Tissue and Cell 47 (2015) 498–505

Contents lists available at ScienceDirect

Tissue and Cell

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

# Effect of metformin and adriamycin on transplantable tumor model

Ahmed M. Kabel[a,b,*], Mohamed S. Omar[c,d], Mohamed F. Balaha[b], Hany M. Borg[e]

[a] Department of Clinical Pharmacy, College of Pharmacy, Taif University, Taif, Saudi Arabia
[b] Pharmacology Department, Faculty of Medicine, Tanta University, Tanta, Egypt
[c] Division of Biochemistry, Pharmacology and Toxicology Department, College of Pharmacy, Taif University, Taif, Saudi Arabia
[d] Chemistry Department, Faculty of Science, Benha University, Benha, Egypt
[e] Department of Physiology, Faculty of Medicine, Kafrelsheikh University, Egypt

ARTICLE INFO

*Article history:*
Received 5 May 2015
Received in revised form 20 June 2015
Accepted 6 July 2015
Available online 26 July 2015

*Keywords:*
Adriamycin
Metformin
Transplantable tumor
Mice

ABSTRACT

Adriamycin is a cytotoxic anthracycline antibiotic used in treatment of many types of cancer. Metformin is antidiabetic drug and is under investigation for treatment of cancer. The aim of this work was to study the effect of each of adriamycin and metformin alone and in combination on solid Ehrlich carcinoma (SEC) in mice. Eighty BALB/C mice were divided into four equal groups: SEC group, SEC + adriamycin, SEC + metformin, SEC + adriamycin + metformin. Tumor volume, survival rate, tissue catalase, tissue reduced glutathione, tissue malondialdehyde, tissue sphingosine kinase 1 activity, tissue caspase 3 activity and tissue tumor necrosis factor alpha were determined. A part of the tumor was examined for histopathological and immunohistochemical study. Adriamycin or metformin alone or in combination induced significant increase in the survival rate, tissue catalase, reduced glutathione and tissue caspase 3 activity with significant decrease in tumor volume, tissue malondialdehyde, tissue sphingosine kinase 1 activity and tumor necrosis factor alpha and alleviated the histopathological changes with significant increase in Trp53 expression and apoptotic index compared to SEC group. In conclusion, the combination of adriamycin and metformin had a better effect than each of these drugs alone against transplantable tumor model in mice.



## 1. Introduction

Ehrlich carcinoma is one of the commonest transplantable tumor models that appeared firstly as a spontaneous breast cancer in a female mouse, and then used as an experimental tumor model (Ozaslan et al., 2011). Advantages of these models include their low cost and reproducibility. Imaging was not needed for assessment of tumor response in these easily accessible and observable tumors (Cespedes et al., 2006). In addition, the tumor grows in an immune-competent host, making these models appropriate for the study of immune modulation and vaccine approaches (Kabel and Abd Elmaaboud, 2014).

Adriamycin (ADR) is an anthracycline antibiotic isolated from *Streptomyces peucetius* var. *caesius* (Ivic et al., 2005). It is given as a treatment for many cancer types such as leukemia, breast cancer, sarcoma, lymphoma, neuroblastoma, ovarian cancer, Hodgkin's disease and lung cancer (Auersperg et al., 2006). ADR damages DNA by intercalation into DNA and inhibition of topoisomerase II resulting in DNA strand breaks (Mazzotta et al., 2001). The drug is a cell cycle non-specific agent but cells in the S-phase are the most sensitive to the cytotoxic action of ADR since it inhibits both DNA polymerase and RNA polymerase (Gümüşhan and Musa, 2008).

Metformin is an antidiabetic agent that decreases intestinal absorption of glucose, increases its anaerobic metabolism, improves insulin sensitivity and decreases glucagon release (El Messaoudi et al., 2011). Studies on animal tumor models and cancer cell lines have demonstrated that metformin prevents tumor development and inhibits cell proliferation. In addition, a recent clinical trial has shown that metformin reduces aberrant crypt foci (ACF) formation in non-diabetic patients with ACF (Li, 2011). The antitumor activity of metformin may be mediated through its regulatory effect on hormonal, metabolic and immune functions. The major molecular targets of metformin are the liver kinase B1 (LKB1)–AMP-activated protein kinase (AMPK) signaling and mammalian target of rapamycin (mTOR) pathways, which regulate cellular energy homeostasis and play a crucial role in the control of cell division and proliferation (Hosono et al., 2010; Kalender et al., 2010). Moreover, metformin has been shown to improve endothelial function, decrease inflammatory response, and regulate immune functions which have a major role in the pathogenesis

* Corresponding author at: Department of Clinical Pharmacy, College of Pharmacy, Taif University, Al-Haweiah, P.O. Box 888, Zip Code 21974, Saudi Arabia
Tel.: +201009041488; +966564833526.
*E-mail address:* drakabel@gmail.com (A.M. Kabel).

http://dx.doi.org/10.1016/j.tice.2015.07.003
0040-8166/

of cancer (Li, 2011). Also, metformin was proven to inhibit p-glycoprotein expression via the nuclear factor-κB pathway through AMP-kinase activation (Garofalo et al., 2013; Rosilio et al., 2014).

