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Plasma Rhenium and Selenium Concentrations After Repeated Daily Oral Administration of Rhenium(I)-diselenoether in 4T1 Breast Tumor-bearing Mice

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Abstract. *Background/Aim: Rhenium(I)-diselenoether (Re-diSe) is a compound combining a rhenium tricarbonyl(I) core with a diselenide ligand. A high dose of 60 mg/kg had a pro-tumor effect in a previous study, in non-immune deficient 4T1 tumor-bearing mice, while doses of 1 and 10 mg/kg did not affect tumor growth, after repeated oral administrations. This study aimed to examine the tumor effects of a lower dose of 0.1 mg/kg with the same experimental design and to assay plasma Re and Se concentrations. Materials and Methods: Syngenic BALB/cByJ (JAX) mice were orthotopically inoculated with 4T1 mammary breast cancer cells. Re-diSe was daily administered orally for 23 days at doses of 0.1, 1, and 10 mg/kg, whereas controls received no treatment. Tumor and mice weights were measured at the end of the experiment. Plasma Re and Se concentrations were assayed by an inductively coupled plasma sector field mass spectrometry instrument (ICP-sf-MS). Results: The weight of the tumors did not vary in treated versus non-treated mice. The limit of detection (LOD) of Re was 0.34 nmol/l. Plasma Re concentrations were 14±20 nmol/l at doses of 0.1 mg/kg, and increased at higher doses, up to 792±167 nmol/l at doses of 10 mg/kg. Plasma Se concentrations were significantly increased in mice treated with the dose of 0.1 mg/kg (4,262±1,511 nmol/l) versus controls (1,262±888 nmol/l), but not from 0.1 to 1 mg/kg, nor from 1 to 10 mg/kg. Conclusion: The 0.1 mg/kg dose of Re-diSe resulted in detectable plasma Re concentrations and significantly increased plasma Se concentrations. In the future, doses as low as 0.1 mg/kg of Re-diSe will be tested, exploring its potential immune interest as a metronomic schedule of treatment, but in mouse models that readily develop extensive metastatic disease.*

Rhenium(I)-diselenoether (Re-diSe) is a metal-based drug, combining a Re(I)tricarbonyl as d^6*fac*-$[Re(CO)_3]^+$ moiety with a diselenide ligand (two atoms of selenium, Se). This $(CO)_3Re(I)(Se^\wedge Se)$ diselenoether complex, with the wide range of oxidation states of the Re metal (–3 to +7) and of the Se element (–2 to +6) may modulate the redox status of cancer cells. Different tricarbonyl $(CO)_3Re(I)$ derivatives have shown anticancer effects (1). Re-diSe showed excellent selective inhibitory effects on malignant cells in culture (2-4), but both pro- and anti-tumor effects of this compound have been observed in animal experiments (5-8). The pro-tumor effect was observed in an experimental model of 4T1 triple-negative breast cancer at the highest dose of 60 mg/kg Re-diSe, but the weight of the tumors was not affected at doses of 1 and 10 mg/kg (8). In this new experiment, with the same design, we tested the dose of 0.1 mg/kg Re-diSe to know if a lower dose could have a beneficial effect, as it is known that anti-oxidants, like Re-diSe may have paradoxical opposite effects according to the dose (9).

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On the other hand, the use of frequent and uninterrupted administration of low, but active doses of chemotherapeutic agents over prolonged periods of time, as a low-dose metronomic (LDM) schedule may be more beneficial than the administration of high doses, with different mechanisms of action, including anti-angiogenic, anti-proliferative, and immunomodulatory activities (10-14). However, optimal drug dosages and dosing intervals are not always easy to identify. In a 4T1 model, a metronomic treatment with nanocaged doxorubicin with targeted H-ferritin delivery had a more potent antitumor activity than standard doses of free or liposomal doxorubicin (15). Moreover, the schedule of treatment with low repeated doses avoided the cardiotoxicity, without decrease of reduced glutathione in heart tissues, in contrast with free or liposomal doxorubicin. The most important differences between standard chemotherapies at the maximal tolerated doses (MTD) and the metronomic approach could be on the effects on the tumor-microenvironment (TME) with a selective antitumor immune response allowed by the prolonged low doses (16).

We compared the efficacy of Re-diSe dose of 0.1 mg/kg to doses of 1 and 10 mg/kg after daily repeated oral administrations. We also assayed plasma Re and Se concentrations by inductively coupled plasma -sector field-mass spectrometry (ICP-sf-MS) at the end of the experiment.

