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Original Research Article

Effect of mebendazole on fibrosarcoma in hamsters

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Abstract

Purpose: *To investigate the effect of mebendazole on an in vivo solid tumor model of fibrosarcoma in hamsters.*
Methods: *24 Syrian golden hamsters of both sexes with the approximate body weight of 100g were randomly distributed in 2 experimental and 2 control groups, with 6 animals in each group. BHK-21/C13 cells (2 x 10^6) in 1 mL Glasgow Minimum Essential Medium (GMEM) were injected subcutaneously into the back of each animal in 3 groups. The experimental groups were treated with mebendazole (460 mg/kg) via a gastric tube on a daily basis, immediately after tumor inoculation. In addition, one experimental group received deoxycholic acid 20 mg/kg once a day. After 2 weeks, when the tumors were approximately 1 - 2 cm in the control group, all the animals were sacrificed, and their blood collected for laboratory analysis. The tumors were excised, their weight and diameters measured, and the volumes calculated. The tumor samples were histopathologically assessed and the main organs toxicologically analyzed. Images were taken and processed by an imaging software, and Ki-67-positive cells in the tumor samples were quantified.*
Results: *Mebendazole diminished tumor mitosis from 18.5 ± 3.02 to 13.5 ± 3.45 ($p < 0.05$), vasculature and tissue penetration, and increased necroses in tumor slices. Tumor volume and weight were insignificantly attenuated. Toxicity was not observed.*
Conclusion: *Mebendazole might be an effective non-toxic agent in sarcoma therapy.*

Keywords: *Mebendazole, Hamsters, BHK-21/C13 cells, Fibrosarcoma therapy, Tumor mitosis*

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INTRODUCTION

Published results of preclinical investigations show that mebendazole, a registered antiparasitic drug widely used in endemic tropical regions, is effective against cancer cell proliferation. Clinical trials of mebendazole's anticancer effect have not yet been completed. [1]. Mebendazole exhibits anticancer activity on: osteosarcoma and soft tissue sarcoma [2]; melanoma [3,4]; meduloblastoma [5,6]; glioblastoma multiforme [7]; acute myeloid leukaemia [8,9]; lung [10], breast [11], hepatocellular [12] colorectal [2,13,14], adrenocortical [15,16], and ovarian [17] carcinoma.

Although mebendazole has shown a significant antiproliferative effect *in vitro*, its therapeutic antitumor value *in vivo* is still uninvestigated and the mechanism of action has not been clarified jet. Only a few papers have been published about mebendazole's anticancer effect on solid tumors, including sarcomas, in experimental animals. Therefore, the aim of this study is to investigate if mebendazole (given alone or in

combination with deoxycholic acid), can suppress fibrosarcoma growth in hamsters.

BHK (Baby Hamster Kidney) cells were used in our study. The primary cultures from which the Syrian hamster fibroblast cell line BHK-21 was produced were prepared in March 1961 from the kidneys of 5 one-day-old hamsters from litter No. 21. Nineteen days after a change in growth rate was first observed, 8 clones were grown from single cells in a small drop of medium under mineral oil. One of these designated BHK-21/13 (or C13) cells has been extensively examined and is the source of the majority of BHK cells distributed in laboratories all over the world. Rapid growth and high cloning efficiency of BHK-21/C13 cells combined with low chromosome numbers make them especially suitable for various preclinical experiments [18].

The BHK-21 line of Syrian hamster fibroblasts is widely used for research on viruses and neoplastic cell transformations *in vitro* [19-22]. Some BHK-21 clones (for example C-13) induce hamster tumors [22]. As previously described in the literature [22,23], BHK cells are capable of both reproducing themselves in hamster tissues and provoking tumors.

Subcutaneous (s.c.) inoculation of 10^7 BHK-21 clone 13 cells produced a fibrosarcoma that killed the hamsters in the forty day span [22].

