Journal of Trace Elements in Medicine and Biology 71 (2022) 126931

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Relationship between the oxidative status and the tumor growth in transplanted triple-negative 4T1 breast tumor mice after oral administration of rhenium(I)-diselenoether

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ARTICLE INFO

Keywords:
Rhenium
Selenium
Oxidative status
Breast cancer
TGFβ1
TNFα
ROS
VEGF
AOPP
DNPH test
selenoprotein P

ABSTRACT

Background: Selective inhibitory effects of rhenium(I)-diselenoether (Re-diSe) were observed in cultured breast malignant cells. They were attributed to a decrease in Reactive Oxygen Species (ROS) production. A concomitant decrease in the production of Transforming Growth Factor-beta (TGFβ1), Insulin Growth Factor 1 (IGF1), and Vascular Endothelial Growth Factor A (VEGFA) by the malignant cells was also observed.
Aim: The study aimed to investigate the anti-tumor effects of Re-diSe on mice bearing 4T1 breast tumors, an experimental model of triple-negative breast cancer, and correlate them with several biomarkers.
Material and methods: 4T1 mammary breast cancer cells were orthotopically inoculated into syngenic BALB/c Jack mice. Different doses of Re-diSe (1, 10, and 60 mg/kg) were administered orally for 23 consecutive days to assess the efficacy and toxicity. The oxidative status was evaluated by assaying Advanced Oxidative Protein Products (AOPP), and by the dinitrophenylhydrazone (DNPH) test in plasma of healthy mice, non-treated tumor-bearing mice (controls), treated tumor-bearing mice, and tumors in all tumor-bearing mice. Tumor necrosis factor (TNFα), VEGFA, VEGFB, TGFβ1, Interferon, and selenoprotein P (selenoP) were selected as biomarkers.
Results: Doses of 1 and 10 mg/kg did not affect the tumor weights. There was a significant increase in the tumor weights in mice treated with the maximum dose of 60 mg/kg, concomitantly with a significant decrease in AOPP, TNFα, and TGFβ1 in the tumors. SelenoP concentrations increased in the plasma but not in the tumors.
Conclusion: We did not confirm the anti-tumor activity of the Re-diSe compound in this experiment. However, the transplantation of the tumor cells did not induce an expected pro-oxidative status without any increase of the oxidative biomarkers in the plasma of controls compared to healthy mice. This condition could be essential to evaluate the effect of an antioxidant drug. The choice of the experimental model will be primordial to assess the effects of the Re-diSe compound in further studies.

Abbreviations: Re-diSe, rhenium(I)-diselenoether; ROS, Reactive Oxygen Species; TGFβ1, Transforming Growth Factor-beta 1; IGF1, Insulin Growth Factor 1; VEGF, Vascular Endothelial Growth Factor; EM, enriched medium; TNFα, Tumor Necrosis Factorα; IL, Interleukin; AOPP, Advanced Oxidative Protein Products; DNPH, dinitrophenylhydrazone; I.R, infrared spectroscopy; SEPP-1, selenoprotein 1; selenoP, selenoprotein P; TME, tumor microenvironment; PCa, prostate cancer; CAFs, cancer-associated fibroblasts; Cys/CySS, cysteine/cystine; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; SH, reduced thiol.

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https://doi.org/10.1016/j.jtemb.2022.126931
Received 31 March 2021; Received in revised form 12 January 2022; Accepted 14 January 2022
Available online 17 January 2022

1. Introduction

Rhenium(I)-diselenoether (Re-diSe) is a metal-based drug that combines a Re(I) tricarbonyl core with a diselenide ligand [1]. Both the Re metal and the Se elements have proven anti-cancer properties. Re-diSe showed selective dose-dependent and time-dependent inhibitory effects on different malignant cells in culture compared to healthy cells, thereby decreasing the production of Reactive Oxygen Species (ROS), Transforming Growth Factor-beta (TGFβ1), Insulin Growth Factor 1 (IGF1), and Vascular Endothelial Growth Factor A (VEGFA) by the cancer cells [2]. These effects were also observed in cancer cells in culture in an enriched medium (EM) containing the inflammatory cytokines Tumor Necrosis Factor (TNFα) and Interleukins (IL-6 and IL-1β) [3]. The anti-tumor effects of Re-diSe were observed in human-derived MDA-MB231 tumor-bearing mice [4,5], a model of triple-negative breast cancer, at a safe dose of 10 mg/kg/24 h for 28 days. However, in another study with the same model of MDA-MB231 tumor-bearing mice, but a total body irradiation of the mice before the inoculation of the tumor cells, a pro-tumor effect of Re-diSe was observed at doses of 5 and 10 mg/kg/24 h for 28 days [6].

