Drug repurposing: mebendazole as effective antitumor agent. Are we seeing the whole story?

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Summary

Purpose: To examine the antitumor effects of Mebendazole (MZ) in a model of experimental fibrosarcoma induced by inoculation of BHK-21/C13 cells in Syrian golden hamster.

Methods: Hamsters were inoculated with a suspension of BHK cells by subcutaneous injection and randomly divided into 5 experimental and 2 control groups. Treatment started on the 10th day after inoculation, when the tumor grew to a diameter of 5mm. The experimental design was based on distributing the total amount of drug MZ(z) in different protocols and approaches (oral/intraperitoneal) to the 5 experimental groups. The positive control group received doxorubicin intraperitoneally. Negative control group received olive oil orally. The total amount of MZ(z) was chosen to be the highest for the animal to survive during the experiment. For antitumor effect evaluation, the main parameters were tu-

mor size, number of mitoses, cytochrome-C immunopositivity and tumor tissue morphology incuding cytoarchitecture and percentage of preserved tumor tissue in stereologically reconstructed tumor mass.

Results: The results of this study showed absence of objective MZ antitumor effect on experimental fibrosarcoma. MZ does not exhibit activity similar to DNA-damaging agents on the fibrosarcoma model.

Conclusions: It might be postulated that soft tissue tumors on animal models could show high level of resistance to MZ effect.

Key words: BHK21 cells, drug repurposing, fibrosarcoma, hamster, mebendazole, tumor

Introduction

Forty years after declaring the War on Cancer [1] the concept of the magic bullet drug defined by Paul Ehrlich in 1906 is still not developed in clinical practice. Therefore, a need for further research and development of cancer prevention and treatment is present worldwide. The main problem is that investments often do not result in expected quantities of novel effective antitumor drugs [2]. Furthermore, the majority of currently new avail-

able antitumor agents are expensive and provide minimal increase in the overall survival [3].

Researchers and clinicians adopted a strategy to evaluate the potential antitumor effect of drugs which are already approved for treating noncancerous diseases. This approach, called "drug repurposing", has gained wide attention in the last 20 years [4]. The biggest advantage of the drug repurposing strategy is the fact that the pharmacodynamics,

pharmacokinetics and toxicity of established drugs are often well known. Repurposed drugs could be instantly translated into Phase II and III clinical studies, and- as a result- the cost of a new antitumor agent would be significantly reduced [5].

Mebendazole (methyl 5-benzoyl-2-benzimidazole-carbamate (MZ) is an oral anthelmintic drug used in clinical practice since 1971 worldwide. Some authors argued that benzimidazole derivatives can inhibit the polymerization of tubulins which leads to microtubular damage in parasitic cells [6,7]. Tubulin has a significant role in cellular division and therefore is a crucial target for chemotherapeutic drugs like colchicine, paclitaxel and vincristine. In the last decade MZ has been recognised as a potential antitumor agent. Its effect, demonstrated in both *in vitro* and *in vivo* experiments, was regarded as impressive [8-14].

The MZ mechanism of action is often described as the selective ability to bring tumor cells to G2-M phase arrest and to consequently cause apoptosis of the cell by binding to colchicine-binding domain of the tubulin [8,11]. Currently there are two clinical case reports in which MZ was used as an antitumor agent in humans, the result of which supports the previously mentioned mechanisms [12,15]. Researchers reported an antiangiogenic effect of MZ on human lung cancer cell line xenograft models [11]. In contrast, other research groups in their *in vivo* analysis of adrenocortical cancer models found opposing results and argued that there was no significant antiangiogenic effect of MZ compared to controls [10], while others suggest that MZ can be used successfully in combination with MEK inhibitors in cases of non-V600E BRAF mutant melanomas [16].

Reports from *in vitro* studies stated that MZ is effective in inducing apoptosis via p53-dependent and p53-independent pathways, where in p53-null lung cancer cells MZ induced cytochrome C accumulation, activation of caspase 8 and 9, and PARP and procaspase 3 cleavage [9,11].

The aim of this study was to examine the potential antitumor effects of MZ in an *in vivo* model of experimental fibrosarcoma induced by inoculation of BHK-21/C13 cells in Syrian golden hamster.

Methods

Buffers

Phosphate buffers (pH 6.8 and pH 7.4) were prepared in the laboratory using deonised water, disodium phosphate dihydrate and sodium hydroxide.

Citrate buffer (pH 6.0) was prepared in the laboratory using deonised water, sodium hydroxide and citric acid.