EXPERIMENTAL

Animal model

The study was carried out on 24 adult Syrian golden hamsters about 12 weeks old (approximate body weight 100 g), male and female. The investigation complies with "Principles of Laboratory Animal Care" [24]. Ethical approval for the animal studies ref. No. 01-78/18-5 was obtained on 26th April 2016 by the Ethics Committee of the University of Novi Sad, Faculty of Medicine, Republic of Serbia.

The hamsters were randomised into 2 treated and 2 control groups (with 6 animals in each group). The BHK-21/C13 cell culture (2×10^6 in 1 mL Glasgow Minimum Essential Medium - GMEM) was inoculated s.c. with an insulin syringe, at a 45 degree angle on the right side of hamsters' loin for the production of the BHK fibrosarcoma. The treatment in two experimental groups was initiated immediately after the s.c. inoculation of BHK-21/C13 cells and continued for 14 days (until tumor diameters were approximately 1 - 2 cm in the untreated control group). At the end of the experiment, all animals were sacrificed, including the second control group without tumor inoculation, which was treated with the same dose of mebendazole as the experimental groups for 14 days. The animals were sacrificed by 10 - 15 minutes prolonged anesthesia, with pentobarbital 30 mg/kg administered intraperitoneally.

Mebendazole, suspended in pure olive oleum, was administered once a day via a gastric tube at a dose of 460 mg/kg (in 1 mL per 100 g weight). One experimental group received additional deoxycholic acid as an adjuvant, 20 mg/kg once a day (in 1 mL of water per 100 g weight). The control group with tumors only received isovolemic vehicle (1 ml/100 g per animal).

The tumors were excided after the sacrifice. Tumor weight and size were measured. Tumor volume was calculated as $L \times S^2/2$ (where L was the longest, and S the shortest diameter). Tumor slices were analysed histopathologically and immunohistochemically (Ki-67) for the verification of tumor growth. Blood samples were collected for conventional blood tests (erythrocytes, leucocytes, lymphocytes, monocytes, granulocytes, platelets, hemoglobin, hematocrit, MCV, MCH, MCHC, serum proteins, albumins, sedimentation, partial thromboplastin time). Body weights were measured for hamsters daily to evaluate possible side effects. The main organs of animals (liver, kidneys, lungs) were toxicologically analysed.

Ki-67 staining

The tumor samples were treated with methanol, 4 % BSA/PBS and anti-Ki-67 pAb at 25 °C. The secondary antibody was polyclonal anti-rabbit FITC. Hoechst 33256 (Sigma) was used for nuclei counterstaining. Leica MC190HD camera was used for taking images, which were processed by software UTHSCSA Image Tools for Windows Version 3.00 [25]. The numbers of Ki-67-positive cells were counted on 20 tumor images of each animal and the mean values were determined and compared between the groups.

Statistical analysis

Mean ± SD was calculated for experimental data. The differences between the treated and control groups of animals were determined using Student´s *t*-test (for small independent samples) for tumor weight, volume, mean number of Ki-67-positive cells marked on images and other measured parameters. Statistical analysis was

performed by Statistica 13.0. Statistically significant difference was set at $p < 0.05$.

RESULTS

The subcutaneous inoculation of BHK-21/C13 cells into hamsters resulted in fibrosarcoma formation at the site of injection in all inoculated animals. Treatments with mebendazole and mebendazole with deoxycholic acid significantly reduced the proliferation status of tumor cells ($p < 0.05$), as shown by Ki-67 staining on hamster tumor sections (Table 1). In our experiment, tumor volume and weight were not significantly changed in either of the treated groups, relative to the control group ($p > 0.05$).

The histopathological evaluation of investigated tumors (Figure 1, Figure 2, Figure 3 and Figure 4) revealed reduction of vasculature, decrease of tissue penetration and expansion of necroses with mebendazole treatment.

Mebendazole treatment had a significant effect on the body weight of the animals during the course of the study (Table 1).

