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Cytostatic effect of ricobendazole on primary cultures of soft tissue sarcomas *in vitro*

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ABSTRACT

Soft tissue sarcomas (STS) are often resistant to treatment. The search for new antitumor compounds against STS remains an urgent task.

Purpose of the study. To assess the sensitivity of primary STS cultures of various histological subtypes to albendazole sulfoxide (ricobendazole) and doxorubicin, the primary metabolite of albendazole.

Materials and methods. STS tumor samples were used. The enzymatic dissociation method was used using 300 units/ml collagenase I (Thermo Fisher Scientific, USA). Ricasol® (NITA-PHARM, Russia) and Doxorubicin-LENS® (VEROPHARM, Russia) were used as test substances. Sensitivity to ricobendazole and doxorubicin was tested using the MTT test. The cultures were seeded in a 96-well plate at 7,000 cells in DMEM medium with 10 % FSC added. After 24 h, the medium was replaced with PPS with ricobendazole in a series of two-fold dilutions from 35.5 μmol/l to 0.0347 μmol/l or doxorubicin from 10 μmol/l to 0.009 μmol/l. After 72 hours of incubation, the MTT test was performed. The cells were seeded in a 24-well plate and cultured in PPS with 2 μmol/l ricabendazole for 72 h. Hoechst 33342 dye (Life Technologies, USA) was added to the culture at a concentration of 1 μg/ml, and photographs were taken using a LionHeart FX digital automatic microscope (BioTek Instruments Inc., USA).

Results. Four primary sarcoma cultures were obtained: SAR-1, SAR-2, SAR-3, and SAR-4. SAR-1 and SAR-4. Cultures demonstrated the most rapid growth, with doubling times of 38 and 27 hours, respectively. The slowest proliferation was observed in the SAR-2 culture (doubling time 156 hours), while SAR-3 showed a doubling time of 45 hours. According to the MTT assay, the IC_{50} values for ricobendazole were 4.54 ± 1.2 μmol/L for SAR-1, 3.31 ± 0.7 μmol/L for SAR-3, and 1.51 ± 0.2 μmol/L for SAR-4, whereas the slowly dividing SAR-2 culture proved to be insensitive to ricobendazole. The cytostatic activity of doxorubicin was higher than that of ricobendazole. The SAR-2 culture was the least sensitive (IC_{50} could not be determined), and SAR-4 (IC_{50} SAR-4 = 0.16 ± 0.01 μmol/l) was the most sensitive to the action of doxorubicin. The IC_{50} value of SAR-1 = 0.64 ± 0.02 μmol/l and IC_{50} SAR-3 = 1.8 ± 0.1 μmol/l. The effect of ricobendazole caused pronounced disturbances in the nuclei of SAR-1 and SAR-4 cultures, in SAR-2 and SAR-3 they were less pronounced.

Conclusion. Ricobenzale had a cytostatic effect on primary STS cultures characterized by rapid cell growth, but the activity was lower than that of doxorubicin. Changes in the morphology of cells and nuclei indicated probable disturbances in the functioning of the spindle and cytoskeleton occurring under the action of this compound. Of particular interest for further research is the combination of ricobendazole with taxanes and other tubulin inhibitors

Keywords: primary cell culture, soft tissue sarcoma, albendazole sulfoxide, chemotherapy, ricobendazole

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Compliance with ethical standards: patients were informed about their participation in the scientific study and provided written informed consent for the collection of biological material. The study was approved by the Local Ethics Committee of the National Medical Research Center for Oncology (Protocol No. 6/1 dated February 10, 2020).

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ОРИГИНАЛЬНАЯ СТАТЬЯ

Цитостатическое действие рикобензадола на первичные культуры сарком мягких тканей *in vitro*

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РЕЗЮМЕ

Саркомы мягких тканей (СМТ) часто резистентны к лечению. Поиск новых противоопухолевых соединений в отношении СМТ остается актуальной задачей.

Цель исследования. Изучить чувствительность к основному метаболиту альбендазола сульфоксида (рикобендазол) и доксорубицину первичных культур СМТ различных гистологических подтипов.

