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In vivo combined treatment of rats with ivermectin and aged garlic extract attenuates ivermectin-induced cytogenotoxicity in bone marrow cells

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ABSTRACT

Ivermectin (IVM) is widely used in human and veterinary medicine for the control of parasitic infections. Researches revealed new avenues of medicinal applications of IVM as an antiviral and an anticancer agent. Very little is known about the genotoxic potential of IVM and the available literature is contradictory. The objective of this study was to evaluate the possible genetic damage caused by IVM. Male Sprague Dawley rats were intraperitoneally given IVM at doses between 0.2 mg and 3.2 mg/kg body weight (b. w). Percentages of mitotic and aberrant bone marrow cells were followed. The results indicated that IVM by itself, at doses higher than the recommended dose, induced significant levels of cytogenetic toxicity. To this end, we decided to investigate the potential use of combination of varying doses of aged garlic extract (AGE); 300, 600 and 1200 mg/kg b w and the minimum detectable toxic (MDT) dose of IVM; 0.4 mg/kg. A powerful capacity of AGE to reduce IVM cytogenetic effects was demonstrated. Overall, the data prove the safety of IVM at the recommended dose and provide a strong scientific evidence for superior protection of AGE against possible cytogenotoxic side effects of IVM, confirming the existence of a meaningful therapeutic window.

1. Introduction

Ivermectin (IVM), due to its broad spectrum of activity, is one of the most effective and widely used antiparasitic agents ever discovered (Sharmeen et al., 2010). In humans, IVM is well known as treatment for head lice (Pediculosis) (Sharmeen et al., 2010; Chhaiya et al., 2013) and Scabies (Mites) (Sharmeen et al., 2010; Worth et al., 2012; Banerji, 2015). Ivermectin has a strong antiviral (Azeem et al., 2015; Croci et al., 2016) and anticancer (Sharmeen et al., 2010; Melotti et al., 2014; Dou et al., 2016; Zhu et al., 2017; Dominguez-Gomez et al., 2018; Juarez et al., 2018) activities.

Significant elevations in both aspartate transaminase (AST) and alanine transaminase (ALT(activities were observed in rats (Ashang, 2009), swine and cattle (Slantna et al., 1989), as well as rabbits (Arise and Malomo, 2009; Seddiek et al., 2013; Mahmoud et al., 2014; El-Sawy et al., 2016) indicating hepatic injuries due to IVM medication. Also, IVM induced reactive oxygen species (ROS) not only result in cytotoxic effect on the parasite (Behera et al., 2011) but also in buffaloes (Mahmoud et al., 2014) as evidenced by a significant increase in malondialdehyde (MAD) concentration as well as significant decrease in the concentrations of serum ascorbate, glutathione, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase, catalase.

Several documentary studies concluded that there was no concrete evidence of a clear clastogenic effect exerted by therapeutic doses of IVM in either bacterial or mammalian cells (Lankas and Gordon, 1989; Aleksić et al., 1996; Molinari et al., 2010; Molinari et al., 2013). However, González et al. (2008) showed that IVM causes in vitro genotoxic and cytotoxic effects. Other studies indicated that IVM is teratogenic in mice, rats, and rabbits at human materno-toxic dose levels (El-Ashmawy et al., 2011). In vivo, Sweify et al. (2015) reported that IVM caused high levels of chromosome aberrations (CA) and micronuclei (MN) in mice bone marrow cells. More recent results demonstrated the likely DNA damaging potential of the use of IVM in cattle as evidenced by the comet assay (Montes-Vergara et al., 2017).

The possible molecular disturbances that may occur in normal cells following IVM administration suggested that the use of this drug must be done with caution (Hutchinson et al., 2009). One of the best candidates that may minimize or get rid of these side effects is treatment with garlic extract. It has been shown to maintain the cellular homeostasis through mechanisms which are caspase dependent and/or independent induction of apoptosis, anti-proliferative, anti-metastasis, antioxidant and immunomodulative properties (Raghu et al., 2012; Arreola et al., 2015; Bertrand et al., 2016). The chemical constituents of the extract include sulfur compounds, some amino acids and their

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glycosides, as well as minerals and enzymes (Rana et al., 2011)). Garlic essential oil, obtained by steam distillation, is commercially available in capsules because of pungent odors (Lawson and Bauer, 1998). The oil-soluble organosulfur compounds in garlic, including allicin, sulfides, and vinyldithiins are not found in the blood or urine after garlic consumption and they are not likely to be the active compounds (Gardner et al., 2007). The irritating, acidic, oxidizing and toxic compounds in raw garlic can be eliminated or modified by extraction. Garlic powder, resulting from crushing, drying and pulverization of garlic cloves, is thought to retain the same ingredients as raw garlic; however, the proportions and amounts of various constituents differ significantly (Bayan et al., 2014), contributing to inconsistent clinical results. Aged garlic extract is prepared by soaking garlic slices in an extracting solution (purified water and diluted alcohol) for varying amounts of time, up to 20 months. Over this time, the harsh and irritating compounds in garlic are converted naturally into more stable, more efficient and safer sulfur compounds (Bayan et al., 2014).