Table 1: Characteristics of animals and tumors in the control and mebendazole treated groups

Hamster No	Weight (g)		Sex	Tumor		
	start	end		Weight mg	Volume cm^3	Ki-67 $\overline{X}$ for 20
Control group without mebendazole treatment, with inoculated tumor						
1	91	95	F	2060	1.50000	17
2	105	117	F	6880	5.00000	23
3	93	94	F	260	0.18750	14
4	94	110	F	1720	1.00000	20
5	105	101	M	2910	2.11981	18
6	97	95	M	1400	1.25025	19
$\overline{X}$	97.5	102		2538	1.84293	18.5
±SD	6.12	9.51		2297	1.67114	3.02
Control group without tumor inoculation, treated with 460 mg/kg mebendazole						
1	90	92	F	0	0	0
2	102	112	M	0	0	0
3	100	110	M	0	0	0
4	88	90	F	0	0	0
5	91	90	M	0	0	0
6	102	105	F	0	0	0
$\overline{X}$	95.50	99.83				
±SD	6.50	10.32				
Treatment with 460 mg/kg mebendazole						
1	110	90	M	730	0.448	12
2	116	86	M	980	0.567	13
3	114	92	M	2110	1.608	14
4	105	95	F	2570	1.710	16
5	110	88	F	690	0.395	8
6	102	100	M	3010	2.280	18
$\overline{X}$	109.5	91.83		1682	1.168	13.5
±SD	5.25	5.08		1012	0.8001	3.45
p (t-test)	< 0.01			> 0.05	> 0.05	< 0.05
Treatment with 460 mg/kg mebendazole and 20 mg/kg deoxycholic acid						
1	103	100	F	1250	0.690	13
2	95	85	M	3120	2.490	18
3	110	98	M	620	0.377	8
4	117	105	F	1320	0.710	13
5	102	90	M	765	0.480	12
6	98	84	F	2415	1.860	15
$\overline{X}$	104.2	93.7		1582	1.1012	13.17
±SD	8.08	8.60		983	0.8645	3.31
p (t-test)	> 0.05			> 0.05	> 0.05	< 0.02

