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Research Article

Evaluation of the pharmacological activity of hybrid organotin compounds in a B16 melanoma model in the classical and metronomic administration modes

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Abstract

Introduction: In modern medical chemistry, much attention is paid to the search for new antimetastatic agents based on metal compounds. Organotin compounds promise to be good candidates as the treatment of malignant neoplasms. In order to reduce a possible nonspecific toxic effect of tin compounds and to expand the intended therapeutic use, the paper presents hybrid tin (IV) complexes with Sn-S bond containing a fragment of 2,6-di-tert-butylphenol. The aim of the study was to evaluate the antitumor and antimetastatic effects of bis (3,5-di-tert-butyl-4-hydroxyphenylthiolate) dimethylolol (Me3) and (3,5-di-tert-butyl-4-hydroxyphenylthiolate) triphenylolol (Me5) in a model of transplanted melanoma tumor in B16 mice in classical and metronomic administration mode.

Materials and methods: The efficacy of organotin compounds was studied in a model of a transplanted tumor with spontaneous metastasis of C57Bl/6 (female) melanoma B16 mice using the following indicators: average life expectancy, inhibition of tumor growth by weight, tumor mass, and metastasis inhibition index.

Results and discussion: The most pronounced antimetastatic effect (54% and 36%) is achieved with a five-fold intraperitoneal injection of Me3 and Me5 at the total doses of 375 mg/kg and 250 mg/kg. The comparable results of the efficacy were obtained in the classical and metronomic modes of the injection of hybrid organotin compounds. With an increase in the injected dose, there is an effect of activating the tumor process with the generalized metastasis.

Conclusion: Bis dimethylolol (Me3) and triphenylolol (Me5) compounds demonstrate both a pronounced antimetastatic activity and a multidirectional effect on the growth of the primary focus and the metastasis in lungs, depending on an injected dose.

Keywords

organotin compounds, melanoma B16, 2,6-di-tert-butylphenol, antitumorigenic activity, metastasis inhibition, metronomic drug administration.

Introduction

Antitumor drugs are among the most popular groups of drugs. Nowadays there are more than 120 names of antitumor substances (The Russian Ministry of Health 2021), which are widely used both in monochemotherapy and combined treatment of malignant neoplasms in Russia. However, the efficacy of most of them is limited by excessive toxicity and the development of resistance during a course administration. So the issue of developing new antitumor drugs with an optimal activity-toxicity ratio and a multifactorial effect remains relevant. This is why, an active search for new organometallic compounds, highly targeted, with minimal toxicity to the body and maximum cytotoxicity to the tumor is still under way. In this research, while creating physiologically active metal compounds with an alleged antitumor activity, we suggest modifying the molecule in order to reduce the overall nonspecific toxicity to non-tumor cells, which is achieved by adding protective organic groups.

One of the most promising directions in this sphere is the study of organic compounds based on tin Sn(IV). Organotin compounds have unique characteristics, such as catalytic and redox abilities, a tendency to exchange ligands and a variety of available interactions with biological targets. Organotin compounds have showed a pronounced antitumor effect in various models *in vitro* and *in vivo* (Banti et al. 2019). There are several antiproliferative mechanisms of organotin compounds leading to the induction of apoptosis (Syed Annuar et al. 2021). Various studies show that organotin compounds affect the macromolecules of the cell (DNA or proteins), as well as the energy of the cell and the functions of mitochondria; they interact with cell membranes and increase the concentration of Ca^{2+} in the cytoplasm. Apoptosis is caused either by the influence of organotin compounds on the redox signaling pathways of cells (accumulation of active oxygen metabolites (AOM)), or by the permeability violation of mitochondrial membranes, activation of caspases or by interaction with DNA, and a decrease in the production of anti-apoptotic protein Bcl-2 (Zhang et al. 2016; Giuliano et al. 2021).