We aimed to study the anti-tumor effects of different doses of Re-diSe after inoculation of mice-derived breast triple-negative 4T1 malignant cells in BALB/c syngenic mice. It is also a recognized model of triple-negative breast cancer, but the mice are not immune-deficient [7]. Re-diSe is an antioxidant, and the oxidative status was evaluated by assaying Advanced Oxidative Protein Products (AOPP), and the protein carbonyl content was measured by the dinitrophenylhydrazone (DNPH) test, which reflects the ROS production. The plasma levels of AOPP and DNPH were measured in non-treated tumor-bearing mice versus healthy mice to determine the effect of tumor cell transplantation on the oxidative status of the host.

Re-diSe was tested at the dose of 10 mg/kg, the active dose in previous studies [4,5], and compared to a lower dose of 1 mg/kg and a higher dose of 60 mg/kg.

Tumor weights and toxicity were evaluated in tumor-bearing mice after the daily oral administration of Re-diSe for 23 consecutive days versus controls (non-treated tumor-bearing mice).

The plasma and tumor levels of AOPP and DNPH were assayed by colorimetric tests, and the biomarkers of inflammation (TNFα), angiogenesis (VEGF), and Se status (Selenoprotein P or selenoP) by Elisa tests. The tumor levels of the markers of tumor proliferation (TGFβ1), immunity (Interferon), TNF, and VEGF were assayed by transcriptomics. All of these biomarkers were compared in treated and non-treated tumor-bearing mice to correlate the efficacy and the toxicity with biological changes.

2. Material and methods

2.1. The procedure of synthesis and analytical structure evaluation

2.1.1. Synthesis

The procedure of synthesis of Re-diSe and its structure has already been described [2,4,8]. The synthesis requires five steps, as is mentioned in the patent on "Rhenium complexes and their pharmaceutical use" with international publication number WO 2011/151399 A1.

All reagents and solvents were purchased from commercial sources and used as received without further purification. Carlo Erba (France) supplied the solvents (anhydrous acetone, dichloromethane, anhydrous diethyl ether, petroleum ether 40−60 °C, ethyl acetate, methanol, ethanol, tetrahydrofuran, diisopropyl ether, cyclohexane, and water) The reagents came from various companies: selenium powder, 1,3-dibromopropane and lithium hydroxide monohydrate from Alfa Aesar, potassium cyanide and methyl bromoacetate from Acros Organics, and aqueous hydrochloric acid from Thermo Fisher Scientific. Mineral salts like magnesium sulfate and sodium carbonate were ordered from Carlo Erba and rhenium pentacarbonyl chloride from Strem Chemicals. All moisture-sensitive reactions were performed in oven-dried glassware under a nitrogen or argon atmosphere.

Synthenova laboratory, Hérouville Saint-Clair, France, synthesized the Re-diSe according to previous recommendations but improved the final step by freeze-drying. This last treatment makes it possible to eliminate residual traces of organic solvents and have a product free of water, less hygroscopic, and thus easier to handle (weighing) and more stable (conservation). Freeze-drying was done using a Virtis brand freeze-dryer (benchtop K) at a temperature of −85 °C under a high vacuum after dissolving the product in water and freezing it in liquid nitrogen.

2.1.2. Analysis

The structure was verified by infrared spectroscopy (I.R) using a Shimadzu 8400 S spectrophotometer coupled with a diamond ATR. The molecular weight of the Re-diSe drug was 668.5.

2.2. Quality control and stability

LC/MS/MS on a Thermo Fisher Q Exactive Plus coupled with a Dionex Ultimate 3000 liquid chromatography in Esi positive mode at the mass spectrometry platform of Nice was used to determine the quality control and verify the stability of the compound. The column used was a Phenomenex Synergy 4 μm Hydro RP 80 Å 250 mm × 3 mm, gradient 80/20 H2O/ACN with 0.1 % formic acid to 20/80 in 25 mn.

2.3. Animals, cell line and reagents

Female Balb/C J mice aged 6–7 weeks were purchased from Charles River (Charles River Laboratories, Saint-Germain-Nuelles, France). The average weight of the mice was 22 g. The mouse triple-negative 4T1 breast cancer cell lines (CRL-2539, ATCC, USA) derived from the Balb/c mice were maintained in a DMEM medium (Lonza, Switzerland) supplemented with 10 % heat-inactivated Hyclone FCS (GE Healthcare, USA), 50 U/mL streptomycin, 50 U/mL penicillin and 1 mg/mL neomycin (Lonza, Switzerland). The growth factor reduced Matrigel was obtained from Corning (Thermo Fisher Scientific, France).