Материалы и методы. Первичные культуры были получены из образцов СМТ, полученных от ранее не леченых пациентов в ходе хирургического удаления опухоли. В качестве исследуемых веществ использовали Риказол® (НИТА-ФАРМ, Россия) и Доксорубицин-ЛЭНС® (ВЕРОФАРМ, Россия). Чувствительность к рикобендазолу и доксорубицину проверяли с использованием МТТ-теста после культивирования с рикобендазолом в серии двукратных разведений от 35,5 мкмоль/л до 0,0347 мкмоль/л или доксорубицином от 10 мкмоль/л до 0,009 мкмоль/л в течение 72 ч. Для изучения морфологических изменений клеточных ядер клетки культивировали с 2 мкмоль/л рикабендазола в течение 72 ч, после чего проводили окрашивание 1 мкг/мл Hoechst 33342 (Life Technologies, США), и фотографировали в цифровом автоматическом микроскопе LionHeart FX (BioTek Instruments Inc., США).

Результаты. Получены 4 первичные культуры сарком. Культуры SAR-1 и SAR-4 характеризовались наиболее быстрым ростом (время удвоения 38 и 27 ч соответственно). Наиболее медленным ростом характеризовалась культура SAR-2 (время удвоения 156 ч), в культуре SAR-3 время удвоения составило 45 ч. По данным МТТ-теста для рикобендазола для SAR-1 IC_{50}–4,54 ± 1,2 мкмоль/л, для SAR-3–3,31 ± 0,7 мкмоль/л и для SAR-4–1,51 ± 0,2 мкмоль/л соответственно, медленно делящаяся SAR-2 оказалась нечувствительной к рикобендазолу. Цитостатическая активность доксорубицина была выше, чем у рикобендазола. Культура SAR-2 была наименее чувствительной (IC_{50} определить не удалось), а SAR-4 (IC_{50} SAR-4 = 0,16 ± 0,01 мкмоль/л) наиболее чувствительной к действию доксорубицина. Значение IC_{50} SAR-1 = 0,64±0,02 мкмоль/л и IC_{50} SAR-3 = 1,8 ± 0,1 мкмоль/л. Воздействие рикобендазола вызвало выраженные нарушения в ядрах в культурах SAR-1 и SAR-4, в SAR-2 и SAR-3 они были менее выражены.

Заключение. Рикобендазол оказал цитостатическое действие на первичные культуры СМТ, характеризующиеся быстрым клеточным ростом, но активность была ниже, чем у доксорубицина. Изменения в морфологии клеток и ядер свидетельствовали о вероятных нарушениях в работе веретена деления и цитоскелета, происходящих под действием данного соединения. Особенный интерес для дальнейшего исследования представляет комбинирование рикобендазола с таксанами и другими ингибиторами тубулинов.

Ключевые слова: первичная клеточная культура, саркома мягких тканей, сульфоксид альбендазола, химиотерапия, рикобендазол

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BACKGROUND

Soft tissue sarcomas (STS) are rare malignant tumors of mesenchymal origin, accounting for approximately 1 % of all human malignancies. In Russia, about 3,000–3,500 new STS cases are diagnosed annually, with a prevalence of 22.1 cases per 100,000 population [1]. STS are characterized by aggressive behavior, rapid recurrence, early dissemination, and high resistance to treatment. Doxorubicin remains the standard first-line chemotherapeutic drug for advanced and unresectable STS; however, the response rate is only around 15 %.

Thus, the search for new compounds with antitumor activity against STS remains an important task. One promising direction is the exploration of therapeutic approaches already tested in other oncologic diseases. Nitrogen-containing heterocyclic compounds – benzimidazole derivatives – are considered a promising basis for anticancer agents [2, 3]. In addition to synthesizing new compounds, repurposing approved benzimidazole-based agents is of great interest, since their safety profile and pharmacological properties are already known, potentially accelerating their introduction as anticancer agents. Some benzimidazole-based anthelmintic drugs used in veterinary medicine exhibit antitumor activity in animals; among them, albendazole and mebendazole are also approved for treating parasitic infections in humans [4]. These compounds were later shown to have cytostatic activity and selectivity toward human cancer cells. Albendazole, for example, suppresses tumor cell growth *in vitro* and *in vivo* through induction of oxidative stress and inhibition of β-tubulin polymerization, leading to impaired glucose uptake, metabolic starvation, cell-cycle arrest, and apoptosis in malignant cell cultures, while its inhibitory effect on normal cells is less pronounced [5]. Currently, several clinical trials of albendazole and mebendazole in various cancers are ongoing, although the number of enrolled patients remains limited [4]. Researchers note relatively good tolerability, with only occasional symptoms of myelosuppression. In isolated cases of patients receiving mebendazole, a clinical effect manifested as disease stabilization has been observed [5].