Organic ligands in the molecule contribute to a significant modulation of the organotin compounds biological effect on the cell. It is known that the presence of one or more covalent C-Sn bonds affects the activity of the compound and depends on the number and nature of alkyl substituents (R) associated with the Sn-center. The effect of RnSnX4-n compounds containing various R groups (R = Me, Et, Pr and Bu), as shown by a meta-analysis, depends on the type and number of R groups, and the biological activity decreases in the following series: n-Bu > Ph and Et > Me (Ghani and Yousif 2021). The chemical structure also explains the selectivity of the pharmacological action on various test systems *in vitro*. For example, Sn(IV) carboxylates based on ortho- or para-hydroxybenzoic acids showed a high cytotoxic activity in relation to sarcoma cancer cells in studying the cytotoxic activity *in vitro* (Balas et al. 2011). Tributyl- and triphenylol derivatives with 2-thiobarbituric acid demonstrated a higher cytotoxic activity than cisplatin in relation to human breast adenocarcinoma cells (MCF-7), and their IC50 values are 272 and 179 times lower than those for cisplatin (Balas et al. 2008).

As antitumor and antimetastatic agents, we have proposed hybrid organotin compounds, which have two components of the molecule: cytotoxic, containing tin (Sn, IV) and a protective complex radical 2,6-di-tret-butylphenyl. The combination of dual properties within a single molecule makes up the uniqueness of this structure. The protective effect of the 2,6-di-tret-butylphenyl radical has been proven by us in the previous studies, in which we have noticed a decrease in the indicators of general toxicity compared to analogous compounds of a simpler structure (Dodokhova et al. 2021a). A fragment of 2,6-di-tret-butylphenol definitely has antioxidant properties, which can contribute an additional mechanism to a biological effect at the level of both the primary focus and the metastasis process. Literature data on the effect of antioxidants of various chemical structures on the growth of malignant neoplasms and the activity of metastatic lesions are very contradictory: from complete or partial inhibition to activation of tumor growth and development at all stages, including the process of malignant cell desymination (Gill et al. 2016; Assi 2017; Gacche 2021). In our opinion, the combination of an organotin base and an antioxidant ligand in one molecule could be used in chemotherapy malignant neoplasms.

Primary *in vitro* screening was performed for compounds of similar structure. The antiproliferative activity of Sn(IV) complexes containing a fragment of 2,6-di-tert-butylphenol with heterocyclic thioamides on breast adenocarcinoma cells (MCF7 cell line) and cervical carcinoma (HeLa) was studied. IC50 value for the $R_2Sn(MPMT)2$ (MPMT = 2-mercapto-4-methylpyrimidine, R = 3,5-di-tert-butyl-4-hydroxyphenyl) complex was 32 times lower than for cisplatin in relation to MCF-7 cells. The complexes showed significantly lower cytotoxic in the normal MRC-5 cell line than MCF-7 and HeLa tumor cell lines (Shpakovsky et al. 2012). The ability of complexes to reduce the content of SH groups in tubulin allows us to consider these compounds as potential antimitotic agents (Antonenko et al. 2018). It has been established that, in addition to antioxidant and detoxifying effects (Milaeva et al. 2006), phenols and polyphenols can independently exhibit selective toxicity against tumor cells, the data was obtained from *in vitro* experiments (Gaynutdinov et al. 2018). Synthetic monophenolic antioxidants activate autophagy and apoptotic processes in tumor cells (Menshchikova et al. 2018).

Currently, new regimes for the use of antitumor drugs related to metronomic chemotherapy are being actively developed (Houy and Grand 2018; Tryakin et al. 2018; Zhong et al. 2020). The fundamental difference between this type of chemotherapy is the regular use of cytotox-

ic drugs for a long period of time in doses significantly lower than the maximum tolerated dose (Fedyanin et al. 2016). The supposed multifactorial effect of the hybrid organotin compounds makes it possible to study them in the metronomic mode of injection.