Sphingosine kinase 1 (SphK1) is an enzyme that catalyzes the phosphorylation of sphingosine to sphingosine-1-phosphate (Yao et al., 2012). In vivo and in vitro studies have proven that SphK1 is associated with cancer cell survival, proliferation, angiogenesis, resistance to chemotherapy and prevention of apoptosis (Shida et al., 2008). A number of studies demonstrated that SphK1 is over-expressed in many types of tumors. Therefore, inhibition of SphK1 may increase the sensitivity of tumors to chemotherapy and may represent a new hope in cancer therapy (Shida et al., 2008; Vadas et al., 2008). The aim of this work was to study the effect of each of metformin and adriamycin alone and in combination on transplantable tumor model in mice.

## 2. Materials and methods

### 2.1. Solid Ehrlich carcinoma (SEC) tumor model

A model of SEC was used, where $1 \times 10^6$ of the Ehrlich carcinoma cells (ECC) obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Cairo), were implanted subcutaneously into the right thigh of the hind limb of mice. A palpable solid tumor mass (about 100 $mm^3$) was developed within 10 days (Kabel et al., 2013).

### 2.2. Drugs used

Metformin was dissolved in drinking water and was typically added to 200 microgram/ml (μg/ml). Adriamycin was dissolved in dimethyl sulfoxide and given by intratumoral injection of 4 mg/kg body weight on days 10, 15, 20, 25, 30 and 35 after implantation of Ehrlich carcinoma cells (Iliopoulos et al., 2011). All chemicals were obtained from Sigma–Aldrich.

### 2.3. Classification of animals

In this study, 80 male BALB/C mice weighing about 20–25 g were used. All the experiments were conducted according to the National Research Council's guidelines. Animal handling was followed according to Helsinki declaration of animal ethics. The animals were divided into 4 equal groups of twenty mice each as follows:

Group (1): Ehrlich tumor cells were implanted subcutaneously into the right thigh of the hind limb of mice (Kabel et al., 2013).

Group (2): Adriamycin was given to mice by intratumoral injection of 4 mg/kg body weight on days 10, 15, 20, 25, 30 and 35 after implantation of Ehrlich carcinoma cells.

Group (3): Metformin was given to mice orally in drinking water in a concentration of 200 μg/ml throughout the experiment starting 10 days after implantation of Ehrlich carcinoma cells and continued for 32 days (Iliopoulos et al., 2011).

Group (4): Adriamycin and metformin were given to mice together starting 10 days after implantation of Ehrlich carcinoma cells and continued for 32 days by the above regimens.

### 2.4. Assessment of the effects of different treatments on tumor volume (TV) of SEC

Tumor volume was measured on days 15, 20, 25, 30, 35 and 40 after implantation of ECC using a Vernier caliper (Tricle Brand, Shanghai, China). The following formula was used to calculate the volume of the developed tumor mass (Attia and Weiss, 1966):

$$\text{tumor volume}\,(\text{mm}^3) = 4\pi\left(\frac{A}{2}\right)^2 \times \left(\frac{B}{2}\right)$$

where $A$ is the minor tumor axis; $B$ is the major tumor axis and $\pi$ equals to 3.14.

### 2.5. Assessment of the survival rate

The day of implantation of ECC was considered zero point of the experiment for recording and analysis of the survival rate weekly for 6 weeks (by recording number of the survived mice in each group at the end of each week).

### 2.6. Assessment of the biochemical parameters

In the 42nd day after implantation of ECC, mice were sacrificed. The tumor was excised and divided into two portions: one for homogenization and the other for histopathological and immunohistochemical examination. The tumor was homogenized for determination of tissue catalase (CAT) according to the method described by Higgins et al. (1978), tissue malondialdehyde (MDA) according to Uchiyama and Mihara (1978), tissue reduced glutathione (GSH) according to the method of Ellman (1959) and tissue tumor necrosis factor alpha (TNF-α) using mouse TNF-α ELISA kits supplied by RayBiotech, Inc. according to Luster and Rothenberg (1997).

### 2.7. Assay of tissue sphingosine kinase 1 (SphK1) activity

50 mg piece from the tumor tissue was homogenized in lysis buffer (50 mM Hepes–NaOH (pH 7.5), 150 mM NaCl, 1 mM DTT, 1 mM EDTA, 1 mM PMSF, 10% glycerol) using a Teflon homogenizer (five strokes) and brief sonication (3–15 s), then centrifuged at $1000 \times g$ for 3 min at 4 °C to remove debris. The homogenates were immediately stored at −80 °C. Then,10 μl homogenate sample was processed using sphingosine kinase 1 activity ELISA kits (Echelon Biosciences Inc., K-3500) following the manufacture's protocol. SphK1 activity was expressed as pmol/min/mg protein.

### 2.8. Assay of tissue caspase-3 activity

A piece of the tumor tissue was homogenized and proteins were extracted and stored at −80 °C. Activity of caspase-3 was determined spectrophotometrically as described by Gurtu et al. (1997). 100 μg of tumor tissue extracts in the assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS,10 mM dithiothreitol,1 mM EDTA,10% glycerol) was added to 100 μM of the peptide substrate N-acetyl–Asp–Glu–Val–Asp–ρ-nitroanilide (Ac–DEVD–ρNA) and incubated at 37 °C for 1 h. Cleavage of the substrate was monitored every 30 min up to 2 h at 405 nm and the enzyme activity was expressed as nmol/min/mg protein.

### 2.9. Histopathological examination

The SEC sections were prepared and stained with hematoxyline and eosin (H&E) and examined by light microscope and apoptotic indices were determined. Apoptotic index was defined as aggregate percentages of apoptotic cells and/or apoptotic bodies per total number of cells (1000 cells counted) in 10 randomly selected high power fields (×400). The morphological criteria for apoptotic bodies were applied in our study according to Staunton and Gaffney (1995).