Several studies have evaluated the antitumor properties of benzimidazole derivatives in sarcoma cell cultures. Michaelis M. et al. demonstrated high sensitivity of Ewing sarcoma cell lines to flubendazole, with relatively low sensitivity observed in osteosarcoma and rhabdomyosarcoma cultures; other sarcoma histotypes were not investigated [6]. There is also evidence of successful use of albendazole to enhance the antitumor effect of doxorubicin when incorporated into composite nanoparticles against osteosarcoma cell lines [7].

Purpose of the study: to assess the sensitivity of primary STS cultures of various histological subtypes to albendazole sulfoxide (ricobendazole) and doxorubicin , the primary metabolite of albendazole.

MATERIALS AND METHODS

Primary cultures of sarcomas of various histological subtypes, obtained intraoperatively in the Department of Bone, Skin, Soft Tissue, and Breast Tumors of the National Medical Research Center for Oncology (Ministry of Health of the Russian Federation) in 2023–2024, served as the material for this study. The histological diagnosis was confirmed in the Department of Pathology of the same institution. The study included treatment-naive patients with soft tissue sarcomas. Exclusion criteria comprised prior chemoradiotherapy for soft tissue sarcomas as well as the presence of blood-borne infectious diseases.

Tumor samples were transferred from the operating room in Hank's Balanced Salt Solution (HBSS, Gibco, USA) supplemented with 1 % penicillin–streptomycin (Biolot, Russia) at +4–8 °C and delivered to the Laboratory of Cell Technologies within 20 minutes after surgical excision. The tissue was minced with a scalpel into fragments of 1–2 mm³ and placed into a culture tube containing DMEM medium (Gibco, USA) with 1 % gentamicin (Biolot, Russia) and collagenase type I (300 U/mL; Thermo Fisher Scientific, USA). The material was incubated for 2 hours at 37 °C on a shaker.

The resulting cell suspension was passed through a sterile nylon filter (70 μm; Becton Dickinson, USA)

and washed twice with DMEM (Gibco, USA). Cell counting and viability assessment were performed in a Goryaev chamber using 0.4 % trypan blue solution (Biolot, Russia). Primary sarcoma cultures were maintained in complete growth medium based on DMEM (Gibco, USA) supplemented with 10 % FBS (HyClone, USA), 1 % insulin–transferrin–selenium (Biolot, Russia), 1 % NEAA (Gibco, USA), and 1 % gentamicin (Biolot, Russia). At each passage, cells were counted and the doubling time was calculated using the formula $DT = t \times ln(2) / ln(n_1/n_2)$, where DT is doubling time, t is the time interval between two measurements, and n_1 and n_2 are cell numbers at the first and second time points, respectively.

Before conducting the main experiments, the presence of malignant cells in the cultures was confirmed in the Pathology Department of the NMRC for oncology, using standard cytological examination with azure–eosin staining according to the Romanowsky-Giemsa method.

Assessment of the Cytostatic Properties of Ricobendazole and Doxorubicin

As stock solutions of the test compounds, we used the anthelmintic drug Rikazol® (NITA-PHARM, Russia) (ricobendazole, albendazole-sulphoxide, 100 mg/mL) and the antitumor drug Doxorubicin-LENS® (VEROPHARM, Russia) (50 mg/25 mL). The sensitivity of primary sarcoma cultures to ricobendazole and doxorubicin was assessed by constructing dose–response curves using indirect quantification of viable cells via the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide). Cells were seeded into 96-well plates at 7,000 cells per well in complete growth medium. After 24 hours, the culture medium was replaced with medium containing ricobendazole in a series of two-fold dilutions ranging from 35.5 μmol/L to 0.0347 μmol/L, or doxorubicin in a two-fold dilution series from 10 μmol/L to 0.009 μmol/L. The concentration range for ricobendazole was selected to include previously reported IC50 values for the structurally related compound flubendazole obtained for a broad panel of malignant cell lines in the study by Michaelis M. et al. (2015) [6]. Plates were incubated for 72 hours, after which the MTT assay was performed according to the standard protocol [8]. Cell viability was determined as the optical density measured at 570 nm in treated wells relative to control wells, expressed as a percentage. Each experimental condition was assessed in 10 technical replicates, and the experiment was repeated in three biological replicates. Viability values are presented as mean ± SD. Data processing and graph construction were performed in Microsoft Excel. The half-maximal inhibitory concentration (IC50) was determined using the drc package in the R programming language [9]. For curve fitting, a three-parameter logistic model with a fixed lower limit (c = 0) was applied, without imposing constraints on the estimated parameters.

$$y = \frac{d}{(1+exp\,(b(log\,(x)-log\,(e))))},$$

where b – is the slope, d – is the upper limit, and e – is the half-maximal effective dose. In cases where hormesis was observed, data points lying above the upper asymptote were excluded from model fitting. IC_{50} values are presented as the mean ± 95 % confidence interval.