The aim of the paper is to evaluate the antitumor and antimetastatic effects of hybrid organotin compounds – bis-(3,5-di-tert-butyl-4-hydroxyphenyl) dimethylolol (Me3) and (3,5-di-tert-butyl-4-hydroxyphenyl) triphenylolol (Me5) thiol containing a protective fragment of 2,6-di-tert-butylphenol in a model of a transplanted melanoma tumor in mice in the classical and metronomic administration mode.

Materials and methods

Investigated substances

The compounds of tin M3 and M5 (Fig. 1), we synthesized earlier (Mukhatova et al. 2013), were used as a pharmaceutical substance (PS).

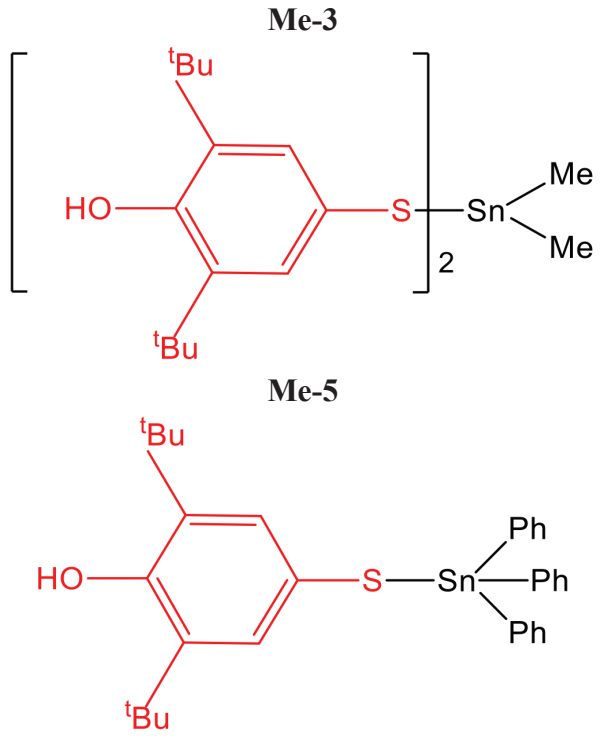

Figure 1. Structural formulas of organotin compounds Me3, Me5. **Note:** Designation of radicals: ᵗBu – tert-butyl, Me – methyl, Ph – phenyl.

The information about organotin compounds had been selected in a preliminary study (Dodokhova et al. 2021a) after determining the toxicity class and the average lethal dose (LD50) from the line of similar compounds by "fixed dose" and "up-and-down" methods according to the OECD protocols (OECD, 2001, 2008). The tested compounds were suspended in a 1% aqueous gelatin solution.

Animals

The animals were obtained from the breeding of the National Research Center Kurchatov Institute – Rappolovo Nursery. The studies were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 Sep 2010 "On the Protection of Animals Used for Scientific Purposes". The animals were kept in cages of 6 animals each, with free access to food and water; they were on a balanced diet corresponding to this type of animal. The ambient temperature was about 20–25 °C, with a relative humidity of 60–65%. Sawdust, which had undergone preliminary UV sterilization, was used as a litter. After the end of the quarantine isolation period (14 days), the animals (females of 8 weeks of age, weighing 21–22 g) were examined for the absence of external signs of diseases, standardized by sex, age and weight, and randomized using a random number table. Melanoma B16 with possible spontaneous metastasis to the lungs had been chosen as a model of the transplanted tumor for the study. The strain was obtained from the bank of tumor materials of the Research Institute of Experimental Diagnostics and Therapy of Tumors of the N.N. Blokhin Russian Oncology Research Center. The maintenance and inoculation of the tumor strain were carried out in accordance with the generally accepted methods in mice of the C57Bl/6 line, described in detail in (Kit et al. 2020). Tumor cells were inoculated subcutaneously into the right axillary region of each mouse with 50 mg of tumor suspension in saline solution at a dilution of 1:10. Forty-eight hours after transplanting melanoma B16 to the female mice of the C57B1/6 line, the studied compounds were injected intraperitoneally once per day for 5 days, according to the classical method used for screening the compounds with a suspected antitumor effect (Sofina et al. 1980).