Materials and Methods

Synthesis and analytical structure evaluation. The synthesis of the Re-diSe was performed by Synthenova laboratory, Herouville Saint-Clair, France, according to the already described procedure of synthesis, by ligand exchange reaction of pentacarbonylchlororhenium (I) with 3,7-diselena nonanedioic acid (8). The structure was verified by LC/MS/MS on a Thermo Fisher Q Exactive Plus (Thermo Scientific) and a Dionex Ultimate 3000 liquid chromatography (Thermo Fisher Scientific) in Esi positive mode on a Synergi 4 µm Hydro-RP 80 Å, 250×2.0 mm column (Phenomenex). The molecular weight of the Re-diSe drug was 668.5.

Animals, cell line and reagents. Female Balb/C J mice, 6-7 weeks old, were purchased from Charles River (Charles River Laboratories, Saint-Germain-Nuelles, France). The syngeneic 4T1 cancer cell line [mouse triple-negative breast cancer, CRL-2539, (ATCC, Manavas, VA, USA)] was maintained in DMEM (Lonza BioWhittakker DMEM with 4.5g/l Glucose and L glutamine; Lonza, Visp, 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).

Tumor induction in mice. All the procedure 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 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.

Experimental triple-negative breast cancer tumors were induced by injection of 4T1 cells (5×10^4) into mammary fat pads as previously described (8). The Re-diSe treatments were administered daily by oral gavage for 23 consecutive days after tumor cell injection. There were 48 mice divided in 4 groups of 12 mice per group: the control group received water placebo gavage and the three Re-diSe-treated groups received doses of 0.1 mg/kg, 1 mg/kg, and 10 mg/kg. Toxicity was evaluated twice a week (clinical examination, mice weights).

Twenty-three days after tumor cell injection, mice were sacrificed by a lethal injection of Dolethal (Vetiquinol, Bern, Switzerland). Plasma samples were collected and stored at –80˚C until use. After dissection, the extent of tumor development was assessed by weighing the tumor.

Plasma Re and Se concentrations. The inductively coupled plasma sector field mass spectrometry instrument (ICP-sf-MS) "ELEMENT 2" from Thermo Fisher Scientific (Bremen, Germany) was employed for the determination of ^{77}Se and ^{187}Re. Plasma samples were diluted 1:5 with Milli-Q water. No HNO_3 was added to plasma samples as this could have precipitated plasma proteins, leading to loss of analytes, and clogging of the nebulizer. ^{103}Rh and ^{193}Ir were administered to each sample at a concentration of 1 µg/l as internal standards. Sample injection was carried out using a peristaltic pump connected to a Seaspray nebulizer with a cyclon spray chamber. The RF power was set to 1300 W, the plasma gas was 15 l Ar/min, whereas the nebulizer gas was approximately 0.9 l Ar/min after daily optimization.

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) corresponding to the effects for the linear part of the model were estimated. 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 $p<0.05$.

Results

Quality-control of the compound. The quality-control was satisfying. The schematic structure is depicted in Figure 1, as previously published (5).

Plasma Re and Se concentrations. The limit of detection (LOD) of plasma Re concentrations was 0.34 nmol/l. The plasma Re concentrations were below the LOD in non-treated mice. However, in mice treated at the dose of 0.1 mg/kg, the plasma Re concentration was 14±20 nmol/l. It was significantly increased ($p=0.0032$) to 92±29 nMol/l at the dose of 1 mg/kg. The plasma Re concentrations were 10 times higher at the dose of 10 mg/kg, reaching values of 792±167 nMol/l, which were significant compared to those measured mice treated with the dose of 1 mg/kg ($p<0.0001$). The results are shown in Figure 2.

The plasma Se concentrations were of 1,268±888 nMol/l in controls. They significantly increased ($p=0.0017$) to

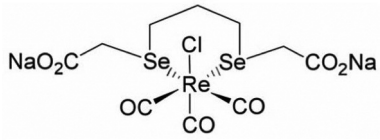

Figure 1. *Chemical structure of the Rhenium(I)-diselenoether (Re-diSe) complex.*

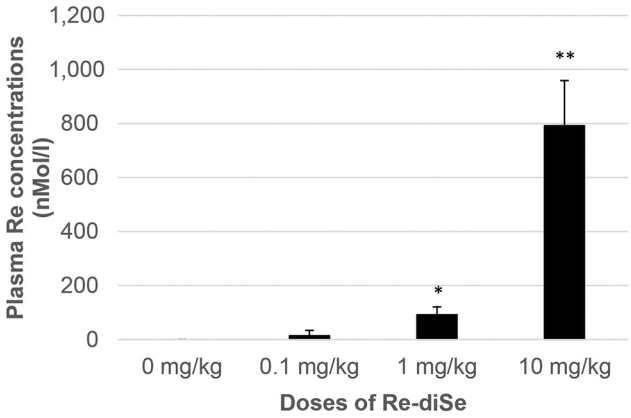

Figure 2. *Dose-dependent effect of Rhenium(I)-diselenoether (Re-diSe) on plasma Re concentrations. Plasma Re concentrations significantly increased in mice treated with doses of 1 mg/kg versus 0.1 mg/kg (*p=0.0032) and in mice treated with doses of 10 mg/kg versus 1 mg/kg (**p<0.0001).*