Analysis of Morphological Features of Cells and Nuclei

Cells of the investigated cultures were seeded into 24-well plates and cultured in complete medium supplemented with 2 μmol/L ricobendazole under standard conditions for 72 hours. A single concentration of ricobendazole was chosen to allow direct comparison of culture sensitivity to the compound. Hoechst 33342 dye (Life Technologies, USA) was then added to the cultures at a final concentration of 1 μg/mL, followed by a 20-minute incubation under standard conditions. Subsequently, the cultures were imaged using a LionHeart FX automated digital microscope (BioTek Instruments Inc., USA).

STUDY RESULTS

Four primary soft tissue sarcoma cultures of different histological subtypes were obtained and demonstrated varying doubling times (Table 1). At the time of the experiment, cultures SAR-1 and SAR-4 exhibited the most rapid growth (doubling

times of 38 and 27 hours, respectively). The slowest growth rate was observed in SAR-2 (doubling time 156 hours), while SAR-3 had a doubling time of 45 hours. Consequently, the number of passages completed prior to the experiment differed between cultures, with the faster-growing cultures reaching later passages by the start of testing (Table 1).

The assessment of ricobendazole's impact on the viability of primary soft tissue sarcoma cultures demonstrated that the slow-growing pleomorphic rhabdomyosarcoma culture SAR-2 was insensitive to the compound. In contrast, the remaining three cultures showed a pronounced decrease in viability in response to increasing concentrations of ricobendazole (Fig. 1A). Among them, the undifferentiated pleomorphic sarcoma culture SAR-4 exhibited the lowest half-maximal inhibitory concentration (IC_{50} SAR-4 = 1.51 ± 0.2 μmol/L) and a clear hormesis effect – an increase in cell viability relative to the untreated control under low concentrations of the toxicant. The IC_{50} values for the other two cultures were IC_{50} SAR-1 = 4.54 ± 1.2 μmol/L and IC_{50} SAR-3 = 3.31 ± 0.7 μmol/L.

The cytostatic activity of doxorubicin in the studied cultures was higher than that of ricobendazole; however, the overall response patterns were similar for both compounds. The SAR-2 culture was the least sensitive (IC_{50} could not be determined within the tested concentration range), whereas SAR-4 was the most sensitive to doxorubicin (IC_{50} SAR-4 = 0.16 ± 0.01 μmol/L). The half-maximal inhibitory concentrations for the remaining two cultures were IC_{50} SAR-1 = 0.64 ± 0.02 μmol/L and IC_{50} SAR-3 = 1.8 ± 0.1 μmol/L (Fig. 1B).

Table 1. Characteristics of primary soft-tissue sarcoma cultures

№	Culture ID	Histological diagnosis	Treatment	Passage at the time of experiment	Doubling time at the time of experiment, h
1	SAR-1	Epithelioid sarcoma	Surgical treatment only	Passage 5	38
2	SAR-2	Pleomorphic rhabdomyosarcoma	Surgical treatment only	Passage 3	156
3	SAR-3	Spindle cell/synovial sarcoma	Surgical treatment only	Passage 5	45
4	SAR-4	Undifferentiated pleomorphic sarcoma with infiltrative growth and areas of high-grade extraosseous osteosarcoma	Surgical treatment only	Passage 7	27