Tris-EDTA buffer (pH 8.4) was prepared in the laboratory using deonised water, EDTA, Tris base and sodium hydroxide.

Counting mitoses

Counting of mitoses was done using Leica DM LB microscope at 400x magnification on 10 random and separate high power visual fields (HPF) on the tumor periphery (the invasion front). The total number of mitoses of one tumor tissue section was then divided with the number of total visual fields and expressed as mean value of that tumor sample.

Drug

Mebendazole (Soltrik®) was donated from Galenika AD (Belgrade, Serbia) in the form of 100% pure powder. Doxorubicin (Adriblastina CS ®) was donated from the Hematology Department of Clinical Centre of Vojvodina.

Experimental animals

Seventy adult male and female Syrian golden hamsters (Mesocricetus auratus) with relative heterogeneity

Table 1. Simplified protocol of MZ administration

Group	Sex	Number of doses	Single dose of MZ (mg/kg)	Total dose (mg/kg)
E1	male	2	3010 p.o.	6020
	female		1498 p.o.	2996
E2	male	14	430 p.o.	6020
	female		214 p.o.	2996
E3	male	28	215 p.o.	6020
	female		107 p.o.	2996
E4	male	70	86 p.o.	6020
	female		42 p.o.	2996
E5	male	4	1505 i.p.	6020
	female		749 i.p.	2996

p.o.:per os ; i.p:intraperitoneal

of inbreed strains were used. The animals were housed in a vivarium with controlled temperature (25±2°C), with 12h dark and 12h light periods and *ad libitum* access to food and water.

Experimental protocol

All animals received subcutaneously 1 ml inoculate that contained freshly trypsinized 1,500,000 BHK-21/C13 cells supplied from the European collection of authenticated cell cultures (Salisbury, UK). The animals were later randomly divided into 5 experimental and 2 control groups. Each group consisted of 10 animals. Treatment started 10 days following the BHK inoculation. At this time point, the BHK-induced tumor grew to a diameter of approximately 5mm.

The experimental design was based on distributing the even total amount of MZ(z) in different protocols and approaches. The total amount of MZ(z) was chosen to be the highest possible for animals to survive during the experiment, based on our pilot study (not published yet). MZ(z) for males was 6020mg/kg, and for females 2996mg/kg. In that way we could eliminate the factor of low drug concentration in plasma and it was possible to influence to final result outcomes. The experimental groups were organised as follows: E1 received MZ(z) divided in 2 doses (1 dose/week; E1= MZ(z)/2); E2 received MZ(z) divided in 14 doses (1 dose/day; E2 = MZ(z)/14); E3 received MZ(z) divided in 28 doses (2 doses/day; E3=MZ(z)/28); E4 received MZ(z) divided in 70 doses (5 doses/day; E4= MZ(z)/70); E5 received MZ(z) divided in 4 doses (2 dose/week; E5= MZ(z)/4). E1-E4 received MZ orally, emulged in olive oil for maximal absorption [17]. E5 received MZ intraperitoneally suspended in 10% DMSO. A simplified protocol of MZ administration is shown in Table 1.

The positive control group (CTRL+) received doxorubicin intraperitoneally. The dosage was adopted according to protocols that used doxorubicin as monotherapy for soft tissue sarcomas treatment [18]. In order to keep the animal dose of doxorubicin equivalent to a human dose recommended by guideline, conversion protocol was used [19]. Each animal in the CTRL+ group received intraperitoneally a total dose of 15mg/kg doxorubicin divided in 4 doses diluted in 0.9% NaCl on the 1st, 4th, 9th and 13th day after tumor masses reached a diameter of 5mm. The negative control group (CTRL-) received orally 1ml of olive oil daily. The experiment lasted for 2 weeks following the beginning of treatment, and 24 days in total since the inoculation of BHK-21 cells. At the end of the experiment all animals were euthanized using chloroform.

Experimental tumor

A continuous culture of BHK-21/13 cells was made from kidney of 1-day-old Syrian golden hamster. BHK-21 cells are tumorigenic for all Syrian golden hamsters. After subcutaneous, intramuscular or intracerebral inoculation in Syrian golden hamster, BHK-21/C13 cells were continuing multiplying with autonomous locally invasive growth, inducing fibrosarcoma *in vivo* with identical morphology as spontaneous well differentiated fibrosarcoma in humans or animals but without forming metastases and noticeable immune response.

Fixatives

Neutral buffered formalin, 10%.