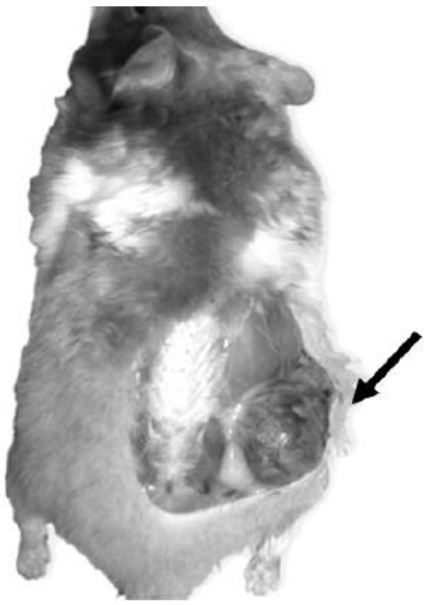


Figure 1: BHK fibrosarcoma: subcutaneous localization in a hamster

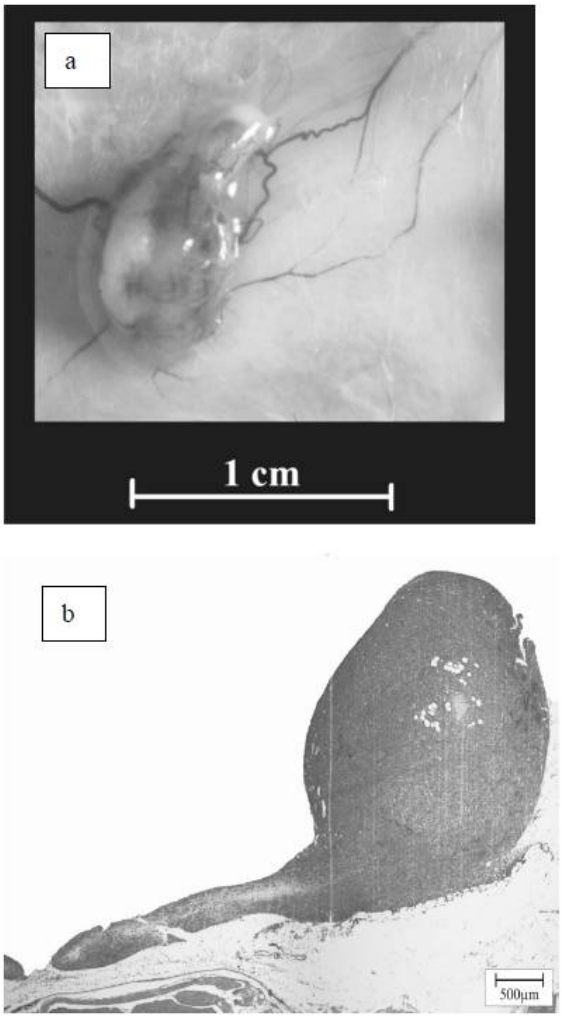
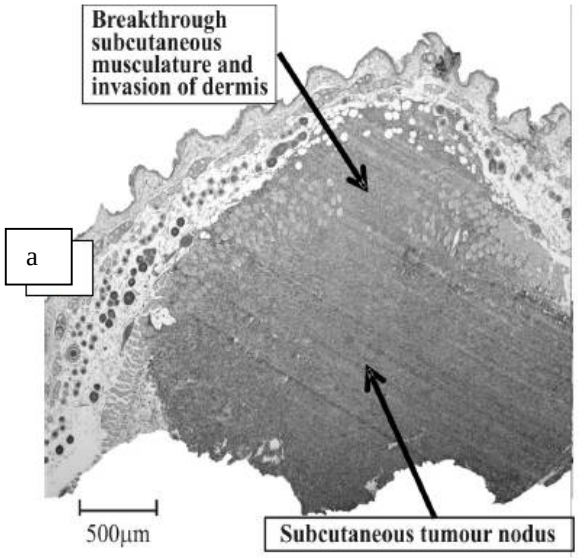


Figure 2: BHK fibrosarcoma: a) subcutaneous localization; b) hypodermal infiltration