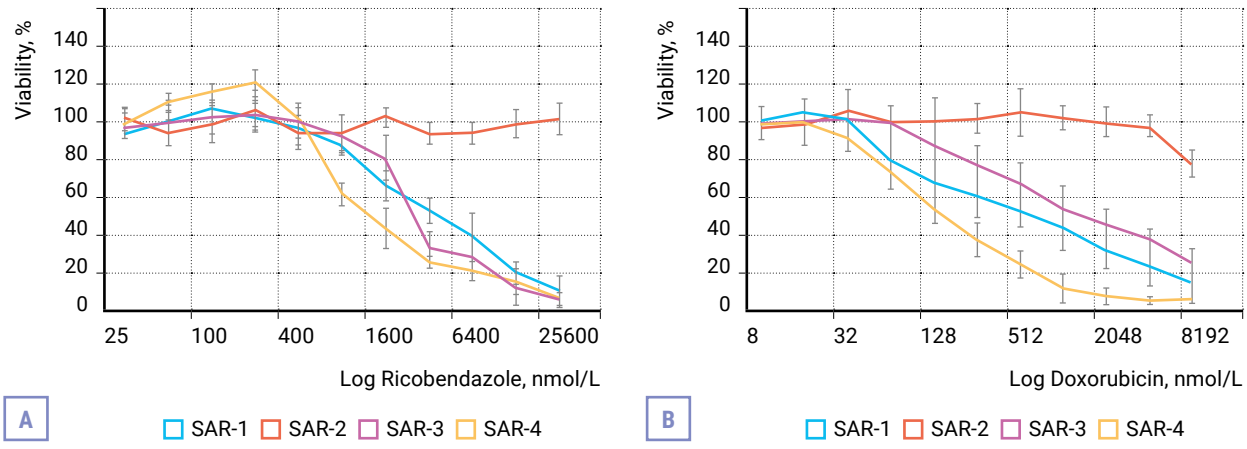

Fig. 1. Cytostatic activity of ricobendazole and doxorubicin against primary sarcoma cultures. A – dose–response curve for ricobendazole; B – dose–response curve for doxorubicin.

A pairwise comparison of the two compounds indicates that in SAR-1 and SAR-4 cultures, the cytostatic activity of ricobendazole was, on average, an order of magnitude lower than that of doxorubicin. In the SAR-3 culture, the difference was less pronounced: the IC_{50} for doxorubicin was only approximately twofold lower than the corresponding value for ricobendazole.

Without exposure to the tested compound, cells of SAR-1 and SAR-2 cultures exhibited an elongated, spindle-shaped morphology (Fig. 2A, C), whereas the SAR-3 and SAR-4 cultures consisted predominantly of cells with an epithelioid appearance (Fig. 2D, G). Cultivation in the presence of 2 μmol/L ricobendazole induced changes in monolayer confluence, cell morphology and the appearance of cytopathic features of varying severity. In the SAR-1 culture, a marked reduction in confluence was observed, along with a decrease in the number of cytoplasmic processes and general compaction of cells (Fig. 2B). A characteristic feature of treated samples was the noticeable increase in the number of rounded cells in metaphase (visible metaphase plate) (Fig. 2B, black arrows). In the SAR-2 culture, ricobendazole exposure induced changes in cell shape – similar to SAR-1, the cells became more compact – however, overall monolayer confluence did not undergo substantial alterations, and no pronounced cytopathic features were observed (Fig. 2B). In the SAR-3 culture, in contrast to the other cultures, a sharp shift in the growth pattern was documented: instead of forming a two-dimensional monolayer, the cells began forming distinct three-dimensional spheroid-like colonies attached to the well bottom (Fig. 2F). Finally, in the SAR-4 culture, in addition to reduced monolayer confluence, all cells demonstrated increased cytoplasmic granularity, a higher number of cells in metaphase (Fig. 2H, black arrows), and an increased proportion of multinucleated cells (Fig. 2H, white arrows).

Ricobendazole exposure induced pronounced nuclear abnormalities in the SAR-1 and SAR-4 cultures. In both cultures, apoptotic features were observed, including hyperchromatic fragmented nuclei (Fig. 3B, H, red arrows).

Alongside these changes, multinucleated cells with polymorphic nuclei and micronuclei were observed in these cultures. Unlike the fragmented nuclei characteristic of late-stage apoptosis, these structures were stained with an intensity typical of normal nuclei, displayed normal chromatin architecture, and had a smooth, rounded shape (Fig. 3B, H, blue arrows). In the SAR-2 and SAR-3 cultures, such features were not detected (Fig. 3D, F).

DISCUSSION

The half-maximal inhibitory concentration values obtained in our study for three of the four primary