Histological and histochemical stains

Mayer's hemalum (MHS32) and Eosin Y (HT110116) as a 0.5% (w/v) alcoholic solution (Fluka Sigma-Aldrich Chemie GmbH, Germany).

Histological staining

Tissue sections were stained with eriochrome cyanin R and eosine according to Stefanovic et al.[20].

Immunohistochemistry

For immunohistochemical analysis, 5 µm-thin sections were cut from the tumor's periphery (invasion front). After usual ethanol-xylene sequence, the samples were embedded in paraffin (Histowax, Sweden) and cut on a rotary microtome RT350 (Leica, Germany).

Methods for immunohistochemical staining included primary antibody against cytochrome C [7H8.2C12] (ab13575) in a 1:500 dilution (Lab Vision; Thermo Scientific, Rockford, IL) using the appropriate visualization system: UltraVision LP Detection System HRP Polymer & AEC Chromogen (Lab Vision; Thermo Scientific). Cytocrome C immunostaining required antigen retrieval with citrate buffer (pH 6.0) in a microwave oven at 850 W for 20 min. Antibodies were applied for 30 min at room temperature. Dehydratation in graded alcohols, xylene wash and Mayer's hemalum was used as a counterstain, followed by mounting and coverslipping (Bio-Optica, Italy) for slides. Prepared slides were analyzed using an Leica DM LB microscope (Leica, Germany) and photographed on an Leica MC 190 HD camera (Leica, Germany). As positive and negative controls for immunostaining, tumor samples from CTRL+ and CTRL- groups were used.

Solvents, emulgants and other chemichals

Virgin Olive Oil (Monini, Spoleto PG, Italy);

Dimethylsulphoxide (DMSO), DPX Mountant for histology, Tris-Base and EDTA (Fluka Sigma-Aldrich Chemie GmbH, Germany);

Citric acid, formaldehyde 37%, sodium phosphate dihydrate ($NaH_2PO_4 \times 2H_2O$), sodium chloride (NaCl), sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), hydrochloric acid (HCl), absolute ethanol and xylene (Centrohem Stara Pazova, Serbia);

Histowax special 52-54°C (Histolab, Göteborg, Sweden);

Distilled water was prepared in the laboratory of Pasteur Institute, cooled at 25°C, and later purified with ion exchange resins.

Stereology

Each tumor was divided in 20 equal cross-sections and every 2nd section was used for creating histological sample. Samples were later photographed with camera connected on microscope with grade scale. Photomicro-

graphs were imported in the ImageJ program for mesuring and reconstructing the volume of whole tumor sample and the volume of necrosis and haemorrhage fields via Cavalieri estimator.

Statistics

Comparisons between volumes of tumor masses, volumes of preserved/necrotic tumor tissue and average mitotic indexes were preformed using paired 2-tailed Student's t-test. Data were listed as the mean±standard deviation (SD). Statistically significant difference was considered for a p value <0.05. For statistical analyses Microsoft Excel 2013 v.15.0 included in Office 2013 package was used.

Results

The five key results of this experimental study are the following:

Effect of mebendazole on tumor growth

After 24 days of MZ treatment, tumor volumes of experimental groups E1 (1031.25±748 mm³, p=0.211, p=0.027), E2 (512.5±419.19 mm³, p=0.081, p=0.042), E3 (1397.33±730.43 mm³, p=0.231, p=0.023), E4 (1300.5±398.10 mm³, p=0.303, p=0.025) and E5 (972.54±525.71 mm³, p=0.183) compared to CTRL- group (1847.978±612.18 mm³) did not show any statistically significant reduction. In addition, there was significant difference in tumor volumes between CTRL+ group (4.45 mm³±1.254 mm³)

and all experimental groups. There were some isolated cases of tumor growth reduction in the experimental groups, and the same phenomenon was noticed in one animal in the CTRL- group. More often tumor growth seemed to be undisputed, therefore great standard deviation was present (Figure 1).

Mebendazole effect on tumor morphology

Located subcutaneously, the tumor showed expansive growth in a form of fleshy grayish pink mass. Visual examination of the experimental and control tumor samples revealed clearly distinctive and well preserved peripheral whitish parts and central necrotic parts with areas of fresh haemorrhage.

Histologically, the picture observed in the control group resembles a well differentiated fibrosarcoma. Tumor samples of animals treated with MZ did not show any signs of cytoarchitecture disruptions or morphological changes, while diffuse areas of destructed tumor tissue and haemorrhage was present in the CTRL+ group (Figure 2).