2.4. Experimental breast tumor in mice

All the procedures involving animals and their care were conducted following institutional guidelines in the IRCAN animal facility (French Ministry agreement N° AO6-08115) and with the approval of the national and local (N°28) ethics committee. The mice were acclimatized for at least one week before the start of the experiments. There were four animals housed per cage in a climate and light-controlled environment. They were fed with SAFE®AO3, a complete breeding diet for mice, purchased from SAFE DIETS PRODUCTION, Augy (France). It contains arginine, cystine, lysine, methionine, tryptophane, glycine, fatty acids, vitamins, and many minerals, but no Se. Sterilized water was available ad libitum. The animals were numbered and given an identification ear notch mark. The mice were first anesthetized with an isoflurane mask before the N°2 mammary fat pads were exposed using an aseptic surgical procedure. 4T1 cells (5.10^4) were suspended in a volume of 11 μl of PBS, mixed with 11 μL of Matrigel, and immediately injected directly into the mammary fat pad using a 0.5 mL insulin syringe. One suture (nonabsorbable nylon 4.0; Surgical Specialties, USA) was used to close the incision. The mice were treated with pediatric paracetamol (100 mg/kg/day) for two days via drinking water.

The Re-diSe treatments were administered daily by oral gavage for 23 consecutive days following the tumor day induction. There was an inclusion of control groups corresponding to tumor-bearing mice receiving water placebo gavage. Toxicity was evaluated twice a week (clinical examination, weights of the mice). Throughout the experiments, the weight of the animals was controlled, and their behavior was studied. The behavioral study focused on the condition of the coat, the

possible isolation from other mice, whether the eyes were closed or not, whether the back was hunched, and whether the animal was prostrate or listless.

The sacrifice of the mice took place on day 23 after the lethal injection of Dolethal (Vetiquinol). The efficacy was evaluated by the tumor weights, the day of sacrifice. The plasma and tumor samples were collected and stored at −80 °C until use.

There were 60 mice in total, with 8 healthy mice, 12 tumor-bearing mice receiving placebo (controls), and 40 tumor-bearing mice receiving Re-diSe at the dose of 1 mg/kg (n = 12), 10 mg/kg (n = 16) or 60 mg/kg (n = 12). The group treated with 10 mg/kg had more mice because it was expected to be the most effective dose. The plasma samples of healthy mice were primarily used to evaluate the effects of the transplantation of the tumor cells on biological parameters and thus validate the success of the tumor model.

2.5. Biological assays

2.5.1. Sample preparation

Heparinized blood samples were centrifuged at 1500×*g* for 15 min at 4 °C. The plasma was harvested and stored at −20 °C. Frozen tumor tissue samples were thawed, precisely weighed, ground, and crushed with a Precellys® homogenizer (Bertin-Instruments, Montigny-le-Bretonneux, France) at a final concentration of 100 mg tissue/mL of precellys lysis buffer. The mixture was centrifuged at 5000×*g* for 10 min at 4 °C. Supernatants were collected and kept in an ice bath until the protein concentration determination. The protein concentration of the plasma and tumor extracts was measured using the BCA protein assay kit (Thermo Scientific, Rockford, IL). All measurements were conducted on the same day of sample processing.

2.5.2. Gene expression analysis

Total RNAs were extracted from tumors collected from the sacrifice using a NucleoSpin RNA II kit (Macherey-Nagel, Germany) and reverse-transcribed into cDNA using the Superscript III enzyme (Invitrogen, USA) in a Flexicycler (Analytik Jena, Germany). Quantitative real-time PCR (qPCR) was performed in a StepOnePlus Real-Time PCR System (Life Technologies, USA) and carried out using SYBR Premix Ex Taq II, Tli RNase H Plus (Takara, Japan). Relative levels of mRNA expression were determined using ΔC_T values obtained by subtracting C_T control (actin) from C_T target gene, measured in the same RNA preparation.

The primer sequences for selection target genes were:

F: CAGCAACAGCAAGGCGAAA, **R**: GCTGGATTCCGGCAACAG for Interferon γ,

F: TTTACTGCTGTACCTCCACCA, **R**: ATCTCTCCTATGTGCTGGCTTT for VEGFA,

F: CCTGGAAGAACACAGCCAAT, **R:** GGAGTGGGATGGATGATGTC for VEGFB,

F: AGGCGGTGCCTATGTCTCA, **R**: GGGTCTGGGCCATAGAACTG for TNFα,

F: GGACCCTGCCCCTATATTTG, **R**: GCTTGCGACCCACGTAGTAG for TGFβ1,

F: AGATCTGGCACCACACCTTCT, **R:** TTTTCACGGTTGGCCTTAGG for actine.

2.5.3. ELISA quantification

Selenoprotein 1 (SEPP-1; RK03184), tumor necrosis factor-alpha (TNFα; Picokine EK0527), and vascular endothelial growth factor (VEGF; RK0028) were measured using the sandwich ELISA technique according to the instructions of the manufacturer (CliniSciences, Nanterre, France). The detection assay was based on the colorimetric reaction of horseradish peroxidase with TMB substrate. Immediately, the absorbance was measured at 450 nm. Selenoprotein 1 and VEGF were assayed in the tumor protein extract and in the plasma, and TNFα in the tumors. TNFα and VEGFA concentrations were expressed in pg/mL while selenoP concentrations were in ng/mL. The ELISA quantification could not be performed in mice treated at doses of 1 mg/kg for limited volumes of blood samples.