4,262±1,511 nMol/l at the dose of 0.1 mg/kg. At the dose of 1 mg/kg Re-diSe, the plasma Se concentrations were 5,735±1,728 nMol/l and this increase was not significant. The concentration was 6,253±2,017 nmol/l in mice treated at the dose of 10 mg/kg Re-diSe and this value was not significantly different compared to that at the dose of 1 mg/kg. The results are shown in Figure 3.

Tumor and mice weights. The tumor weights did not significantly vary between groups, but large interindividual variations were noted. The treatment had to be interrupted after 23 days, as a consequence of an unacceptable tumor weight in one mouse of the group treated with a dose of 10 mg/kg. Results are shown in Figure 4. There was no death related to the treatment, as well as no clinical sign of toxicity or loss of weight. Mice weights are shown in Figure 5.

Discussion

Doses of 0.1, 1, and 10 mg/kg Re-diSe did not affect tumor growth, but a longer duration of treatment should be considered for further experiments. It was previously shown that high doses of 60 mg/kg Re-diSe orally administered

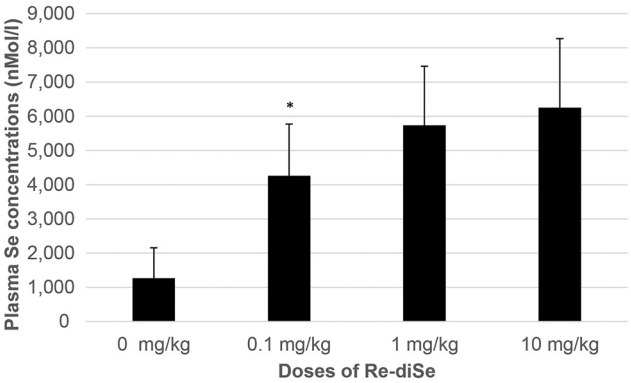

Figure 3. *Dose-dependent effect of Rhenium(I)-diselenoether (Re-diSe) on plasma Se concentrations. There was a significant increase of the plasma Se concentrations in mice treated with the low dose of 0.1 mg/kg versus controls (*p=0.0017), but no difference was observed between the treated groups.*

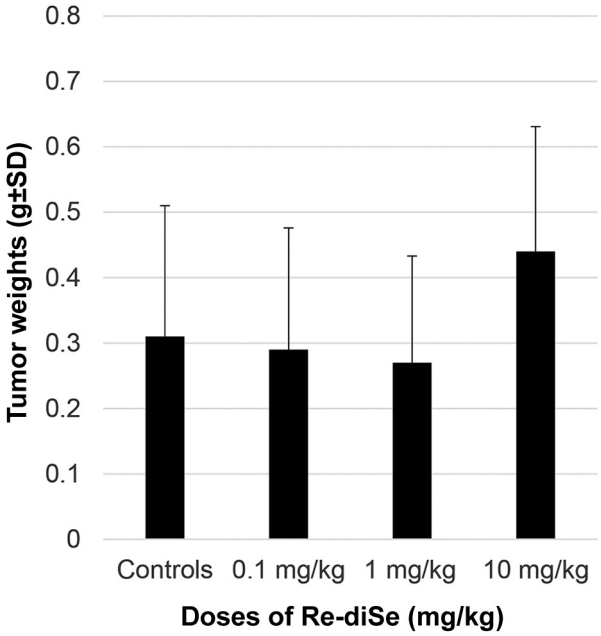

Figure 4. *Dose-dependent effect of Rhenium(I)-diselenoether (Re-diSe) on tumor weights. No statistically significant difference was observed between treated mice and controls.*

daily for 23 days induced a protumor effect in the same 4T1 breast tumor experimental model (8). Lower doses of 1 and 10 mg/kg did not affect tumor growth. In this new study with the same design, we examined a low dose of 0.1 mg/kg Re-diSe, as it is known that anti-oxidants may have opposite effects *via* dual roles in oxidative stress, either inhibiting or favoring tumor growth (9). The low dose of 0.1 mg/kg was compared to doses of 1 and 10 mg/kg. We did not observe any significant effect on tumor growth. The treatment was

