given one day before MMC [33]; in the post-treatment group, a single dose of rhEPO was given five days after MMC [34]. All injections were given intraperitoneally (i.p.). Animals within different treatment groups were divided into three subgroups of six animals each: A (for the micronucleus assay), B (for the chromosome aberration assay) and C (for the comet assay) and received their respective treatment. Experimental design is detailed in Table 1. Animals were sacrificed by cervical dislocation after the different treatments.

2.3. Rat bone-marrow micronucleus assay

Immediately after sacrifice of the animals, the femur and tibia of the rats in subgroup A were removed and bone marrow was flushed out by injection of filtered foetal calf serum by use of a syringe. The suspension of bone-marrow cells was centrifuged at 390 × *g* for five min and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried, fixed with absolute methanol for five min, then air-dried for conservation at room temperature or directly stained with Acridine orange. The working solution of acridine orange was freshly prepared in sodium-phosphate buffer (0.15 M; pH 7.2) at a concentration of 0.01 mg/ml. Slides were kept for ten min in this staining solution, then rinsed in the same buffer for 15 min and allowed to dry in the dark at room temperature. As a result of the acridine-orange staining, red fluorescence is observed in polychromatic erythrocytes (PCE), while normochromatic erythrocytes (NCE) appear slightly green or opaque, and micro-nucleated polychromatic erythrocytes (PCEMN) appear red with one or more yellow-fluorescent corpuscles, which are micronuclei (MN). Scoring of micronuclei was performed according to criteria described by Hayashi et al. [35]. These criteria are based essentially on the diameter and the shape of the micronucleus.

2.4. Rat bone-marrow chromosome aberration assay

Bone marrow cells were obtained according to the technique of Yosida and Amano [36]. Briefly, femur and tibia of rats in subgroup B were removed immediately after animal sacrifice and bone marrow was flushed out with KCl solution (0.075 M, 37 °C) by use of a syringe. The suspension of the bone-marrow cells was incubated for 20 min at 37 °C and centrifuged at 390 × *g* for ten min. The supernatant was discarded, the pellet was re-suspended in five ml of a fixative solution (acetic acid/methanol, 1:3 v/v), centrifuged (390 × *g* for ten min) and the supernatant was discarded again. This step was repeated three times in order to clean the pellet. Finally, the pellet was re-suspended in one ml of the above-mentioned fixative solution and used for chromosome preparations.

2.5. Analysis of slides

2.5.1. For the micronucleus assay

The slides, randomly coded, were observed under 1000× magnification with a fluorescence microscope (Nikon Eclipse E 400). The number of PCEMN among 2000 PCE per rat was determined to calculate the induction of MN. The number of PCE among 1000 (NCE + PCE) was determined in order to evaluate the cytotoxic effect of MMC and a possible cyto-protective effect of rhEPO. This required 1000 cells to be counted.

2.5.2. For the chromosome aberration assay

The slides were examined under 1000× magnification with an optical microscope (Carl Zeiss, Germany). Three hundred well-spread metaphases per group were analyzed for abnormalities. Metaphases with chromosome breaks, gaps, rings and centric fusions (robertsonian translocation) were recorded and expressed as percentage of total metaphases per group.

2.6. Single-cell gel electrophoresis (SCGE, the comet assay)

After sacrifice of the animals in subgroup C, both femurs were removed and the content was directly flushed out with a 24-gauge needle into a micro-centrifuge tube. The cell suspension was prepared in PBS containing 20 mM EDTA and 1% DMSO. From the suspension, five μl of sample (containing approximately $2–5 \times 10^4$ cells/ml) were added to 95 μl of 0.5% low-melting agar (LMA) (in PBS) to prepare the final cell–agarose suspension. The comet assay was performed as described by Singh et al. [37] with some modification as suggested. From the final cell-agarose suspension, 80 μl was spread over the microscope slide, which was pre-coated with 1% normal-melting agar (NMA). The cells were then lysed in a buffer containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10.0), with freshly prepared 1% Triton X-100 and 10% DMSO for 24 h at 4 °C. After lysis, slides were rinsed three times in de-ionized water to remove salt and detergent. Slides were placed in a horizontal electrophoresis unit and DNA was allowed to unwind for 20 min in alkaline solution containing 300 mM NaOH and 1 mM EDTA, pH > 13. The DNA was electrophoresed for 15 min at 300 mA and 25 V (0.90 V/cm). The slides were neutralized with 0.4 M Tris (pH 7.5), stained with Acridine Orange (20 μg/ml) before examination with a

Table 1
Animal groups and treatments in the experimental design of this study.

	Day 0	Day 1	Day 5	Day 6
Control group	saline solution injection		sacrifice	
rhEPO group (3000 IU/kg bw)	rhEPO injection	sacrifice		
MMC Group (3 mg/kg bw)	MMC injection		sacrifice	
Co-treatment group	MMC + rhEPO injection		sacrifice	
Pre-treatment group	rhEPO injection	MMC injection		sacrifice
Post-treatment group	MMC injection		rhEPO injection	sacrifice

Download English Version:

https://daneshyari.com/en/article/2148045

Download Persian Version:

https://daneshyari.com/article/2148045

Daneshyari.com