Research design

At the first stage of the study, the injection of M3 and M5 was carried out in the classical mode in a wide range of doses; the animals from the control groups were injected with 1% aqueous gelatin solution in equivalent regimens and volumes. For both the substances, the single dose (SD) was from 30 to 100 mg/kg; the total dose (TD) was from 150 to 500 mg/kg. At the second stage of the study, with the metronomic administration mode, the SD of the studied compounds was 25 and 37.5 mg/kg, which was injected to the animals for 10 days, once per day, intraperitoneally' TD was 250 mg/kg and 375 mg/kg for Me3 and Me5, respectively. The most effective doses identified at the first stage of the study were investigated in the metronomic mode.

For each group, the number of animals at all stages of the experiment was 12.

The experimental groups at the first stage were formed as follows (Table 1).

Table 1. Distribution of test animals into groups at the first stage of the experiment

Injected compounds and doses	Groups of animals (female mice of the C57Bl/6 line)
Me3 in SD of 30 mg/kg (TD 150 mg/kg)	I, Ia
Me3 in SD of 50 mg/kg (TD 250 mg/kg)	II, IIa
Me3 in SD of 75 mg/kg (TD 375 mg/kg)	III, IIIa
Me3 in SD of 100 mg/kg (TD 500 mg/kg)	IV, IVa
Me5 in SD of 30 mg/kg (TD 150 mg/kg)	V, Va
Me5 in SD of 50 mg/kg (TD 250 mg/kg)	VI, VIa
Me5 in SD of 75 mg/kg (TD 375 mg/kg)	VII, VIIa
Me5 in SD of 100 mg/kg (TD 500 mg/kg)	VIII
Control group	IX, IXa

Note: Me3 – bis-(3,5-di-tert-butyl-4-hydroxyphenylthiolate) dimethylol, Me5 – (3,5-di-tert-butyl-4-hydroxyphenylthiolate) triphenylol, SD – single dose, TD – total dose.

Registration of the changes in the general condition of the animals and their deaths was carried out daily until all the individuals had died (groups I–IX). To assess TRO (inhibition of tumor growth) (%) by tumor mass and MIR (metastasis inhibition rate) (%), euthanasia of the animals of groups Ia–IXa was performed on a guillotine on the 18th day after transplantation. Group VIIIa was excluded from the second series of the experiment due to the pronounced toxic effect in the animals of group VIII.

The experimental groups at stage II were formed as follows: A – injection of Me3 in a SD of 37.5 mg/kg, B – injection of Me5 in a SD of 25 mg/kg, C – control group without any pharmacological correction. The animals were removed from the experiment by the guillotine on the 18th day after the inoculation.

The efficacy was evaluated according to the generally accepted indicators: average life expectancy, inhibition of tumor growth by tumor mass, and metastasis inhibition rate (Habriev 2005).

The change in the average life expectancy (Δτ, %) was defined as (Formula 1):

$$\Delta\tau,\% = \frac{(\tau C - \tau T)}{\tau C} \quad (1),$$

where τC and τT – average life expectancy (ALE, days) of mice in the groups of control (C) and treated (T) animals.

The inhibition of tumor growth coefficient (TGI, %) by tumor mass was determined from the ratio (Formula 2):

$$TGI,\% = \frac{(RC - RT)}{RC} \quad (2),$$

where RC and RT – average tumor mass of mice in the groups of control (C) and treated (T) animals.

The metastasis inhibition rate (MIR, %) was calculated using Formula 3:

$$MIR,\% = \frac{((Ac \times Bc) - (A \times B)) \times 100\%}{Ac \times Bc} \quad (3),$$

where Ac, A – frequency of metastasis in the control and experimental groups; Bc, B – average number of metastases in animals of the control and experimental groups.