2.5.4. Colorimetric enzymatic dosages

They could not be performed in mice treated at doses of 1 mg/kg for the same reasons (limited volumes of blood samples).

2.5.4.1. AOPP. The ROS production was studied by assaying advanced oxidative protein products (AOPP) in plasma and tumors using the AOPP assay kit from Abcam (ab242295). The samples were treated with a chloramine reaction initiator. After five minutes, the reaction was stopped, and the absorbance was measured at 340 nm. The sample AOPP content was calculated by reference to the chloramine standard curve. The AOPPs concentrations were expressed in μmol/liter of chloramine-T equivalents.

2.5.4.2. DNPH. Carbonyl content was measured by the mean of the DNPH assay kit (ab126287) from Abcam (Cambridge, UK). Protein carbonyls were first tagged with 2,4-Dinitrophenylhydrazine (DNPH). The DNP hydrazones formed from the reaction were easily quantifiable at 375 nm. The carbonyl protein content was expressed in nmol/mg.

2.6. Statistical analysis

The statistical analysis was performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). A generalized linear model (GLM) was applied to evaluate the differences between the administered doses of Re-diSe. The least-squares means (LS means) that correspond to the specified effects for the linear part of the model were estimated first. The p-values (adjusted with the Tukey-Kramer test for multiple comparisons) with the relative confidence limits were then calculated after performing multiple comparisons. The results were considered statistically significant at $p<0.05$.

3. Results

3.1. Procedure of synthesis

The synthesis required five steps, as described in the patent on "Rhenium complexes and their pharmaceutical use" with international publication number WO 2011/151399 A1. In the end, the compound was lyophilized. The structure was verified by IR spectroscopy.

3.2. Stability of the compound

The stability of the compound was verified by mass spectrometry. The spectra were repeated four times over a year (before, during, and after the experiments), and they did not differ, showing excellent stability of the compound.

3.3. Efficacy

A significant increase of the tumor weights was observed in mice treated at the dose of 60 mg/kg by comparison with controls (95 % CI: (0.018, 0.32), p = 0.02) and also by comparison with the mice treated at the dose of 1 mg/kg (95 % CI: (−0.32, −0.039), p = 0.007).

The tumor weights did not vary in mice treated at doses of 1 and 10 mg/kg compared to controls. The tumor weights increased only at the very high dose of 60 mg/kg.

Results are shown in Fig. 1.

3.4. Toxicity

There was no death due to the treatment, nor was there any clinical sign of toxicity or weight loss.

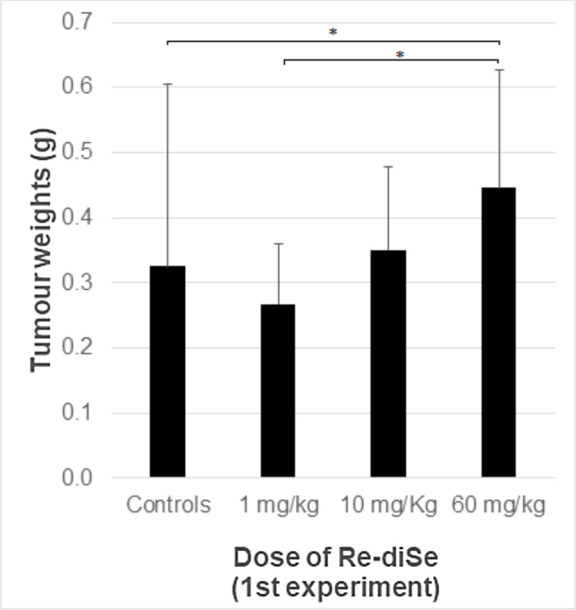

Fig. 1. Dose-effects of Re-diSe on the tumor weights (* $p \leq 0.05$ detected for the comparisons of 60 mg/kg versus controls, and 60 mg/kg vs 1 mg/kg).

The decision to sacrifice the mice on day 23 was made for ethical reasons, as one of the mice in the group treated at the dose of 60 mg/kg had an unacceptable tumor volume.

Results are shown in Fig. 2.

3.5. Biological effects

3.5.1. Transcriptomic assays

There was a significant decrease of TGF-ß1 at the dose of 60 mg/kg Re-diSe versus controls ($p = 0.01$), as well as in mice treated at the dose of 1 mg/kg (95 % CI: (0.16, 1.96), $p = 0.01$) or 10 mg/kg (95 % CI: (0.17, 2.04, $p = 0.01$). No significant changes were observed for VEGFA, VEGFB, TNFα and INF.

Results are expressed in Fig. 3.

3.5.2. ELISA and enzymatic assays

3.5.2.1. Oxidative stress. The AOPP concentrations significantly decreased in tumors at the dose of 60 mg/kg versus controls (95 % CI: (−83.07, −10.31), $p = 0.01$). There were no significant changes observed among the other groups, as well as in the plasma.