Induction of cytochrome C release

Cytosolic cytochrome C (Cyto C) is considered to be necessary for the initiation of the apoptotic process. Cyto C is normally located in the mitochondrial intermembrane space. Elevation of
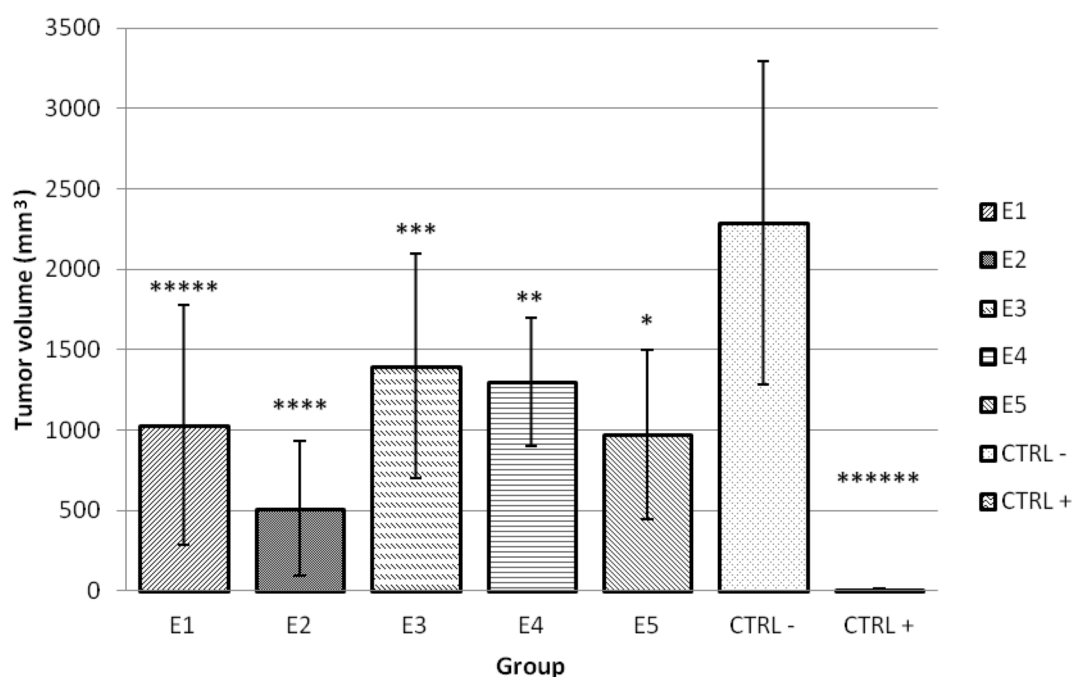

Figure 1. Tumor volume comparison between groups. Compared to CTRL-: *p=0.183, **p=0.303, ***p=0.231, ****p=0.081, *****p=0.211; Only CTRL+ group showed statistically significant volume reduction (******p= 0.004). Compared to CTRL+ all experimental groups showed statistically significant volume difference (*p=0.048, **p=0.025, ***p=0.023, ****p=0.042, *****p=0.027).

cytosolic Cyto C is considered to be an indirect sign that the cell is undergoing apoptosis.

Accumulation of Cyto C was noticed in the control group tumors, mostly equal as in tumors treated with MZ. The intensity of Cyto C accumulation and the size of free Cyto C areas seemed not to be linked with the drug given. In contrast, immunopositivity of Cyto C in the CTRL+ group was intense. Therefore, no evidence was noted for MZ to induce fibrosarcoma cells damage *in vivo* (Figure 3).

Mebendazole induced tumor tissue destruction

Fields of necrosis and haemorrhage in histological cross sections of tumor tissue were analysed using the Cavalieri estimation method. The whole tumor sample was reconstructed using stereology and fraction of preserved tumor tissue within the whole tumor mass was calculated.

Considering experimental groups the most obvious difference from the CTRL- group (79.31±1.84%) showed the E1 group (43.68±14.69%, p=0.0001), E3 (55.11±11.48%, p=0.0003) and E2 (48.18±27.75%, p=0.0056) respectively. The percentage of preserved tumor tissue in E4 (81.44±1.02%, p=0.1739) and E5 group (68.26±16.08%, p=0.0653) showed no statistical difference from the percentage measured in the CTRL- group (Figure 4). The most prominent difference from CTRL- was noticed in the CTRL+ group (30.2±8%, p=0.032).