The efficacy indicators of the studied compounds were determined in comparison with the control group of the animals without any treatment.

Statistical data processing

Pathoanatomic autopsy of animals was performed according to a well-known technique presented in (Koptyaeva et al. 2018).

The study was approved by the local independent ethics committee of Rostov State Medical University of the Ministry of Health of the Russian Federation (Minutes No. 10/20 of 28.05.2020). Descriptive statistic was used for analyzing all the results. Statistical processing of the obtained data was carried out using the Statistica 6.0 computer software package. The normality of the distribution was evaluated using a modified version of the Kolmogorov-Smirnov method, namely, the Anderson-Darling method. The reliability of the differences between the compared parameters was assessed using the Student's t-test. The groups were compared in pairs.

Results and discussion

Compounds Me3 and Me5 have maximum safety indicators for use as drugs. LD50 for both organotin compounds is much more than 300 mg/kg, which makes them suitable for further study as drug candidates. According to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Me3 should be assigned to V toxicity category and Me5 to IV toxicity category (GHS-unece 2019).

Stage I. Administration of the investigated compounds in the classical mode according to Sofina Z.P.

The effect of hybrid organotin compounds on the growth of the main tumor node and on the activity of metastasis of of melanoma B16 to the lungs was studied in the experiment with a wide range of doses. In all the experimental groups, the metastasis frequency was the same as in the control animals, reaching 100%. The minimum and maximum single doses for Me3 are 8 and 2.5 times lower than the maximum tolerated dose, which is 250 mg/kg with its single intraperitoneal administration, while the range of administered doses for Me5 was chosen from 2.5 times lower than the maximum tolerated dose (80 mg/kg with a single intraperitoneal administration) to 100 mg/kg. The results are shown in Table 2.

With the administration of Me3 and Me5 in the total dose of 150 mg/kg, the life expectancy of animals in the control and experimental groups did not significantly differ. There was a tendency to increase the life expectancy of animals in experimental group II; the maximum increase in this indicator for compound Me5 was revealed with the administration of a similar dose in group VI. When compound Me3 was administered at a total dose of 375 mg/

Table 2. Effect of compounds M3 and M5 on the development of melanoma B16 (C57Bl/6 mice, females) in the classical mode of administration according to Sofina Z.P.

Experimental groups	Administered doses SD*/ TD**, mg/kg	Average life expectancy, days	Inhibition (+) or stimulation (-) of tumor growth by mass, %	Index of inhibition (+) or stimulation (-) of metastasis, %
Compound Me3				
I, Ia	30/150	21.58±0.98	6.1	9.7
			p≤0.05	p≤0.05
II, IIa	50/250	23.48±0.92	17.6	31.4
			p≤0.05	p≤0.05
III, IIIa	75/375	30.75±1.64	27	54
		p≤0.05	p≤0.05	p≤0.05
IV, IVa	100/500	17.75±1.15	-15	-27.3
		p≤0.05	p≤0.05	p≤0.05
Compound Me5				
V, Va	30/150	21.41±1.25	12.3	17.5
			p≤0.05	p≤0.05
VI, VIa	50/250	25.83±1.56	22.9	36
		p≤0.05	p≤0.05	p≤0.05
VII, VIIa	75/375	18.66±1.42	-10.6	-26.4
			p≤0.05	p≤0.05
VIII	100/500	15.83±0.95	*	*
		p≤0.05		
Non compound				
IX, IXa	Control group	21.83±1.68	-	-

Note: * – single dose; ** – total dose (single dose × 5 days); Me3 – bis-(3,5-di-tert-butyl-4-hydroxyphenylthiolate) dimethyltin; Me5 – (3,5-di-tert-butyl-4-hydroxyphenylthiolate) triphenyltin. The second series of the experiment using 500 mg/kg TD for Me5 was not carried out.

kg, there was a 41% increase in the average life expectancy. Administration of a similar dose of compound Me5, on the contrary, caused a decrease in this indicator. With a further increase in the single dose of both compounds, the average life expectancy decreased sharply.