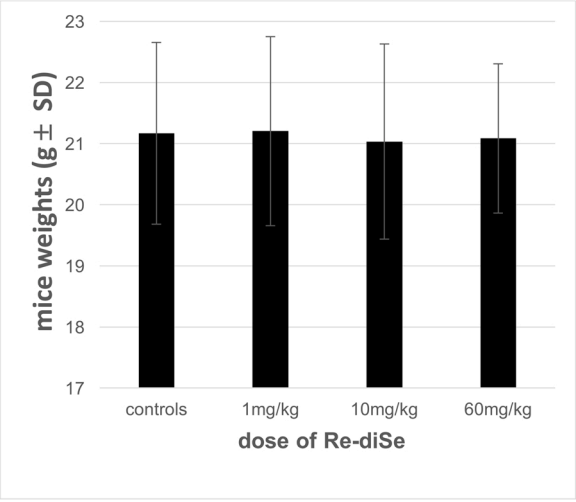

Fig. 2. Dose-effects of Re-diSe on the mice weights (no significant changes).

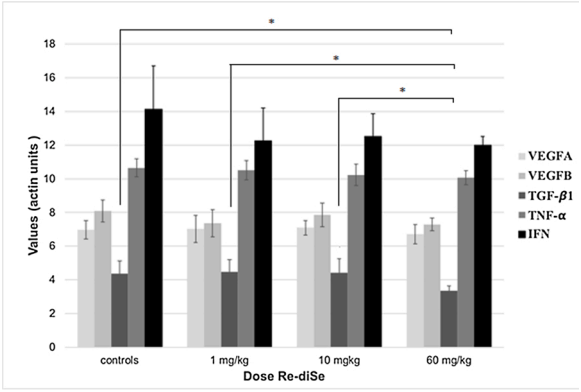

Fig. 3. Dose-effects of Re-diSe on the biomarkers assayed in tumors by transcriptomics. The multiple adjusted comparisons detected the main differences in TGF-ß1 in treated tumors at the dose of 60 mg/kg Re-diSe versus controls, but also versus doses of 1 and 10 mg/kg Re-diSe (* $p \leq 0.05$). No significant changes were noted among the other groups.

The DNPH concentrations in the plasma were significantly lower in controls compared to healthy mice (95 % CI: (−0.69, −0.03), $p = 0.032$), with no significant difference between treated groups and controls. The DNPH levels in tumors were significantly higher in mice treated at the dose of 10 mg/kg versus controls (95 % CI: (0.51, 6.87), $p = 0.021$). The results of all the other multiple comparisons were insignificant.

The results are expressed in Fig. 4.

3.5.2.2. Inflammation. The TNF concentrations were measured in tumors. There was a significant decrease of the TNF values in mice treated at the dose of 60 mg/kg compared to controls (95 % CI: (0.17, 2.04), $p = 0.01$). All the other multiple comparisons were insignificant. The results are expressed in Fig. 5.

3.5.2.3. Angiogenesis. The VEGFA concentrations were measured in the plasma and tumors. No significant effect was observed. The results are expressed in Fig. 6.

3.5.2.4. Selenoprotein P (selenoP). A significant increase in selenoP concentrations was observed in the plasma of mice treated at the dose of 60 mg/kg compared to healthy mice (95 % CI: (0.23, 1.18), $p = 0.002$), as well as in mice treated at the dose of 10 mg/kg versus healthy mice (95 % CI: (0.13, 1.05), $p = 0.008$). The differences were not significant between treated mice and controls or between mice treated at the dose of 60 mg/kg and those treated at 10 mg/kg.

In the tumors, there was no significant difference between the groups. The selenoP concentrations in tumors were lower than in plasma. The selenoP concentrations in the plasma were 2459 ± 2117 ng/mL at a dose of 60 mg/kg, but only 49 ± 11 ng/mL in the tumors. The results are expressed in Fig. 7.

4. Discussion

This study emphasizes the importance of the experimental model. Before evaluating the effects of an antioxidant, it appears essential to verify that the model is suitable with a pro-oxidative status of the host, the tumor, and the tumor microenvironment (TME). We measured markers of the oxidative status in the plasma in tumor-bearing mice versus healthy mice for this purpose without waiting until the study began to see if the tumor cells transplanted to the mice induced such a pro-oxidative status.