Number of mitoses

The CTRL- group showed high mitotic index (3.66±1.18 mitosis per HPF). In contrast, the mitotic index of CTRL+ group was low (1.02±0.3). Compared to CTRL- group, no significant reduction in the number of mitosis was observed in E1 (3.77±0.21 mitoses per HPF, p=0.928), E2 (3.8±2.1
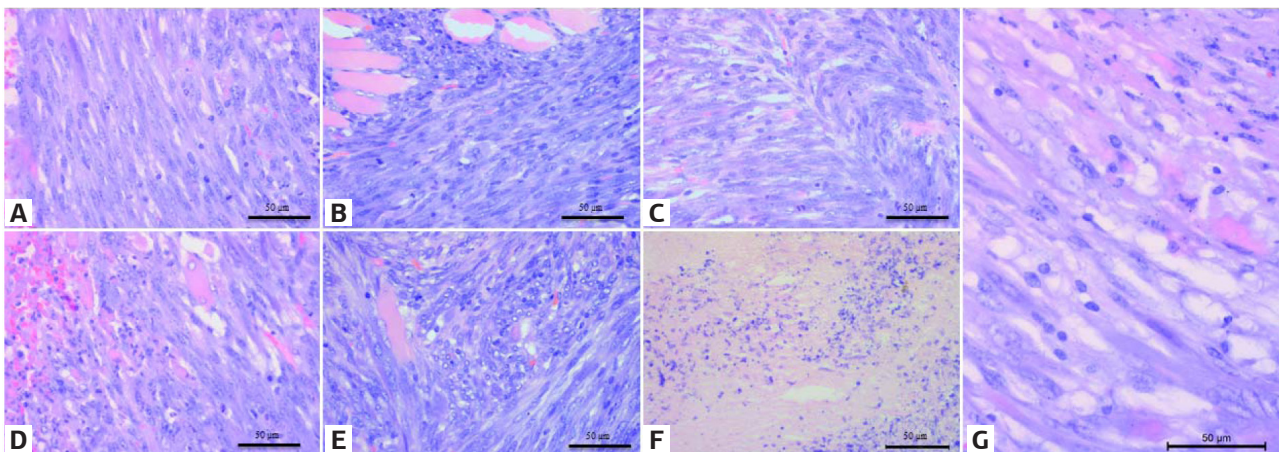

Figure 2. Fibrosarcoma invasion front x400 (ECR&E). **A-E:** Tumors treated with Mebendazole do not show any signs of cytoarchitecture disruptions or morphological changes, many mitotic figures are present. **F:** Cellular destruction observed in tumors treated with doxorubicin (CTRL+). **G:** Preserved tumor tissue (CTRL-).
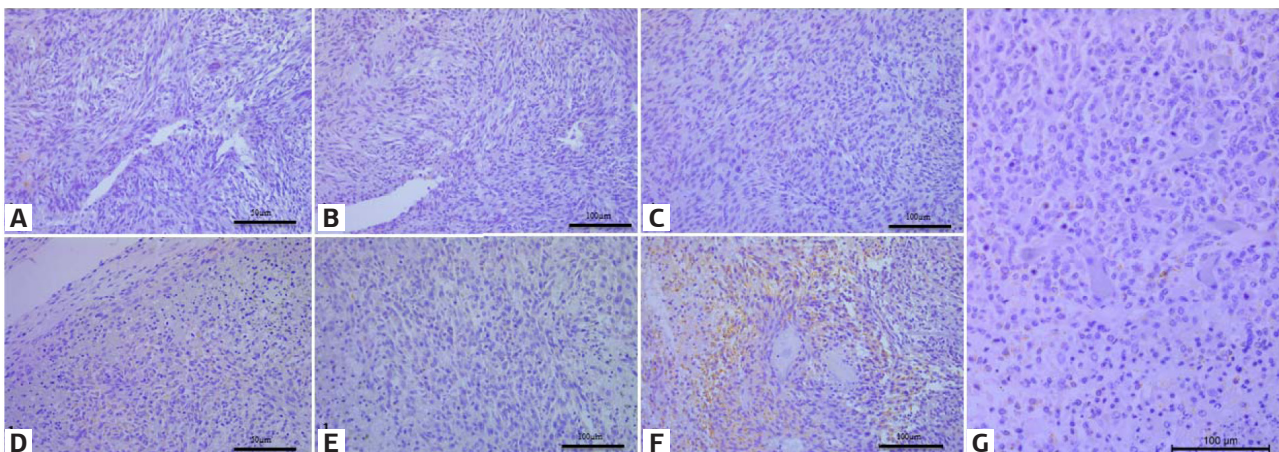

Figure 3. Fibrosarcoma invasion front immunostained for cytochrome C positivity x200. **A-E:** Weak cyto C immunopositivity in tumors treated with Mebendazole. **F:** Intense cyto C immunopositivity in CTRL+ tumor treated with doxorubicin. **G:** Weak cyto C immunopositivity in negative control group.

mitoses per HPF, p=0.903), E3 (2.15±1.36 mitoses per HPF, p=0.098), E4 (3.45±0.70 mitoses per HPF, p=0.753) and E5 (2.85±0.91 mitoses per HPF, p=0.416) (Figure 5).