With the introduction of Me3 in group I, the mass of the tumor and the number of metastases in the lungs tended to decrease (6.1% and 9.7% respectively). With an increase in the dose to 250 mg/kg, the mass of the primary focus decreased by 17.6% and the number of metastases decreased by 31.4%. At a total dose of 375 mg/kg, the tumor mass and the number of metastases in the lungs decreased by 27% and 54%, respectively.

With the administration of Me5, the desired therapeutic effect was observed only in groups Va, VIa of experimental animals and with the administration of a total dose of 150 mg/kg, the tumor mass decreased by 12%, and the metastatic lesion in the lungs decreased by 17.5%. The maximum therapeutic effect was obtained when Me5 was administered at a total dose of 250 mg/kg: the tumor mass decreased by 22.9% and the number of lung metastases decreased by 36%.

The autopsy of the animals of the described groups had no specificities.

The toxic effect was observed when Me3 was administered at a total dose of 500 mg/kg and Me5 – at two doses: 375 mg/kg and 500 mg/kg. Life expectancy significantly decreased for Me3 by 18%, for Me5 – by 14% and 28%, respectively. In groups IVb and VIIb, there were 8 animals in each group by the 18th day after the inoculation, which made it possible to carry out statistical data processing. In group VIIIa, in the preliminary series of the experiment, there was loss of animals (average life expectancy was 16 days); therefore, the second series of the experiment with a total dose of 500 mg/kg for Me5 was not carried out. There was also an increase in the mass of the primary focus by 15% and 11% in the animals, which had been receiving 500 mg/kg of Me3 and 375 mg/kg of Me5, respectively. There was also an increase in metastatic lung damage by 27% (Me3) and 26% (Me5). A decrease in life expectancy, an increase in the mass of the primary tumor focus and the intensity of metastasis to the lungs were accompanied by an additional spread of the tumor not typical for melanoma B16. Metastases were found in the liver, kidneys, spleen, uterus, etc. In addition, there was no linear dependence of the inhibitory effect either on the primary tumor focus or on the process of metastasis of melanoma B16 in the mice with an increase in the administered dose of the studied organotin compounds.

Stage II. Administration of the studied compounds in metronomic mode at the most effective doses for each compound

One of the results of this research was identifying the sensitivity of a universal model of tumor growth with spontaneous lung metastasis of melanoma B16 to metronomic chemotherapy.

Attention should be paid to the fact that the studied compounds were used in the experiment in small (threshold) doses, but the results of specific effectiveness comparable with the antitumor and antimetastatic effects of hybrid organotin compounds in the classical administration mode according to Sofina Z.P.'s method were obtained.

Table 3. Effect of compounds M3 and M5 on the development of melanoma B16 (C57Bl/6 mice, females) in metronomic mode of administration

Experimental groups	Administered doses SD* / TD**, mg/kg	Average life expectancy, days	Inhibition (+) or stimulation (-) of tumor growth by mass, %	Index of inhibition (+) or stimulation (-) of metastasis, %
Compound Me3				
A	37.5/375	31.67±1.6	30	59.4
		p≤0.05	p≤0.05	p≤0.05
Compound Me5				
B	25/250	26.6±1.9	25	37.4
		p≤0.05	p≤0.05	p≤0.05
Non compound				
C	Control group	19.9±1.2	-	-
		p≤0.05		

Note: * – single dose; ** – total dose (single dose × 5 days); Me3 – bis-(3,5-di-tert-butyl-4-hydroxyphenylthiolate) dimethyltin; Me5 – (3,5-di-tert-butyl-4-hydroxyphenylthiolate) triphenyltin.