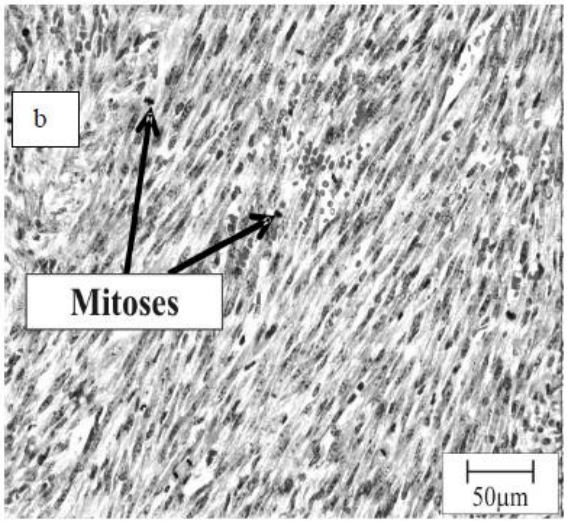


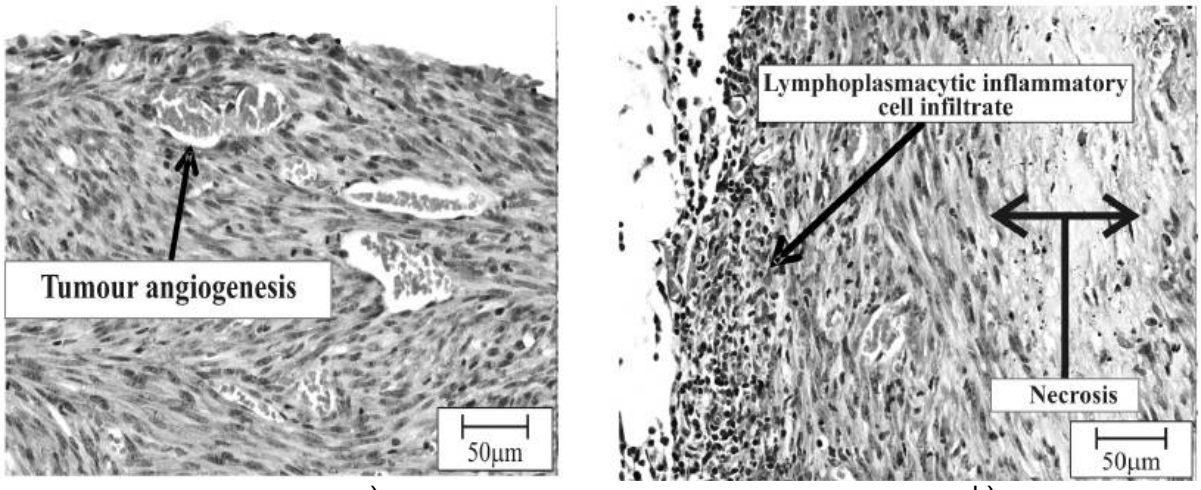
Figure 3: BHK fibrosarcoma: a) penetration into the subcutaneous musculature and the invasion of dermal structures; b) mitoses

Experimentally obtained values: the number of red and white blood cells and platelet, haemoglobin levels, hematocrit, serum proteins, sedimentation, activated partial tromboplastin time and other blood laboratory values, were statistically compared between the treated and control groups, but no significant difference was observed.

An examination of the main organs revealed no pathological or toxicological changes in the control and experimental groups.

DISCUSSION

Mebendazole has been shown to cause tubulin depolymerization in cells of various cancers [1,8,10].



a) b)

Figure 4: BHK fibrosarcoma: a) tumour angiogenesis; b) lymphoplasmacytic inflammatory cell infiltration and tumour necrosis

Microtubules in the lung cancer culture were effective targets for anticancer therapy with mebendazole. This therapy blocked mitosis, induced apoptosis of lung cancer cells, activated caspase and released cytochrome c [10]. Bcl-2 inactivation is one possible explanation for mebendazole's anticancer effect on melanoma cells [3,4].

In human therapeutic concentrations, mebendazole inhibits Hedgehog signaling pathway and reduces the growth of human medulloblastoma cells [26].

Like other antimicrotubular drugs, mebendazole can block tumor supply with blood by depolymerizing microtubules in tumor vasculature cells. [27]. This is in accordance with our histopathological findings on the investigated tumor vasculature.

In this experiment, histopathological improvements of the investigated tumors were observed, but we suppose that the short duration of mebendazole treatment was the main reason that the tumor volume and weight remained insignificantly changed in both treated groups. The combination with deoxycholic acid gave a slight advantage with respect to pathohistological properties probably due to better penetration of mebendazole in tissues. The verification of these preliminary results is ongoing with pretreatment (treatment before tumor inoculation), higher doses (30-50 % LD_{50}), dose dependency evaluation and a longer treatment period on larger groups of experimental animals.

So far, no clinical trials on the anticancer effects of mebendazole have been completed [1]. Regarding tumour control with mebendazole therapy, only one case report of adrenocortical cancer [16] and one case report of colon cancer [14] can be found in the literature.

Future clinical trials will elucidate whether mebendazole has the potential to become an adjuvant to current antitumor and especially antisarcoma, therapies.

CONCLUSION

Since non-toxic mebendazole p.o. doses, given post-BHK21/C13 cell inoculation, inhibits sarcoma cell proliferation in hamsters, mebendazole may be a safe novel candidate for adjuvant human anti-cancer therapy. However, further investigations are required to ascertain its usefulness in a clinical setting.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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