In view of the contradictory cytogenetic findings and taking into account that IVM could be repurposed for the treatment of cancer and could be rapidly advanced into clinical trials, the necessity of re-consideration for a further hazard evaluation of IVM is strongly recommended. The aim of the present study was to evaluate the cytogenotoxic effects in normal rat bone marrow cells using CA and nuclear abnormalities (NA) tests as cytogenetic end-points. Another goal was to examine the ability of AGE to reverse or prevent such side effects.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (10–12 weeks-old, average weight 240 ± 25 g) were used in this study. The study was reviewed and approved by the Local Ethics Committee of Yarmouk University and was performed in accordance with the Declaration of Helsinki with regard to ethical principles for research involving laboratory animals.

2.2. Drug preparation

Ivermectin (Noromectin® 1% injection, Norbrook Laboratories, Northern Ireland), the formula used for cattle and small ruminant injection, was diluted with propylene glycol (PG). Aged garlic extract (Wakunaga, USA) was obtained as liquid suspension containing 240 mg AGE/ml. Mitomycin C (MMC, CAS: 50–07-7) was purchased from Sigma (USA). A 20 mg/ml MMC was diluted with a final DMSO (Santa Cruz, USA) concentration of 0.5%, then the solution was diluted with 0.9% phosphate-buffered saline (PBS).

2.3. Ivermectin treatment

Twenty rats were randomly divided into 5 groups (4 animals each). An animal in each group intraperitoneally (i.p) received 0.5 ml containing one of the following doses of IVM: 0.2, 0.4, 0.8, 1.6, and 3.2 mg/kg b.w. Three additional groups of rats served as control were injected with equivalent volume of PBS, or DMSO or PG. One final group was given MMC at 2 mg/kg and acted as a positive control.

2.4. Ivermectin and garlic combination treatment

Ivermectin at 0.4 mg/kg (the minimum detectable toxic [MDT] dose) was co-administered with various doses of AGE; 300, 600 and 1200 mg/kg. Two other groups were treated with 0.4 mg/kg IVM or PBS and as positive and negative control, respectively.

2.5. Cytogenetic studies

Based on the previous results in mice (Sweify et al., 2015), that CA peaked after three days following the treatment with single of IVM, the present experiments were terminated after 72 h. After this period, the frequencies of CA are reduced due to cytotoxicity of the drug and exclusion of severely affected cells from the cell cycle (Molinari et al., 2013), or the rapid metabolism and elimination of the drug (Lifschitz et al., 1999). Colcemid (0.6 mg/kg; CAS: 477-30-5, Santa Cruse) was i.p. given to each rat 2–3 h prior to the end of experiments. At sacrifice by cervical dislocation, bone marrow was collected from the femurs and chromosome spreads were prepared according to a standard protocol described previously (Khalil and Da'dara (1994).

Mitotic index (MI) was calculated by finding the percentage of dividing cells among 8000 cells/dose. A total of 800 cells for each dose were examined for the appearance of polyploidy, breaks, fragments, bridges, rings or dicentric chromosomes and the percentage of cells with CA (% Abc) was recorded using the following formula (Mahata et al., 2003):

$$\%Abc = C/B \times 100\% \quad (1)$$

where, D: total cells with nuclear abnormalities, B: total number of cells screened.

For nuclear abnormalities (NA), a total of 2000 cells from each treatment were examined for the presence of binucleated (BN) cells, binuncleated cells with micronucleus (BN + MN) or nuclear bridge (BN + bridge), lobed nucleus (LN), micronucleus (MN), nuclear bud (NB), and cells with nuclear fragmentation (NF). The %NA was determined according to the following formula:

$$\%NA = D/B \times 100\% \quad (2)$$

where, D: total cells with nuclear abnormalities, B: total number of cells observed.

Antimutagenicity of AGE was expressed as percentage reduction of IVM-induced % Abc (RCA) and calculated according to Akinboro et al. (2016) formula:

$$RCA = a - b/a - c \times 100\% \quad (3)$$

where, a: percentage of IVM-induced % Abc; b: percentage of AGE plus IVM-induced % Abc; c: percentage of PBS-induced % Abc.

2.6. Statistical analysis

The collected data were analyzed using Minitab statistical software version 14. Data are expressed as mean ± standard error (SE). Student's *t*-test was used to compare the mean values of treated groups with their corresponding control. Differences were regarded as significant at $p \leq .05$.

3. Results

3.1. Cytotoxicity of ivermectin

Table (1) presents the cytotoxicity data on bone marrow cells obtained following treatment of rats with various doses of IVM. The daily recommended dose of IVM (0.2 mg/kg) caused a slight increase in %MI over the negative control (PBS) value (14.1% versus 13.8%). Similarly, PG and DMSO (the IVM diluent and MMC solvent) induced non-sigificant effects on MI values; 13.5 and 12.9, respectively. In contrast, progressive decreases in %MI were noticed with increasing doses of the test chemical. Relative to the negative control, statistically significant ($P \leq .05$) depressions in %MI started to show up at doses that grades from the maximum daily recommended dose (0.4 mg/kg IVM) which reached 13.0%. Hgher doses of IVM; 0.8 mg/kg, 1.6 mg/kg, and 3.2 mg/kg significantly ($P \leq .01$) lowered the control %MI value to 11.9%, 9.6% and 7.8%, respectively). The positive control (MMC) resulted in a highly significant reduction in the mean value of MI (4.2%).