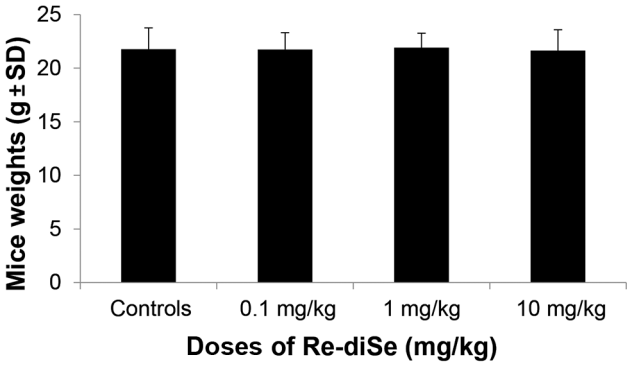

Figure 5. *Dose-dependent effect of Rhenium(I)-diselenoether (Re-diSe) on mice weights. No significant difference was observed between treated mice and controls.*

interrupted after 23 days of administration due to an unacceptable tumor weight in one mouse at the dose of 10 mg/kg, that could have been considered as aberrant. Therefore, a longer duration of treatment should be interesting in these groups as it is known that the exposure time, and not only its dose, is an important factor in evaluating the efficacy of Re-diSe (5).

Increasing the dose of Re-diSe did not correlate with an increase in efficacy. In a previous study with Re-diSe in MDA-MB231 tumor-bearing mice, Re-diSe showed an anti-tumor effect, but there was no difference between doses of 10 and 40 mg/kg (7). In addition, increasing doses of Re compounds did not result in a higher effect in another study published by Konkankit *et al.* (17). They demonstrated that a Re(I) tricarbonyl compound was very effective in an experimental mouse model of ovarian cancer, but efficacy did not differ by increasing the dose from 10 to 40 mg/kg. Interestingly, they also showed that the Re concentrations were lowest in the tumor than in many other healthy tissues, with the highest levels in the kidneys and lungs.

Dose-effects of the Re-diSe on plasma Re and Se concentrations. In this study, there was a clear effect of the dose of Re-diSe on the plasma Re concentrations. We did not assay Re in tumors, but the tumor Re uptake was already evaluated in a previous study, after repeated oral doses of 5 and 10 mg/kg Re-diSe for 6 weeks in MDA-MB231 breast tumor bearing-mice, another model of triple-negative breast cancer (6). In that experiment, tumor uptake of Re was demonstrated with concentration of 63±34 μg/kg at dose of 5 mg/kg/24 h and 91±49 μg/kg at dose of 10 mg/kg/24 h, while no Re was detected in controls. The plasma Se concentrations were significantly increased in mice treated at the dose of 0.1 mg/kg *versus* controls, but not by increasing the dose from 0.1 to 1 mg/kg nor from 1 to 10 mg/kg.

Importance of experimental model. Differences in the study design may affect the results obtained using models of triple-negative breast cancers. The importance of the experimental model has been emphasized in a review by Franca *et al.* (18). Some drugs may be active only when metastases are present. It has been observed that the antioxidant genistein had antimetastatic effects on spontaneous metastases of MDA-MB-435 after a continuous oral administration and not on the primary tumor growth rate (19). In fast growing tumors, such as MDA-MB231 or 4T1 tumors, it may be necessary to surgically remove the primitive tumors when they reach a high volume, before metastases appear. Other examples are given in the aforementioned review. Mice with locally invasive tumors are more likely to develop metastases, and they also tend to have a worse prognosis. The disparity of response to therapy between primary tumors and advanced metastases was attributed to the metronomic schedule of the treatment. Differences observed with Re-diSe along the experimental model could perhaps also be attributed to the immune and the redox status of cancer and TME cells.