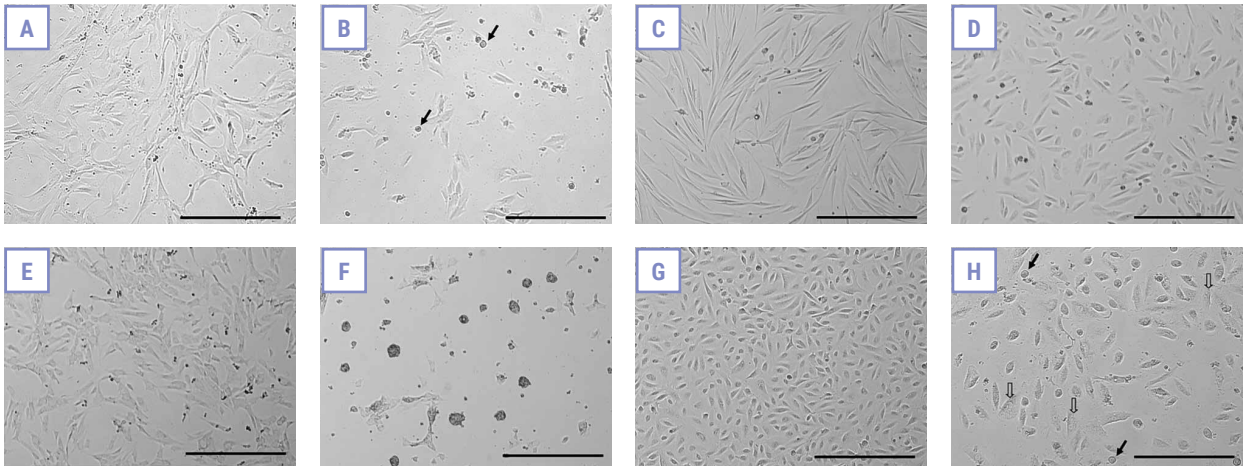

Fig. 2. Effect of ricobendazole at 2 μmol/L on primary sarcoma cultures. Exposure time: 72 h. Objective magnification ×5. A – SAR-1 culture, control; B – SAR-1 culture, ricobendazole; C – SAR-2 culture, control; D – SAR-2 culture, ricobendazole; E – SAR-3 culture, control; F – SAR-3 culture, ricobendazole; G – SAR-4 culture, control; H – SAR-4 culture, ricobendazole.
Annotations: black arrows – cells in metaphase; white arrows – multinucleated cells. Scale bar: 400 μm.

sarcoma cultures ranged from 1.5 to 4.5 μmol/L. According to available data, the maximal blood concentration of albendazole sulphoxide after oral administration of albendazole at a dose of 400 mg (6–8 mg/kg) is 0.16–1.58 mg/L, which corresponds to 0.6–6 μmol/L [10]. Thus, an antitumor effect of ricobendazole in humans may theoretically be achievable at doses considered safe and commonly used for helminthiasis treatment [4].

Based on our findings, it may be hypothesized that sensitivity to ricobendazole, as well as to doxorubicin, is likely more dependent on the proliferation index than on the histotype of soft tissue sarcoma cultures.The observed alterations in cell and nuclear morphology suggest that the target of ricobendazole in primary sarcoma cultures may be the microtubules of the mitotic spindle and cytoskeleton. The action of the compound likely leads to mitotic arrest at metaphase and to unequal segregation of chromosomes, giving rise to micronuclei or mitotic catastrophe and subsequent cell death. Disturbances of the cytoskeleton may also be reflected in the altered cell shape seen across cultures and the shift in growth behavior in the spindle-cell/synovial sarcoma SAR-3 culture – from a two-dimensional monolayer to three-dimensional spheroid-like structures. β-Tubulin has been indicated as a target for benzimidazole derivatives in the literature [5]. The hypothesis that ricobendazole exerts its effect through the cell division machinery is consistent with the observation that the slowly proliferating SAR-2 pleomorphic rhabdomyosarcoma culture was insensitive to the cytostatic action of this compound. However, additional studies are required to verify this hypothesis, as the weak impact on slowly dividing cells is a common feature of all cytostatic agents, regardless of their mechanism of action. For example, doxorubicin, to which SAR-2 was likewise insensitive, inhibits DNA synthesis in proliferating cells without targeting the cell division apparatus.

Tubulin inhibitors such as paclitaxel and vincristine have been well-established anticancer agents since the 1960s. Paclitaxel stabilizes microtubules by shifting the equilibrium toward polymer formation and thereby lowering the critical tubulin concentration, disrupting normal microtubule dynamics essential for spindle function and intracellular transport. Vincristine, in contrast, is a microtubule-destabilizing agent that binds specifically to microtubule plus-ends, preventing polymerization and impairing mitotic spindle assembly and function [11]. Although highly effective, these tubulin inhibitors lack sufficient selectivity for tumor cells, which has stimulated the search for novel agents targeting tubulin isotypes overexpressed in malignant tumors [12, 13]. The β3-tubulin (TUBB3) isotype is most strongly as-

