Food and Chemical Toxicology 106 (2017) 283–291

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Modulatory effects of metformin on mutagenicity and epithelial tumor incidence in doxorubicin-treated *Drosophila melanogaster*

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ARTICLE INFO

Article history:
Received 3 April 2017
Received in revised form
10 May 2017
Accepted 26 May 2017
Available online 29 May 2017

Keywords:
Gene wts
Glucophage
SMART
Somatic Mutation and Recombination Test
Tumor
Warts

ABSTRACT

Metformin (MET) is an anti-diabetic drug used to prevent hepatic glucose release and increase tissue insulin sensitivity. Diabetic cancer patients are on additional therapy with anticancer drugs. Doxorubicin (DXR) is a cancer chemotherapeutic agent that interferes with the topoisomerase II enzyme and generates free radicals. MET (2.5, 5, 10, 25 or 50 mM) alone was examined for mutagenicity, recombinogenicity and carcinogenicity, and combined with DXR (0.4 mM) for antimutagenicity, antirecombinogenicity and anticarcinogenicity, using the Somatic Mutation and Recombination Test and the Test for Detecting Epithelial Tumor Clones in Drosophila melanogaster. MET alone did not induce mutation or recombination. Modulating effects of MET on DXR-induced DNA damage were observed at the highest concentrations. In the evaluation of carcinogenesis, MET alone did not induce tumors. When combined with DXR, MET also reduced the DXR-induced tumors at the highest concentrations. Therefore, in the present experimental conditions, MET alone did not present mutagenic/recombinogenic/carcinogenic effects, but it was able to modulate the effect of DXR in the induction of DNA damage and of tumors in D. melanogaster. It is believed that this modulating effect is mainly related to the antioxidant, anti-inflammatory and apoptotic effects of this drug, although such effects have not been directly evaluated.

1. Introduction

Metformin (MET) (tradename Glucophage) is an oral anti-diabetic drug of the biguanide family widely prescribed as a first choice medication for type 2 diabetes mellitus (T2DM). It prevents hepatic glucose release and increases tissue insulin sensitivity (Malek et al., 2015; Cheki et al., 2016; Nishihama et al., 2016). MET has been widely used in the treatment of polycystic ovary syndrome and gestational T2DM (Amador et al., 2012; Reece et al., 2014). Besides, several studies have even suggested that MET may have further application in anticancer and antiaging therapies, mainly in tumors driven by insulin resistance and obesity (Kasznicki et al., 2014; Marycz et al., 2016; Talaulikar et al., 2016).

In mammals, MET is absorbed predominately from the small intestine and is excreted unchanged in urine (Graham et al., 2011). The mechanisms of MET action are only partially explored and remain controversial (Song, 2016). Several potential mechanisms of action have been proposed: suppression of liver glucose production (hepatic gluconeogenesis) by inhibiting mitochondrial glycerophosphate dehydrogenase (Madiraju et al., 2014); inhibition of the mitochondrial respiratory chain (complex I) (Owen et al., 2000); activation of AMP-activated protein kinase (AMPK) (a major cellular regulator of lipid and glucose metabolism) in hepatocytes, through liver kinase B1 (Zhou et al., 2001); suppression of hepatic glucagon signaling by decreasing production of cyclic adenosine monophosphate (cAMP) (Miller et al., 2013); and changes in the gut microbiota and their metabolic pathways (Lee and Ko, 2014).

MET may exert antineoplastic effects through: AMPK-mediated

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http://dx.doi.org/10.1016/j.fct.2017.05.052
0278-6915/

or AMPK-independent inhibition of mammalian target of rapamycin (mTOR), which is up-regulated in many cancer tissues (Han et al., 2015); or blocking migration and invasion of tumor cells by inhibition of matrix metalloproteinase-9 activation through a calcium and protein kinase Cα-dependent pathway (Hwang and Jeong, 2010).

Several studies indicate that MET has also antioxidant (Hou et al., 2010; Algire et al., 2012; Ashour et al., 2012; Na et al., 2013; Yang et al., 2014b; Vilela et al., 2016), anti-inflammatory (Woo et al., 2014; Jin et al., 2015; Cameron et al., 2016; Zhou et al., 2016), and apoptotic effects (Fang et al., 2014; Takahashi et al., 2014; Han et al., 2015; Sun et al., 2016). Regarding the mutagenic/clastogenic/recombinogenic potential of MET, literature data are conflicting. Some studies have shown that MET is not genotoxic *in vivo* or *in vitro* (Aleisa et al., 2007; Attia et al., 2009; Amador et al., 2012; Malek et al., 2015; Sant'Anna et al., 2013; Cheki et al., 2016; Ullah et al., 2016), non-recombinogenic (Sant'Anna et al., 2013) and may protect from genomic instability (Attia et al., 2009; Cheki et al., 2016; Ullah et al., 2016). Nevertheless, MET induced genotoxicity in rodent cells *in vitro* (Amador et al., 2012) and in T2DM patients *in vivo* (Harishankar et al., 2015).

Doxorubicin (DXR) (also called Adriamycin® or 14-hydroxydaunorubicin) is an anthracycline drug first extracted from *Streptomyces peucetius* ATCC 27952 that is used to treat many different types of cancer (Malla et al., 2010). Nevertheless, its use as an antitumor therapeutic agent is limited due to its cardiotoxic effects (Sheta et al., 2016). DXR may intercalate on DNA and induce formation of DNA adducts at active promoter sites, increasing torsional stress and enhancing nucleosome turnover. Furthermore, it may trap topoisomerase II at breakage sites, causing double strand breaks. Enhanced nucleosome turnover might increase the exposure of DNA to reactive oxygen species (ROS) resulting in DNA damage and cell death (Yang et al., 2014a). Previous studies have demonstrated that MET may have protective effects against DXR-induced cardiotoxicity and clastogenicity (Aleisa et al., 2007; Sheta et al., 2016).