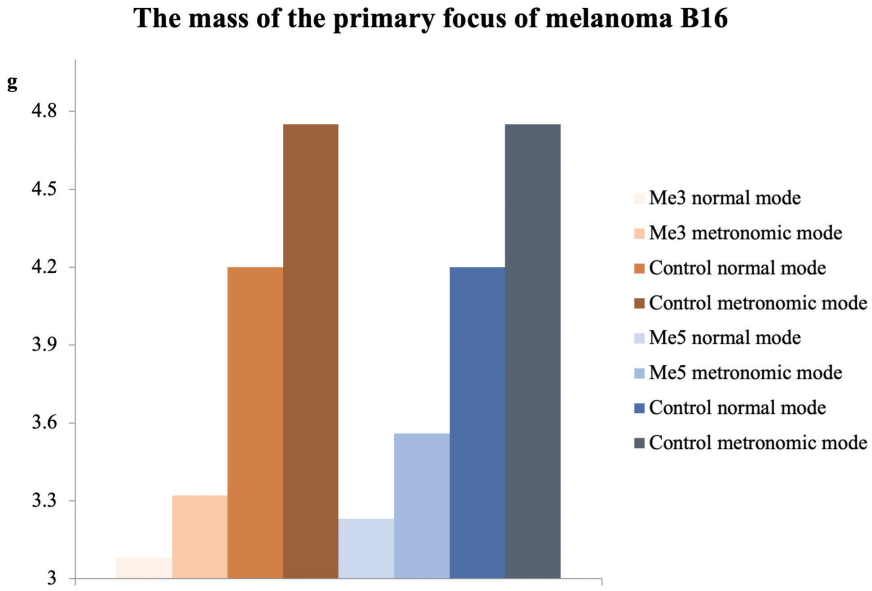

Figure 2. Effects of Me3 and Me5 on the primary tumor weight of melanoma B16 (C57Bl/6 mice, females). The differences are significant in relation to the corresponding control group ($p \leq 0.05$). **Note:** Me3 – bis-(3,5-di-tert-butyl-4-hydroxyphenylthiolate) dimethyltin; Me5 – (3,5-di-tert-butyl-4-hydroxyphenylthiolate) triphenyltin.

It is of note that there is an increase in the absolute mass of the primary focus of the tumor process during experimental metronomic administration in all the groups of animals (Fig. 2) with higher indices of inhibition of tumor growth by mass and metastasis activity.

The obtained experimental results allow us to hypothesize a multifactorial mechanism of action of hybrid organotin compounds on the growth of the primary focus and on the metastatic activity of melanoma B16 of mice of the C57Bl/6 line as well as an increase in tissue resistance to metastasis with the administration of Me3 and Me5.

Cells of many tumors, including melanoma, increase the rate of glycolysis providing an increased flow of substrate for biosynthetic pathways partially carried out in mitochondria to maintain proliferation and survival. As a result of activation of the metabolic flow through the mitochondrial pathways, the production of reactive oxygen species in tumor cells increases, which causes activation of the antioxidant response pathways of cells with inhibition of all antioxidant enzymes (Bandovkina et al. 2017). An imbalance in the rates of lipid peroxidation and the activity of enzymatic and non–enzymatic antioxidant protection is one of the causes of oxidative stress in the body. A tumor in the body creates signaling pathways between metastatic cells and normal ones, which forms a metastatic niche for metastasis (Maru 2015). The creation of a pre-metastatic niche is influenced by many factors that provide innate immunity, control the state of cell membranes and blood vessels, etc.

The studied biologically active compounds based on tin (IV) metal complexes acquire the property of selective permeability into a healthy and malignantly altered cell and actively affect the pro/antioxidant balance in the cell due to a fragment of spatially obstructed phenol (Dodokhova et al. 2021b).