Immune status. A pro-tumor effect has already been observed in MDA-MB231 tumor-bearing nude mice orally treated with Re-diSe (6). In that case, immune-deficient nude mice were treated by a total body irradiation before transplantation of the tumor cells, suggesting a role of immune cells. In fact, it has been demonstrated that Re-diSe may induce a significant dose-dependent decrease in the production of cysteine cathepsins B and S by malignant MDA-MB231 cells in culture, but also by normal cells (4). Cysteine cathepsins are proteolytic enzymes with roles in tumor-associated immune cell functions, and on the degradation of the extracellular matrix (20). Inhibition of the activity of cathepsins B and S in cancer cells could induce antitumor effects (21). Inhibition of these enzymes in immune cells of the tumor microenvironment (TME), dendritic cells, B- and T-cells, and macrophages, may result in an improvement of cell immunity. For example, inhibition of cathepsins B and S could induce a shift from the M2 macrophage phenotype to M1-like phenotype (22), counteracting the immuno-suppressive effects of cancer (23). However, cathepsins must be strictly regulated as they are also essential for an optimal immune function (24). The combination of total body irradiation and Re-diSe in immune-deficient mice could have a deleterious effect on the immune function. The aim should be to normalize the levels of cathepsins, for example, when they are produced in excess, as observed by Wan-Li *et al.* (25). They showed that in gastric cancer patients serum levels of cathepsins S were significantly ($p<0.001$) increased in late stage III/IV (27.77±10.64 μg/l) by comparison with early stage (16.67±6.19 μg/l) and reported that generally, cysteine proteases are overexpressed at late stage of cancer with metastasis (25). Furthermore, in a model of metastatic

triple-negative breast cancer with lung metastases orally administered repeated doses of Re-diSe showed a very significant antitumor activity (5). It may be hypothesized that Re-diSe could be more effective in metastatic models, with an overproduction of cathepsins, than in models with only primary tumors without metastases.

Redox status. In vitro studies comparing the effects of Re-diSe in MDA-MB231 breast malignant cells and in normal cells in culture showed that doses of only 5 μM Re-diSe induced selective cell death after an exposure time of 120 h, but the amount of reactive oxygen species (ROS) produced by the malignant cells was very high in comparison to normal cells (3). Re-diSe significantly decreased the production of ROS in malignant cells, but not in normal cells. Re-diSe could be considered as an anti-oxidant in these conditions.

In a model of 4T1 breast tumor-bearing non-immune deficient mice, transplantation of tumor cells did not induce an increase of oxidative stress plasma markers (8). It was thus proposed that the efficacy of Re-diSe could mainly depend on the redox potential of malignant and TME cells. The status of a cancer type may be oxidative or reductive, and, furthermore, the oxidative status of cancer and TME cells varies with the stage of cancer, with an important relationship between the extracellular redox state and cancer cell aggressiveness (9). The Re-diSe could therefore be more effective in metastatic models, considering that the redox potential is greater in metastases than in primary tumors.

Oxidation status of the targeted proteins. The redox couple ReI/ReII is a characteristic of the fac-Re(I) tricarbonyl compound (26). Metallomic studies performed by microwave plasma atomic emission spectrometry showed that soluble proteins were the main target of cationic tricarbonyl Re compounds, and not DNA or RNA (27). If proteins are the main targets of Re, reducing (ReI/ReII) or oxidizing effects (ReII/ReI) could be observed according to the oxidative status of the substrate. The tumor effects could therefore mainly depend on the oxidation state of the targeted proteins than on the plasma Re and Se concentrations.

Concerning the effects of the Se component of the Re-diSe compound, they may occur in a number of redox-regulated proteins resulting in decreased activity of enzymes (28), and could particularly depend on the redox state of cysteine residues in proteins (29). Transcription factors having regulatory cysteines can form Se adducts inactivating their functions (30). According to Gopalakrishna *et al*. proteins lacking cysteine clusters are unaffected by Se (31). Protein kinase C (PKC) is a protein with cysteine clusters in its catalytic subunit. These authors showed opposite dose-effects of Se on this kinase in prostate cancer cell lines (32). Low concentrations of Se inactivated isoenzymes of PKC (ε and α), which have antiapoptotic functions. In that case, Se reacted with the cysteine-rich regions in the catalytic domain and selectively induced cancer cell death. In contrast, higher concentrations of Se also inactivated other isoenzymes (PKCδ and PKCζ), which are proapoptotic. In that case, Se also reacted with the cysteine residues in the regulatory domain within the zinc-fingers proteins. Consequently, the tumor cells became resistant to apoptosis. This is a very good example demonstrating that low doses of drugs reacting with cysteine residues in proteins may have favorable antitumor effects and that higher doses may have negative effects.

Redox-active compounds (like the Re-diSe drug) may either inhibit tumor progression, cell growth, invasion, and metastasis, or, in contrast, induce resistance to apoptosis (33). Therefore, biomarkers can be very helpful in treatment management.