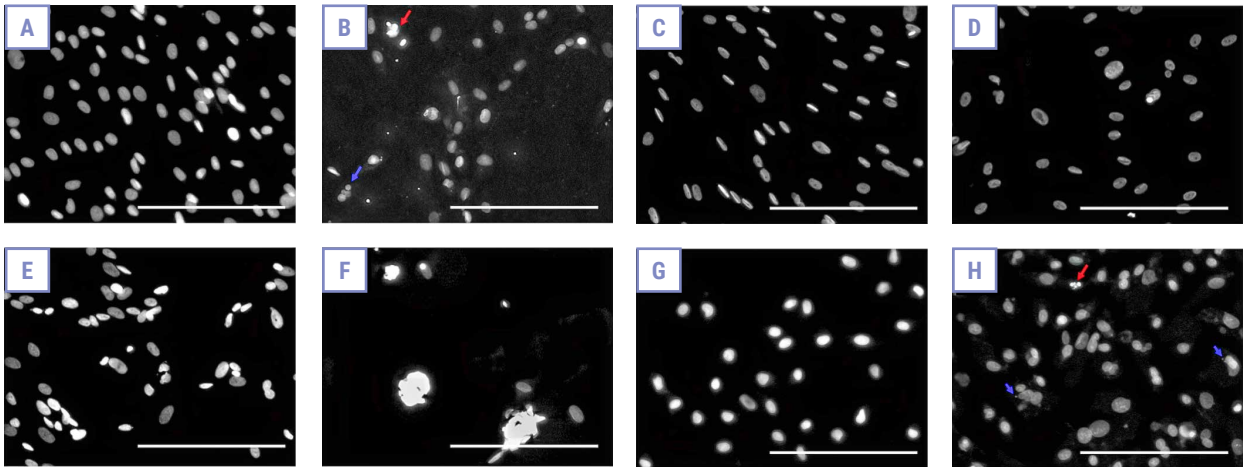

Fig. 3. Effect of ricobendazole at 2 μmol/L on nuclear morphology in primary sarcoma cultures. Exposure: 72 h. Hoechst 33342 staining. Objective magnification ×10. A – SAR-1 culture, control; B – SAR-1 culture, ricobendazole; C – SAR-2 culture, control; D – SAR-2 culture, ricobendazole; E – SAR-3 culture, control; F – SAR-3 culture, ricobendazole; G – SAR-4 culture, control; H – SAR-4 culture, ricobendazole.
Annotations: red arrows – nuclei with apoptotic features; blue arrows – micronuclei. Scale bar: 200 μm.

sociated with tumor progression, metastasis, and chemoresistance [14]. Notably, β3-tubulin overexpression correlates with resistance to eribulin in leiomyosarcoma cells [15]. Its critical role in sarcoma oncogenesis is further underscored by the sensitivity of these tumors to the β3-tubulin inhibitor plocabulin [16]. Current evidence suggests that the pro-oncogenic properties of β3-tubulin are driven by multiple mechanisms. β3-Tubulin-associated activation of the transcription factors Snail and ZEB1 may trigger epithelial-mesenchymal transition [17]. Resistance to taxanes may be linked to the increased dynamic instability of β3-tubulin-containing microtubules, conferring reduced susceptibility to microtubule-stabilizing agents [18]. In addition, β3-tubulin may protect cells from endoplasmic reticulum stress and reactive oxygen species-induced stress, thereby promoting survival during chemotherapy exposure [14, 19]. Possible therapeutic applications of ricobendazole in sarcomas via its potential interaction with β3-tubulin remain an important subject for future investigation.

CONCLUSION

Ricobendazole exerted a pronounced cytostatic effect on primary soft tissue sarcoma cultures characterized by rapid proliferation; however, its activity was lower than that of doxorubicin. Particularly promising is the potential for combining ricobendazole with taxanes and other tubulin-targeting agents in future studies.