Discussion

The main goal of this research was to reveal any potential antitumor effect of MZ on an *in vivo* model of experimental fibrosarcoma.

In this study MZ did not show any significant effect on tumor growth dynamics, tumor mitotic index, cytoarchitecture and morphology of tumor cells. Significant difference compared to negative control group was observed only in the percentage of non-necrotic and non-hemorrhagic tumor tissue inside the tumor mass, but only in 3 experimental groups and without any relationship with the drug administration protocol. Cytoplasmic cytochrome C distribution, a marker of cellular damage, did not
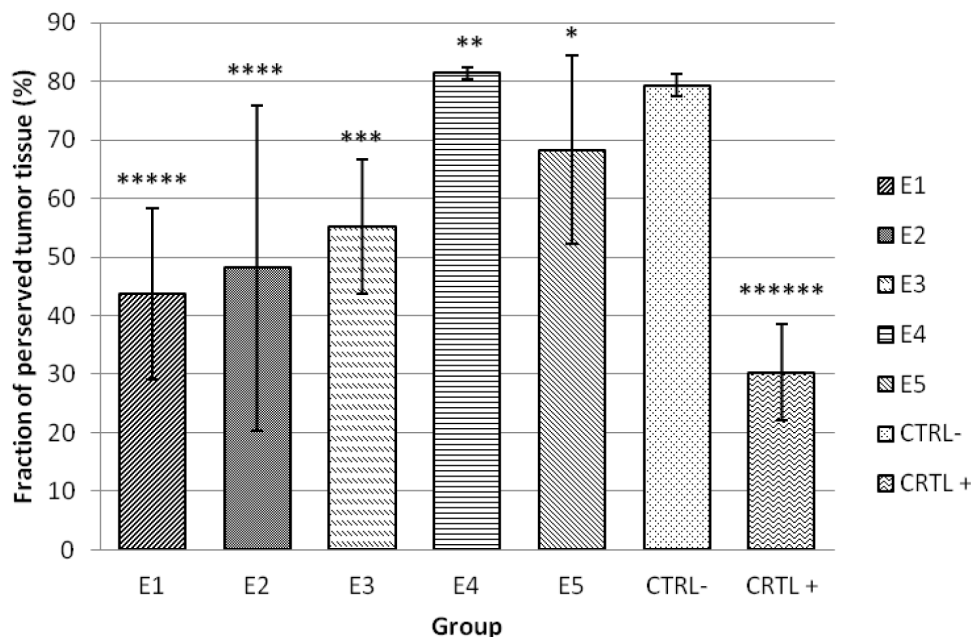

Figure 4. Comparison of preserved tumor area within the tumor mass reconstructed with Cavalieri Estimator. Compared to CTRL-: *p=0.14, **p=0.21, ***p=0.047, ****p=0.045, *****p=0.043, ******p=0.032). A significant percentage of destructed tumor tissue is observed in groups E1-3 compared to CTRL-. Tumor destruction is most prominent in CTRL+ group (doxorubicin-treated).
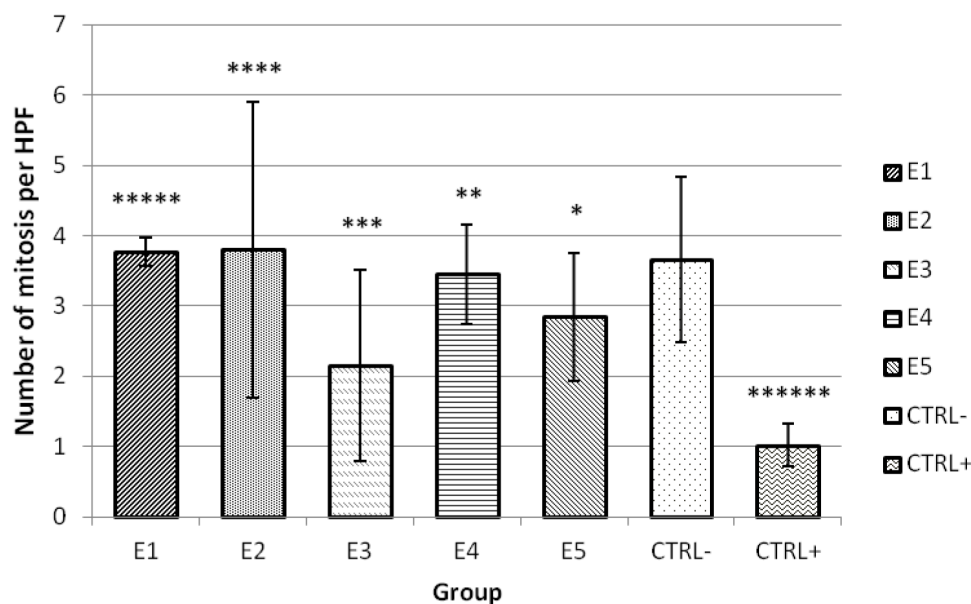