Inside the cell, Me3 and Me5 can be hydrolyzed by the S-Sn bond with the formation of primary metabolites: Me3SnOH or Ph3SnOH hydroxides and 2,6-di-tert-butyl-4-mercaptophenol. The hydrogen atom of the phenolic OH-group is easily split off under the influence of peroxyl radicals, forming a relatively non-reactive phenoxyl radical that can interact with other peroxyl radicals and break off the chain radical process (Milaeva et al. 2020). The tin-containing fragment, along with a direct biocidal action (damage of DNA and tubulin), promotes the formation of reactive oxygen species and promotes the development of apoptosis (Kobliakov 2019; Alfarouk et al. 2020).

A change in the concentration of hydrogen ions with the active growth of primary tumor node and distant foci of malignant growth plays an important part in inhibiting tumor invasion and increasing the permeability of Me3 and Me5 to the tumor cell. The pH levels are different in normal and tumor cells. The average pH level in a normal cell is neutral, and in the intercellular space, it is weakly alkaline (7.35–7.45). The indicators are opposite in a tumor cell: the pH value is slightly alkaline inside the cell (7.12–7.90) and slightly acidic outside the cell (6.2–6.9). It has been shown that invasion occurs from tumor zones with a low pH level, whereas there is no invasion in tumor zones with a normal pH level (Kobliakov 2017). Compounds Me3 and Me5 are more lipophilic in the more acidic environment of the intercellular space of the tumor tissue, which contributes to more effective permeation through biological membranes. Therapeutic doses of Me3 and Me5 due to their antioxidant fragment reduce the level of reactive oxygen species and products of molecular damage under oxidative stress – in the membranes of hydroperoxides, ketoaldehydes and other products that are excessively synthesized in the tissues of the body during the restructuring of metabolism as a whole. The protective effect of hybrid organotin compounds is manifested in the

increased resistance of internal organs to metastasis. The research results by other authors can be cited as confirmation of our hypothesis. The efficacy of antioxidants from the class of spatially hindered phenols in terms of protecting the liver with toxic hepatopathy from metastasis was established in (Menshchikova et al. 2019).

The results demonstrate the importance of the functional state of the organ for its resistance to the development of metastases.

The administration of toxic doses of hybrid organotin compounds causes the excessive formation of reactive oxygen species, which promotes cell proliferation, subsequent tumor invasion and stimulates metastasis (Puzakov et al. 2019; Vostrikova et al. 2020). Vitamin E supplementation stimulated tumor progression and reduced survival in *in vivo* experiments (mice) on lung cancer models caused by BRAF and KRAS gene expression. Antioxidants reduce the amount of reactive oxygen species and DNA damage, but they also reduce the expression of p53 (Teplova et al. 2018; Zenkov et al. 2019). In our opinion, the nonlinear dose-dependent therapeutic and toxic effect may be associated with a change in the pro/antioxidant status of the cell.

Conclusion

Organotin compounds containing a fragment of 2,6-di-tert-butylphenol show a multidirectional effect on the growth and development of the primary focus and the intensity of metastasis in the lungs in a B16 melanoma model depending on a dose administered. The most pronounced antimetastatic effect was achieved with the administration of bis-(3,5-di-tert-butyl-4-hydroxyphenyl) dimethylolol thiolate (Me3) at a total dose of 375 mg/kg.

The administration of hybrid organotin compounds in metronomic mode revealed the sensitivity of a universal model of tumor growth with spontaneous lungs metastasis of melanoma B16 for metronomic chemotherapy. The efficacy of the antitumor and antimetastatic effects of Me3 and Me5 at the maximum effective total dose in the metronomic and classical mode of administration according to Sofina Z.P.'s method was comparable. An increase in the administered dose of more than 375 mg/kg (Me3) and 250 mg/kg (Me5) causes a toxic effect resulting in a decrease in life expectancy, an increase in the mass of the primary tumor focus and the intensity of metastasis in the lungs. Investigation of the detailed mechanism and targets of antitumor and antimetastatic actions of hybrid organotin compounds is one of the main tasks of our further research.

Conflict of interests

The authors declare that there is no conflict of interests.

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