References

1. Shakhzadova AO, Starinsky VV, Lisichnikova IV. Cancer care to the population of Russia in 2022. Siberian Journal of Oncology. 2023;22(5):5–13. (In Russ.). https://doi.org/10.21294/1814-4861-2023-22-5-5-13
2. Komarova EF, Morkovnik AS, Zhukovskaya ON, Verenikina EV, Shevchenko NA, Khodakova DV, et al. A benzimidazole derivative as an effective antitumor agent in terms of syngeneic lung tumors and melanoma treatment. South Russian Journal of Cancer. 2022;3(1):15–21. https://doi.org/10.37748/2686-9039-2022-3-1-2
3. Kit OI, Komarova EF, Verenikina EV, Maksimov AYu, Morkovnik AS, Zhukovskaya ON, et al. Evaluation of antitumor activity of benzimidazole derivative on models of experimental tumors. Yakut Medical Journal 2022;1(77):23–26. (In Russ.). https://doi.org/10.25789/ymj.2022.77.06
4. Chai JY, Jung BK, Hong SJ. Albendazole and Mebendazole as Anti-Parasitic and Anti-Cancer Agents: an Update. Korean J Parasitol. 2021 Jun;59(3):189–225. https://doi.org/10.3347/kjp.2021.59.3.189
5. Son DS, Lee ES, Adunyah SE. The Antitumor Potentials of Benzimidazole Anthelmintics as Repurposing Drugs. Immune Netw. 2020 Aug 4;20(4):e29. https://doi.org/10.4110/in.2020.20.e29
6. Michaelis M, Agha B, Rothweiler F, Löschmann N, Voges Y, Mittelbronn M, et al. Identification of flubendazole as potential anti-neuroblastoma compound in a large cell line screen. Sci Rep. 2015 Feb 3;5:8202. https://doi.org/10.1038/srep08202
7. Zhao TT, Zhou TJ, Zhang C, Liu YX, Wang WJ, Li C, et al. Hypoxia inhibitor combined with chemotherapeutic agents for antitumor and antimetastatic efficacy against osteosarcoma. MolPharm.2023; May1;20(5):2612–2623. https://doi.org/10.1021/acs.molpharmaceut.3c00068
8. van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. Methods Mol Biol. 2011;731:237–245. https://doi.org/10.1007/978-1-61779-080-5_20
9. Ritz C, Baty F, Streibig JC, Gerhard D. Dose-Response Analysis Using R. PLoS One. 2015 Dec 30;10(12):e0146021 https://doi.org/10.1371/journal.pone.0146021
10. Dayan AD. Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. Acta Trop. 2003 May;86(2-3):141–159. https://doi.org/10.1016/s0001-706x(03)00031-7
11. Legátová A, Pelantová M, Rösel D, Brábek J, Škarková A. The emerging role of microtubules in invasion plasticity. Front Oncol. 2023 Feb 13;13:1118171. https://doi.org/10.3389/fonc.2023.1118171
12. Galmarini CM, Martin M, Bouchet BP, Guillen-Navarro MJ, Martínez-Diez M, Martinez-Leal JF, Akhmanova A, Aviles P. Plocabulin, a novel tubulin-binding agent, inhibits angiogenesis by modulation of microtubule dynamics in endothelial cells. BMC Cancer. 2018 Feb 7;18(1):164. https://doi.org/10.1186/s12885-018-4086-2

13. Janke C, Magiera MM. The tubulin code and its role in controlling microtubule properties and functions. Nat Rev Mol Cell Biol. 2020 Jun;21(6):307–326. https://doi.org/10.1038/s41580-020-0214-3
14. Kanakkanthara A, Miller JH. βIII-tubulin overexpression in cancer: Causes, consequences, and potential therapies. Biochim Biophys Acta Rev Cancer. 2021 Dec;1876(2):188607. https://doi.org/10.1016/j.bbcan.2021.188607
15. Yahiro K, Matsumoto Y, Fukushi JI, Kawaguchi KI, Endo M, Setsu N, et al. Class III β-Tubulin Overexpression Induces Chemoresistance to Eribulin in a Leiomyosarcoma Cell Line. Anal Cell Pathol (Amst). 2018 Jun 21;2018:8987568. https://doi.org/10.1155/2018/8987568
16. Wang Y, Wozniak A, Cornillie J, Avilés P, Debiec-Rychter M, Sciot R, Schöffski P. Plocabulin, a Novel Tubulin Inhibitor, Has Potent Antitumour Activity in Patient-Derived Xenograft Models of Soft Tissue Sarcoma. Int J Mol Sci. 2022 Jul 5;23(13):7454. https://doi.org/10.3390/ijms23137454
17. Sobierajska K, Wieczorek K, Ciszewski WM, Sacewicz-Hofman I, Wawro ME, Wiktorska M, et al. β-III tubulin modulates the behavior of Snail overexpressed during the epithelial-to-mesenchymal transition in colon cancer cells. Biochim Biophys Acta. 2016 Sep;1863(9):222–2233. https://doi.org/10.1016/j.bbamcr.2016.05.008
18. Kamath K, Wilson L, Cabral F, Jordan MA. BetaIII-tubulin induces paclitaxel resistance in association with reduced effects on microtubule dynamic instability. J Biol Chem. 2005 Apr 1;280(13):12902–12997. https://doi.org/10.1074/jbc.m414477200
19. Parker AL, Turner N, McCarroll JA, Kavallaris M. βIII-Tubulin alters glucose metabolism and stress response signaling to promote cell survival and proliferation in glucose-starved non-small cell lung cancer cells. Carcinogenesis. 2016 Aug;37(8):787–798. https://doi.org/10.1093/carcin/bgw058

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