Figure 5. Comparison of mean values of mitotic index per group. Compared to CTRL-: *p=0.416, **p=0.753, ***p=0.098, ****p=0.903, *****p=0.928, ******p=0.012). Significant difference present only in CTRL+ group (doxorubicin-treated).

show any difference between negative control and experimental groups. Therefore, compared to the effect of doxorubicin, there could be serious doubt in MZ's antitumor effect on fibrosarcoma cells *in vivo*.

However, this is the first experiment that employed MZ as an antitumor agent on hamsters and if there were some factors in the hamsters' drug metabolism, like low intestinal absorption, liver metabolism, drug distribution, etc, significant effect should be present in the group with intraperitoneal administration where most of those factors are avoided.

Sasaki et al. and Mukhopadhyay et al. showed that MZ *in vitro* can induce time- and dose-dependent apoptosis of lung cancer cell lines. *In vivo* studies based on a model of nu/nu mice inoculated with H460 non-small lung carcinoma, C3H, K1735 and A549 cell lines treated orally with 1mg MZ once in two days reported considerable effect on reducing tumor growth and vascularity in treated tumors [8,11].

Martarelli et al. reported that MZ can substantially inhibit H295R and SW-13 human adrenocarcinoma cells *in vivo* and *in vitro* via induction of apoptosis. The *in vivo* model included nu/nu mice treated with 1mg and 2mg MZ alone and 2mg MZ suspended in 10% DMSO, respectively. The results revealed reduction of tumor growth, however, in a no dose-dependent manner. In their experiment MZ didn't inhibit tumor neoangiogenesis [10].

A study of Vaziri et al. also noted the potential importance of MZ repurposing on medullobastoma models showing increase of overall survival in treated animals, focusing on the MZ antiangiogenic effect [21].

Another *in vitro* study suggested that MZ can inhibit MDR gene expression in malignant ascites cells [14], while De Witt et al. demonstrated antitumor effect on GL261 glioblastoma cells *in vitro* and *in vivo* [13].

MZ's effects demonstrated in our study were not consistent with the findings of the majority of other authors. Though we used much higher doses than other authors and administered various regimens, no antitumor effect on fibrosarcoma was observed. A possible explanation could be the nature of the tumor itself, considering that this is the first experiment that is testing MZ effect on a soft tissue tumor. Almost all other experiments are based on epithelial tumor models. If we consider that the majority of the described pathway of MZ action on tumor cell (inhibition of mitotic spindle leading to arrest in G2/M phase, followed with bcl-2 phosphorylation), it is understandable that fibrosarcoma, primarily sensitive to DNA-damaging agents show resistance to an antimicrotubule drug. Confirmation of experimental fibrosarcoma sensitivity to DNA-damaging agents is shown in the experiment of Milijasevic et al., where a single dose of dacarbazine induced changes in tumor cells morphology and reduced the number of mitoses in a dose-dependent manner [22] and our study agrees with that finding.

The results of this study indicate that MZ does not have a broad spectrum of action as it is believed. Due to the absence of objective evidence of antitumor effect on experimental fibrosarcoma, it seems that MZ does not exhibit activity similar to DNA-damaging agents. Therefore, it might be postulated that although MZ shows significant effect on cell culture lines, soft tissue tumors on animal models could show high level of resistance on its effect.

Ethical standards statement

This investigation has been conducted in accordance with the ethical standards and according to the Declaration of Helsinki and according to national and international guidelines and has been approved by the Serbian Ministry of Agriculture, Forestry and Water Management- Veterinary Directorat.