Biomarkers for the Re-diSe treatment. It has been demonstrated that Re-diSe decreased the production of reactive oxygen species (ROS), Transforming Growth Factor-beta (TGFβ1), Insulin Growth Factor 1 (IGF1) and Vascular Endothelial Growth Factor A (VEGFA) in malignant MDA-MB231 cells in culture (2). TGFβ1, IGF1, VEGFA are redox regulated. The levels of all these markers produced by malignant MDA-MB231 cells, were significantly higher than those produced by HEK-293 cells. However, it is known that serum levels of TGFβ1, IGF1 and VEGFA are increased in triple-negative breast cancer patients and associated with metastasis (34). ROS, TGFβ1, IGF1, VEGFA could thus be considered as biomarkers for Re-diSe treatment, at least in triple-negative breast cancer patients. We also showed the importance of assaying cathepsins cysteine proteases in plasma. All these markers are required for an optimal function of the cells, while an excess of production favors tumor growth. Plasma levels of these markers may be more helpful to manage the Re-diSe treatment than plasma Re and Se concentrations.

Conclusion

Spontaneous metastases are not often tested in preclinical trials and selective antimetastatic drugs could be unexplored (18). To avoid this error, we have thus to repeat the experiment which showed the efficacy of Re-diSe in lung metastatic MDA-MB231 mice (5). Detectable plasma Re concentrations were observed after the daily oral administration of only 0.1 mg/kg Re-diSe for 23 days. However, Re-diSe doses higher than 1 mg/kg did not increase plasma Se concentrations. Furthermore, increasing the doses could not increase the efficacy. We shall thus evaluate the effects of low doses Re-diSe, orally administered, daily, with a longer duration of treatment. We shall focus on the immune and redox effects. Plasma biomarkers should include cathepsins cysteine proteases. They could better help optimize Re-diSe treatment than plasma Re and Se concentrations.

Conflicts of Interest

The Authors declare that they have no conflicts of interest in relation to this study. Philippe Collery has been assigned as inventor on a patent on "Rhenium complexes and their pharmaceutical use". He is the manager of the Society for the Coordination of Therapeutic Researches, which is owner of the Intellectual Property Rights.

Authors' Contributions

Philippe Collery was the initiator of this cooperation. Annie Schmid-Alliana, Heidy Schmid-Antomarchi and Jean-Claude Scimeca of the University of Nice proposed the design of the study. Didier Varlet performed the synthesis of the Re-diSe drug in Synthenova laboratory. Jean-Marie Guigonis verified the structure of the compound several times. Annie Schmid-Alliana and Heidy Schmid-Antomarchi were responsible for the animal experiments. The plasma Re and Se concentrations were assayed by Bernhard Michalke. Marianna Lucio performed the statistical analysis. The making of the manuscript was a collective work.

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References

1 Collery P, Desmaele D and Vijaykumar V: Design of rhenium compounds in targeted anticancer therapeutics. Curr Pharm Des *25(31)*: 3306-3322, 2019. PMID: 31475892. DOI: 10.2174/1381612825666190902161400

2 Collery P, Veena V, Harikrishnan A and Desmaele D: The rhenium(I)-diselenoether anticancer drug targets ROS, TGF-β1, VEGF-A, and IGF-1 in an *in vitro* experimental model of triple-negative breast cancers. Invest New Drugs *37(5)*: 973-983, 2019. PMID: 30632005. DOI: 10.1007/s10637-019-00727-1

3 Veena V, Harikrishnan A, Lakshmi B, Khanna S, Desmaele D and Collery P: A new model applied for evaluating a rhenium-diselenium drug: breast cancer cells stimulated by cytokines induced from polynuclear cells by LPS. Anticancer Res *40(4)*: 1915-1920, 2020. PMID: 32234880. DOI: 10.21873/anticanres.14146

4 Collery P, Veena V, Desmaële D, Harikrishnan A and Lakshmi B: Effects of rhenium(i)-diselenoether and of its diselenide ligand on the production of cathepsins B and S by MDA-MB231 breast malignant cells. Anticancer Res *41(12)*: 5997-6001, 2021. PMID: 34848453. DOI: 10.21873/anticanres.15418

5 Collery P, Mohsen A, Kermagoret A, Corre S, Bastian G, Tomas A, Wei M, Santoni F, Guerra N, Desmaële D and D'Angelo J: Antitumor activity of a rhenium (I)-diselenoether complex in experimental models of human breast cancer. Invest New Drugs 33: 848-860, 2015. PMID: 26108551. DOI: 10.1007/s10637-015-0265-z

6 Collery P, Santoni F, Mohsen A, Mignard C and Desmaele D: Negative impact of total body irradiation on the antitumor activity of rhenium-(I)-diselenoether. Anticancer Res *36(11)*: 5813-5819, 2016. PMID: 27793903. DOI: 10.21873/anticanres.11165

7 Collery P, Santoni F, Ciccolini J, Tran TN, Mohsen A and Desmaele D: Dose effect of rhenium (I)-diselenoether as anticancer drug in resistant breast tumor-bearing mice after repeated administrations. Anticancer Res *36(11)*: 6051-6057, 2016. PMID: 27793932. DOI: 10.21873/anticanres.11194