In the present study, the wing Somatic Mutation and Recombination Test (SMART) was used to assess MET mutagenicity and its anti-mutagenic potential against DXR-induced mutagenicity. We also investigated the carcinogenic potential of MET alone and its anti-carcinogenic potential against DXR-induced carcinogenicity using the Test for Detection of Epithelial Tumor Clones (Warts) in *D. melanogaster*.

2. Material and methods

2.1. Chemical agents

Metformin (N, N′-dimethylbiguanide; CAS 657-24-9) was purchased from Merck, Rio de Janeiro, Brazil. Doxorubicin (DXR; CAS 25316-40-9), commercially known as Adriblastina®, was produced by Actavis Italy, Nerviano, Italy. The solutions were always prepared immediately before use with ultrapure water obtained from a MilliQ system (Millipore; Vimodrome, Milan, Italy). The structural formulas of these substances are shown in Fig. 1.

2.2. Strains and stock

In this study the following strains of *D. Melanogaster* were used: [1] multiple wing hairs (*mwh/mwh*); [2] flare-3 (*flr^3/In(3LR)TM3, ri p^p sep l(3)89Aa bx34^e and Bd^S*); [3] ORR; flare-3 (*ORR/ORR; flr^3/In(3LR)TM3, ri p^p sep l(3)89Aa bx34^e and Bd^S*); and [4] *wtsTM3, Sb^1*. These strains were maintained in glass vials filled with a maintenance medium (i.e., banana, sucrose, yeast and methylparaben) under light/dark cycles (12 h:12 h), at 25 ± 1 °C and approximately 60% humidity in a BOD-type chamber (Model: SL224, SOLAB – Equipamentos para Laboratórios Ltda., São Paulo, SP, Brazil).

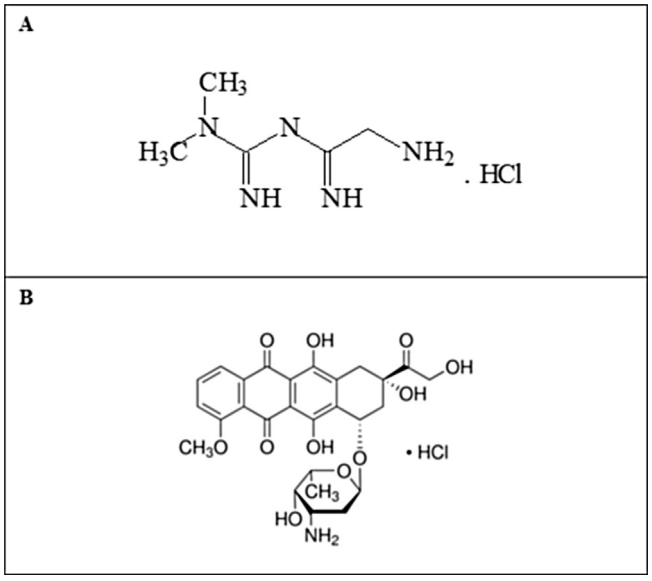

Fig. 1. Structural formulas of the substances used in the present study: A. Metformin (MET); B. Doxorubicin (DXR).

2.3. Somatic Mutation and Recombination Test – SMART

2.3.1. Crosses and treatments

The SMART assay allows the detection of different genetic endpoints, using two different strains of *D. melanogaster* that carry specific genetic markers (*mwh* and *flr^3*) on the left arm of chromosome 3 (Graf et al., 1984).

Two crosses were carried out to produce the experimental larval progeny: (1) Standard (ST) cross: *mwh/mwh* males crossed with *flr^3/In(3LR)TM3, ri p^p sep l(3)89Aa bx34^e and Bd^S* virgin females (Graf et al., 1984, 1989); (2) High bioactivation (HB) cross: *mwh/mwh* males crossed with *ORR/ORR; flr^3/In(3LR)TM3, ri p^p sep l(3)89Aa bx34^e and Bd^S* virgin females (Graf and van Schaik, 1992). The two crosses produce two types of flies: marker *trans*-heterozygous (MH) flies (*mwh +/+ flr^3*) and balancer-heterozygous (BH) flies (*mwh+/+TM3, Bd^S*). Detailed information on genetic symbols can be found in Lindsley and Zimm (1992). The ST cross uses strains carrying basal levels of the metabolizing cytochrome P450 enzyme (Cyp6A2) and is used to detect direct-acting genotoxins. The HB cross uses strains with high levels of Cyp6A2 and is used to detect indirect-acting genotoxins that exert their genotoxic activity only when metabolized (Graf and van Schaik, 1992; Saner et al., 1996; Rezende et al., 2011).

2.3.2. Experimental procedure

Eggs, from both crosses, were collected for 8 h in culture bottles containing a solid agar base (5% w/v agar in water) covered with a thick layer of live baker's yeast supplemented with sucrose. Approximately 72 h after the end of the egg-laying stage, larvae were collected and distributed in four sets of vials for each cross with 1.5 g of mashed potato flakes and 5 ml of different concentrations of MET (2.5, 5, 10, 25 or 50 mM) alone and MET (2.5, 5, 10, 25 or 50 mM) in association with DXR (0.4 mM) (for co-treatments). Negative (ultrapure water) and positive doxorubicin (DXR 0.4 mM) controls were included. The larvae were counted before distribution into two series of these vials. The number of hatched flies was used to calculate the survival rates upon exposure. The experiments