To assess the oxidative status, we measured the AOPP

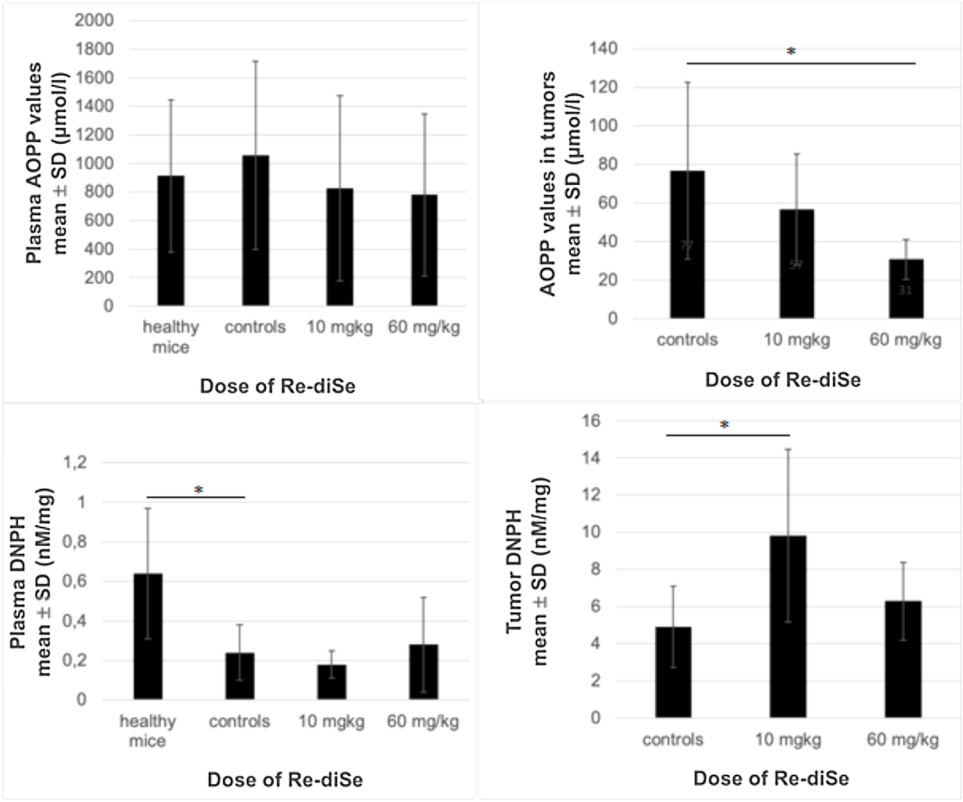

Fig. 4. Significant multiple comparisons values in the AOPP and DNPH (plasma and Tumor) values (* $p \leq 0.05$).

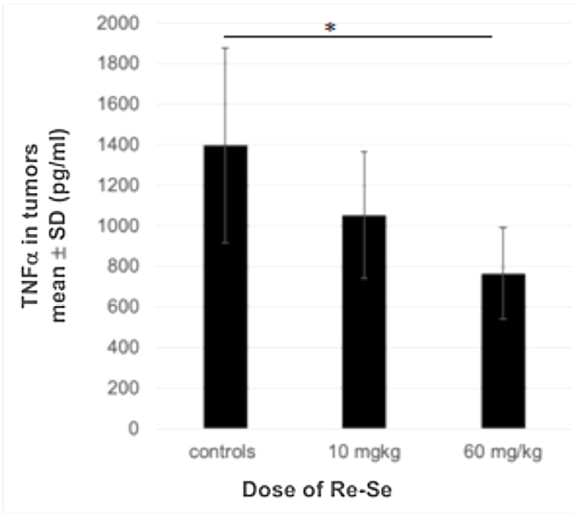

Fig. 5. Dose-effects of Re-diSe on the TNFα in tumors (* $p \leq 0.05$ 60 mg/kg versus controls).

concentrations, which reflect the ROS production. They are used to quantify the amount of oxidative damage in chronic conditions such as cancer, inflammatory status, and immune-mediated inflammatory disease [9]. Patients with bladder, colorectal, and gastric cancer, for example, had higher plasma AOPP levels [10–12]. It could not correspond to a chronic condition in our study because the tumor growth was so rapid that we had to interrupt the treatment after only 23 days of its administration. The DNPH levels were also assayed as a marker of oxidative stress, measuring the levels of protein carbonyls [13], which are frequently increased in cancers [14]. The levels of AOPP, carbonyl proteins, and thiol (-SH) groups in prostate cancer (PCa) patients were assayed, which showed lowered antioxidant defenses, particularly lowered thiol groups, in metastatic PCa patients, and increased levels of oxidative AOPP and DNPH biomarkers [15]. Our findings contradicted these published results. It was expected that the AOPP and DNPH levels in the plasma of transplanted mice would be higher compared to healthy mice, but it was not observed, and there was even a decrease in plasma DNPH levels in transplanted mice. The host status was therefore not pro-oxidative.

The Re-diSe drug, which is an antioxidant, aimed to normalize a pro-oxidant status induced by the transplantation of the tumor [16] but this pro-oxidative status was not achieved. It is important to note that the oxidative stress has a dual role [17]. An excess of ROS/RNS production or an excessive ROS/RNS removal may favor the development of the cancer disease and a faulty immune system [18]. Antioxidants may have pro- or anti-tumor effects [19–24]. Reduced ROS production below a normal limit has a detrimental impact on the immune system and promotes tumor growth. This is the well-known paradoxical effect of antioxidants. The prime reason for the failure of the Re-diSe treatment could thus be the lack of pro-oxidant status after the transplantation of the tumor cells. Without an excess of pro-oxidant status, a deficit in ROS induced by the Re-diSe drug at the highest dose of 60 mg/kg played a negative role.