8 Collery P, Lagadec P, Krossa I, Cohen C, Antomarchi J, Varlet D, Lucio M, Guigonis JM, Scimeca JC, Schmid-Antomarchi H and Schmid-Alliana A: Relationship between the oxidative status and the tumor growth in transplanted triple-negative 4T1 breast tumor mice after oral administration of rhenium(I)-diselenoether. J Trace Elem Med Biol *71*: 126931, 2022. PMID: 35063816. DOI: 10.1016/j.jtemb.2022.126931

9 Collery P: Strategies for the development of selenium-based anticancer drugs. J Trace Elem Med Biol *50*: 498-507, 2018. PMID: 29548612. DOI: 10.1016/j.jtemb.2018.02.024

10 Cazzaniga ME, Biganzoli L, Cortesi L, De Placido S, Donadio M, Fabi A, Ferro A, Generali D, Lorusso V, Milani A, Montagna E, Munzone E, Orlando L, Pizzuti L, Simoncini E, Zamagni C, Pappagallo GL and "Metronomic Chemotherapy in Advanced Breast Cancer" Study Group: Treating advanced breast cancer with metronomic chemotherapy: what is known, what is new and what is the future? Onco Targets Ther *12*: 2989-2997, 2019. PMID: 31114242. DOI: 10.2147/OTT.S189163

11 Romiti A, Cox MC, Sarcina I, Di Rocco R, D'Antonio C, Barucca V and Marchetti P: Metronomic chemotherapy for cancer treatment: a decade of clinical studies. Cancer Chemother Pharmacol *72(1)*: 13-33, 2013. PMID: 23475105. DOI: 10.1007/s00280-013-2125-x

12 Cramarossa G, Lee EK, Sivanathan L, Georgsdottir S, Lien K, Santos KD, Chan K and Emmenegger U: A systematic literature analysis of correlative studies in low-dose metronomic chemotherapy trials. Biomark Med *8(6)*: 893-911, 2014. PMID: 25224945. DOI: 10.2217/bmm.14.14

13 Fares JE, El Tomb P, Khalil LE, Atwani RW, Moukadem HA, Awada A and El Saghir NS: Metronomic chemotherapy for patients with metastatic breast cancer: Review of effectiveness and potential use during pandemics. Cancer Treat Rev *89*: 102066, 2020. PMID: 32769038. DOI: 10.1016/j.ctrv.2020.102066

14 Lien K, Georgsdottir S, Sivanathan L, Chan K and Emmenegger U: Low-dose metronomic chemotherapy: a systematic literature analysis. Eur J Cancer *49(16)*: 3387-3395, 2013. PMID: 23880474. DOI: 10.1016/j.ejca.2013.06.038

15 Mazzucchelli S, Bellini M, Fiandra L, Truffi M, Rizzuto MA, Sorrentino L, Longhi E, Nebuloni M, Prosperi D and Corsi F: Nanometronomic treatment of 4T1 breast cancer with nanocaged doxorubicin prevents drug resistance and circumvents cardiotoxicity. Oncotarget *8(5)*: 8383-8396, 2017. PMID: 28039473. DOI: 10.18632/oncotarget.14204

16 Muñoz R, Girotti A, Hileeto D and Arias FJ: Metronomic anti-cancer therapy: a multimodal therapy governed by the tumor microenvironment. Cancers (Basel) *13(21)*: 5414, 2021. PMID: 34771577. DOI: 10.3390/cancers13215414

17 Konkankit CC, King AP, Knopf KM, Southard TL and Wilson JJ: *In vivo* anticancer activity of a rhenium(I) tricarbonyl complex. ACS Med Chem Lett *10(5)*: 822-827, 2019. PMID: 31098006. DOI: 10.1021/acsmedchemlett.9b00128

18 Francia G, Cruz-Munoz W, Man S, Xu P and Kerbel RS: Mouse models of advanced spontaneous metastasis for experimental therapeutics. Nat Rev Cancer *11(2)*: 135-141, 2011. PMID: 21258397. DOI: 10.1038/nrc3001