Conflict of interests

The authors declare no conflict of interests.

References

1. Hanahan D. Rethinking the war on cancer. Lancet 2014;383:558-63.
2. Gupta SC, Sung B, Prasad S, Webb LJ, Aggarwal BB. Cancer drug discovery by repurposing: teaching new tricks to old dogs. Trends Pharmacol Sci 2013;34: 508-17.
3. Fojo T, Parkinson DR. Biologically Targeted Cancer Therapy and Marginal Benefits: Are We Making Too Much of Too Little or Are We Achieving Too Little by Giving Too Much? Clin Cancer Res 2010;16:5972-80.
4. Boguski MS, Mandl KD, Sukhatme VP. Repurposing with a difference. Science 2009;324:1394-5.

5. Thayer AM. Drug Repurposing. Chem Engineer News 2012;90:15-25.

6. Gull K, Dawson PJ, Davis C, Byard EH. Microtubules as target organelles for benzimidazole anthelmintic chemotherapy. Biochem Soc Transact 1987;15:59-60.

7. Laclette JP, Guerra G, Zetina C. Inhibition of tubulin polymerization by mebendazole. Biochem Biophys Res Commun 1980;92:417-23.

8. Sasaki J, Ramesh R, Chada S, Gomyo Y, Roth JA, Mukhopadhyay T. The Anthelmintic Drug Mebendazole Induces Mitotic Arrest and Apoptosis by Depolymerizing Tubulin in Non-Small Cell Lung Cancer Cells. Mol Cancer Ther 2002;1:1201-9.

9. Doudican N, Rodriguez A, Osman I, Orlow SJ. Mebendazole Induces Apoptosis via Bcl-2 Inactivation in Chemoresistant Melanoma Cells. Mol Cancer Res 2008;6:1308-15.

10. Martarelli D, Pompei P, Baldi C, Mazzoni G. Mebendazole inhibits growth of human adrenocortical carcinoma cell lines implanted in nude mice. Cancer Chemother Pharmacol 2008;61:809-17.

11. Mukhopadhyay T, Sasaki J, Ramesh R, Roth JA. Mebendazole elicits a potent antitumor effect on human cancer cell lines both in vitro and in vivo. Clin Cancer Res 2002;8:2963-9.

12. Dobrosotskaya I, Hammer G, Schteingart D, Maturen K, Worden F. Mebendazole Monotherapy and Long-Term Disease Control in Metastatic Adrenocortical Carcinoma. Endocr Pract 2011;17:59-62.

13. De Witt M, Gamble A, Hanson D et al. Repurposing Mebendazole as a Replacement for Vincristine for the Treatment of Brain Tumors. Mol Med 2017;23:50-6.

14. Celestino Pinto L, de Fátima Aquino Moreira-Nunes C, Soares BM, Burbano RMR, de Lemos JAR, Montenegro RC. Mebendazole, an antiparasitic drug, inhibits drug transporters expression in preclinical model of gastric peritoneal carcinomatosis. Toxicol in Vitro 2017;43:87-91.

15. Nygren P, Fryknäs M, Ågerup B, Larsson R. Repositioning of the anthelmintic drug mebendazole for the treatment for colon cancer. J Cancer Res Clin Oncol 2013;139:2133-40.

16. Simbulan-Rosenthal CM, Dakshanamurthy S, Gaur A et al. The repurposed anthelmintic mebendazole in combination with trametinib suppresses refractory NRASQ61K melanoma. Oncotarget 2017;8:12576-95.

17. Liu C, Zhang H, Jiang B et al. Enhanced bioavailability and cysticidal effect of three mebendazole-oil preparations in mice infected with secondary cysts of Echinococcus granulosus. Parasitol Res 2012;111:1205-11.

18. In GK, Hu JS, Tseng WW. Treatment of advanced, metastatic soft tissue sarcoma: latest evidence and clinical considerations. Ther Adv Med Oncol 2017;9:533-50.

19. Nair A, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 2016;7:27.

20. Stefanović M, Lalošević D. Use of eriochrome cyanine R in routine histology and histopathology: is it time to say goodbye to hematoxylin? Biotech Histochem 2015;90:461-9.

21. Bodhinayake I, Symons M, Boockvar JA. Repurposing mebendazole for the treatment of medulloblastoma. Neurosurgery 2015;76:15-6.

22. Milijašević B, Stefanović D, Lalić-Popović M et al. Acute toxic effects of single dose dacarbazine: hematological and histological changes in an animal model. Biotech Histochem 2014;89:583-90.