The oxidative status of the host, the tumor, and the TME differ greatly depending on the type of cancer and its stage [14,25,26]. In further studies, it would be preferable to choose a metastatic model in which the production of ROS is the highest. Re-diSe showed anti-tumor activity in previous studies such as the MDA-MB231 model of triple-negative breast cancer, as reported in the introduction. The best result was observed in a metastatic model with lung metastases [4].

In the 4T1 model, cells are derived from murine tumors and are inoculated in non-immune deficient mice, while in the MDA-MB231 model, the cells are derived from human tumors and inoculated in nude mice. It could be another explanation for why the 4T1 model was resistant to Re-diSe in this study. Other differences exist between the

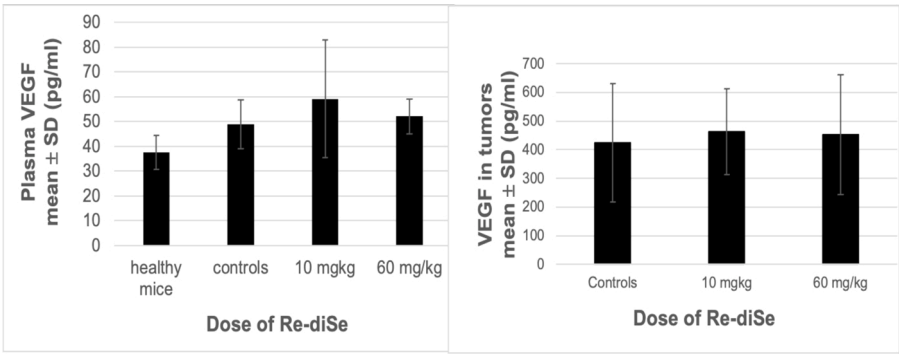

Fig. 6. Dose effects of Re-diSe on the VEGF-A concentrations.

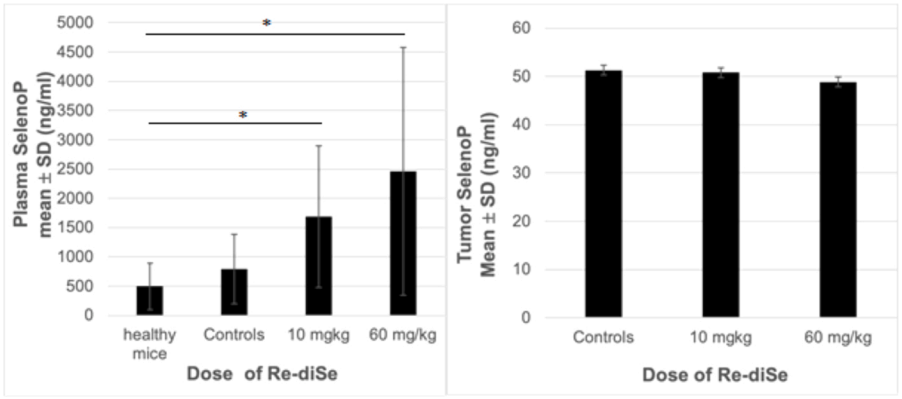

Fig. 7. Dose effects of Re-diSe on the selenoP concentrations (* $p \leq 0.05$ 10 and 60 mg(kg versus healthy mice). The differences were not significant in treated mice compared to controls (non-treated tumor-bearing mice).

MDA-MB231 and 4T1 models, include the crucial role of cancer-associated fibroblasts (CAFs) in the 4T1 tumor microenvironment [27], and the absence of mutation of the Brca1 and Brca2 genes in 4T1 tumors [28].

The influence of the diet has to be elucidated. The diet may have influenced the plasma redox potentials (E_h) of the plasma cysteine/cystine (Cys/CySS) redox couple [29]. The amount of cystine in the diet was 3200 mg/kg. Cystine is reduced to cysteine intracellularly for glutathione (GSH) synthesis, a powerful agent for reducing lipid peroxides. However, cysteine was not directly present in the diet, which may have affected its biodisponiblity. Moreover, the cellular uptake of cystine in exchange for intracellular glutamate from the microenvironment depends on the xCT cystine/glutamate transporter [30]. It would thus be helpful to quantify the levels of this transporter in the tumors. Oxidized glutathione (GSSG) and reduced glutathione (GSH) constitute another redox couple (GSH/GSSG). Plasma GSG/GSSG and Cys/CySS can be assayed by HPLC with electrochemical detection to quantify these thiol/disulfides redox couples, providing an excellent understanding of the oxidative status [31]. They could help study the influence of the diet, the effects of the tumor transplantation, and the consequences of the Re-diSe treatments on the oxidative status.