19 Vantyghem SA, Wilson SM, Postenka CO, Al-Katib W, Tuck AB and Chambers AF: Dietary genistein reduces metastasis in a postsurgical orthotopic breast cancer model. Cancer Res *65(8)*: 3396-3403, 2005. PMID: 15833874. DOI: 10.1158/0008-5472.CAN-04-4109
20 Jakoš T, Pišlar A, Jewett A and Kos J: Cysteine cathepsins in tumor-associated immune cells. Front Immunol *10*: 2037, 2019. PMID: 31555270. DOI: 10.3389/fimmu.2019.02037
21 Pogorzelska A, Żołnowska B and Bartoszewski R: Cysteine cathepsins as a prospective target for anticancer therapies-current progress and prospects. Biochimie *151*: 85-106, 2018. PMID: 29870804. DOI: 10.1016/j.biochi.2018.05.023
22 Oelschlaegel D, Weiss Sadan T, Salpeter S, Krug S, Blum G, Schmitz W, Schulze A and Michl P: Cathepsin inhibition modulates metabolism and polarization of tumor-associated macrophages. Cancers (Basel) *12(9)*: 2579, 2020. PMID: 32927704. DOI: 10.3390/cancers12092579
23 Stout RD and Suttles J: Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J Leukoc Biol *76(3)*: 509-513, 2004. PMID: 15218057. DOI: 10.1189/jlb.0504272
24 Perišić Nanut M, Pečar Fonović U, Jakoš T and Kos J: The role of cysteine peptidases in hematopoietic stem cell differentiation and modulation of immune system function. Front Immunol *12*: 680279, 2021. PMID: 34335582. DOI: 10.3389/fimmu.2021.680279
25 Soond SM, Kozhevnikova MV, Townsend PA and Zamyatnin AA Jr: Cysteine cathepsin protease inhibition: an update on its diagnostic, prognostic and therapeutic potential in cancer. Pharmaceuticals (Basel) *12(2)*: 87, 2019. PMID: 31212661. DOI: 10.3390/ph12020087
26 Rodríguez Arce E, Machado I, Rodríguez B, Lapier M, Zúñiga MC, Maya JD, Olea Azar C, Otero L and Gambino D: Rhenium(I) tricarbonyl compounds of bioactive thiosemicarbazones: Synthesis, characterization and activity against Trypanosoma cruzi. J Inorg Biochem *170*: 125-133, 2017. PMID: 28237731. DOI: 10.1016/j.jinorgbio.2017.01.011
27 Soba M, Scalese G, Pérez-Díaz L, Gambino D and Machado I: Application of microwave plasma atomic emission spectrometry in bioanalytical chemistry of bioactive rhenium compounds. Talanta *244*: 123413, 2022. PMID: 35358896. DOI: 10.1016/j.talanta.2022.123413
28 Ganther HE: Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. Carcinogenesis *20(9)*: 1657-1666, 1999. PMID: 10469608. DOI: 10.1093/carcin/20.9.1657
29 Kim IY and Stadtman TC: Inhibition of NF-kappaB DNA binding and nitric oxide induction in human T cells and lung adenocarcinoma cells by selenite treatment. Proc Natl Acad Sci U.S.A. *94(24)*: 12904-12907, 1997. PMID: 9371773. DOI: 10.1073/pnas.94.24.12904
30 Gopalakrishna R and Gundimeda U: Protein kinase C as a molecular target for cancer prevention by selenocompounds. Nutr Cancer *40(1)*: 55-63, 2001. PMID: 11799924. DOI: 10.1207/S15327914NC401_11
31 Gopalakrishna R, Gundimeda U and Chen ZH: Cancer-preventive selenocompounds induce a specific redox modification of cysteine-rich regions in Ca(2+)-dependent isoenzymes of protein kinase C. Arch Biochem Biophys *348(1)*: 25-36, 1997. PMID: 9390171. DOI: 10.1006/abbi.1997.0334
32 Gopalakrishna R, Gundimeda U, Zhou S, Bui H and Holmgren A: Redox regulation of protein kinase C by selenometabolites and selenoprotein thioredoxin reductase limits cancer prevention by selenium. Free Radic Biol Med *127*: 55-61, 2018. PMID: 29775743. DOI: 10.1016/j.freeradbiomed.2018.05.062
33 Gopalakrishna R, Gundimeda U, Zhou S, Zung K, Forell K and Holmgren A: Imbalance in protein thiol redox regulation and cancer-preventive efficacy of selenium. React Oxyg Species (Apex) *2(4)*: 272-289, 2016. PMID: 29795790. DOI: 10.20455/ros.2016.851
34 Bahhnassy A, Mohanad M, Shaarawy S, Ismail MF, El-Bastawisy A, Ashmawy AM and Zekri AR: Transforming growth factor-β, insulin-like growth factor I/insulin-like growth factor I receptor and vascular endothelial growth factor-A: prognostic and predictive markers in triple-negative and non-triple-negative breast cancer. Mol Med Rep *12(1)*: 851-864, 2015. PMID: 25824321. DOI: 10.3892/mmr.2015.3560

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