The selenoP concentrations did not increase in the tumors. They increased in the plasma as a function of the Re-diSe doses but did not vary significantly in the tumors of treated mice versus controls. Moreover, compared to the plasma concentrations, the selenoP concentrations in the tumors were very low. The selenoP concentrations are markers of the Se status. The selective Se uptake by the tumor cells is usually the most crucial factor for the efficacy of Se-based drugs [16]. The Se uptake depends on the expression of the xCT cystine/glutamate antiport system, but also on the content of Glutathione S-transferase (GST) and its isoform P (GSTP), the presence of reduced thiols (SH) in the extracellular compartment, the cysteine availability, and the GSH content [32]. According to Gopalakrishna et al. [33], more than 90 % of Se is bound in the serum to selenoproteins, which are responsible for the effects of Se when administered at a low dose (0.1 ppm). However, at doses >5 ppm, the effects of Se are mainly mediated by selenometabolites, like methylselenol. A dose of 10 mg/kg Re-diSe represents 2.4 ppm Se. Selenometabolites should have been produced at a 60 mg/kg Re-diSe dose, and could be responsible for some of the adverse effects observed at this very high dose.

The decrease in TGF-ß1 and TNFα in the tumors of mice treated at the high dose of 60 mg/kg Re-diSe may be a consequence of the antioxidant effect of the Re-diSe, as the ROS regulate many of the redox signaling pathways of proliferation and inflammation [34]. On the other hand, TGF-ß1 also has a dual role. In the early stages of cancer, TGF-β exhibits tumor-suppressive, while in the later stages, it exerts tumor-promoting effects, increasing tumor invasiveness and metastasis [35]. In our study, the tumor decrease in TGF-β did not correspond to an anti-tumor effect, but we can assume that the tumors were at an early stage as there was no metastases at the time of the sacrifice.

Finally, it is interesting to note that the Re-diSe drug may have an anti-inflammatory effect via the decrease of TNFα, even though it was not beneficial in this study. There is a strong relationship between oxidative status and inflammation, and this path needs to be explored [36].

5. Conclusion

Re and Se complexes are being more and more extensively studied as potential anticancer agents [37–43]. Among all the described mechanisms of action, their effects on the oxidative status must be considered primordial, and we continue to hypothesize that the anti-tumor activity

of the Re-diSe could depend on the oxidative status of the host and the tumor. There is a need to continue research on the Re-diSe drug, but with different experimental models that consider the types and stages of cancer, as well as the relationships with the host, the tumor, and the oxidative status of TME. Perhaps, Re-diSe and other antioxidants could only be beneficial in the case of pro-oxidative status, with a high production of ROS/RNS and a low antioxidant defense system, including low Se and selenoP concentrations; otherwise, there may be opposite and negative effects on the tumor growth and the immune system.

Ethics approval

All the procedures involving animals and their care were conducted in accordance with institutional guidelines in the IRCAN animal facility (French Ministry agreement N° AO6-08115). The study was approved by the National and Institutional Research Ethics Committees (respective numbers: APAFIS#17571-2018103010485674, PEA538) and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent for publication

All authors have read the final version of the manuscript and agree to its submission and publication.

Availability of data and materials

The data and materials may be available in the corresponding laboratories for each part of the study.

Funding

This study was made possible by the financial support of the “Agence de Développement Economique de la Corse” and the “Collectivité de Corse”.

Authors’ contributions

Philippe Collery first proposed a collaboration to Jean-Claude Scimeca due to a common interest in metal-based drugs and outlined the main objectives to be accomplished to evaluate the effects of a Re-diSe compound. Annie-Schmid Alliana, Heidy Schmid Antomarchi, and Jean-Claude Scimeca of the University of Nice proposed the study’s design. Didier Varlet synthesized the Re-diSe drug in the Synthenova laboratory. Jean-Marie Guigonis verified the structure of the compound several times. The animal experiments and transcriptomic assays were overseen by Annie-Schmid Alliana and Heidy Schmid Antomarchi, with the assistance of Imène Krossa, Charlotte Cohen, and Julie Antomarchi. Patricia Lagadec was in charge of the Elisa quantification and colorimetric enzymatic dosages. Marianna Lucio performed the statistical analysis. The redaction of the manuscript was a collective work.

Author statement

The Authors declare that all animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

The authors clearly indicated in the manuscript that such guidelines have been followed. The sex of animals were indicated, and only female animals were included in the study.

Declaration of Competing Interest

The authors declare that they have no conflict of interest. Philippe Collery has been named an inventor on the patent for “Rhenium complexes and their pharmaceutical use”. He is the manager of the Society for the Coordination of Therapeutic Researches, which owns the Intellectual Property Rights.

Acknowledgments

The authors are thankful for the moral and financial support of “Agence de Développement Economique de la Corse” and the “Collectivité de Corse”.

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