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Uptake and Efflux of Rhenium in Cells Exposed to Rhenium Diseleno-Ether and Tissue Distribution of Rhenium and Selenium After Rhenium Diseleno-Ether Treatment in Mice

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Abstract. *We proposed a new water-soluble rhenium diseleno-ether compound (with one atom of Re and two atoms of Se) and investigated the uptake of Re into the nucleus of malignant cells in culture exposed to the compound for 48 h and its efflux from the nucleus after a post-exposure period of 48 h, as DNA is the main target of Re. We also studied the distribution of both Re and Se in the main organs after an oral administration of 10 or 40 mg/kg Re diseleno-ether to mice for four weeks, five days-a-week. Materials and Methods: Re and Se concentrations were assayed by inductively coupled plasma mass spectrometry (ICP-MS). Statistical analysis was performed using the Wilcoxon signed-rank test, comparing two related groups. Results: We observed that Re was well incorporated into the nucleus of malignant cells in the most sensitive cells MCF-7, derived from human breast cancer, and that there was no efflux of Re. In contrast, in MCF-7 resistant cells (MCF-7 Mdr and MCF-7 R), A549 and HeLa cells, there was significant efflux of Re from the nucleus after the wash-out period. In mice, an important and dose-dependent uptake of both Re and Se was observed in the liver, with lower concentrations in kidneys. The lowest concentrations were observed in blood, lung, spleen and bones. There was a significant increase of Re concentrations in the blood, liver and kidney in mice treated with Re diseleno-ether at the dose of 40 mg/kg/24 h versus those treated at the dose of 10 mg/kg/24 h. There was a significant increase of Se concentrations in all tissues with the dose of Re diseleno-ether of 10 mg/kg/24 h versus controls, and a significant increase in the liver in mice treated with dose of Re diseleno-ether of 40 mg/kg/24h versus those treated with 10 mg/kg/24 h. Conclusion: We are the first to demonstrate that a compound combining Re and Se in a single molecule, is able to deliver Re and Se to the organism via an oral route, for cancer treatment.*

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Rhenium (Re) is a metal able to induce adducts with nucleosides, like cisplatin, with cytotoxic properties on malignant cells. Selenium (Se) has shown dual effects, according to the dose and the schedule of treatment, acting as oxidant or antioxidant, influencing cell signaling pathways, especially the Pi3 kinase/Akt and MTOR pathways, inflammation, through the regulation of NF-Kappa B and immunity, by the modulation of the activity of T-lymphocytes and NK cells. Therefore, Se may in some cases be used as an oxidant to induce cell death in cancer cells, or to protect the cells from oxidative damage *e.g.* due to alkylating agents or toxic metals. We proposed a Re (I) diseleno-ether which is a new innovative compound, soluble in water, combining one atom of Re and two atoms of Se, as a new anticancer agent. The compound can be administered orally and its activity is based on targeting DNA by Re and signaling pathways by Se. The compound was synthesized by ligand exchange from pentacarbonyl-chlororhenium with 3,7-diselenanonanedioic acid followed by disodium salt formation with sodium carbonate and characterized as published elsewhere (1). The molecular weight is 668.5 and the formula is: $Re(CO)_3Cl(NaO_2CCH_2Se(CH_2)_3SeCH_2CO_2Na)$ (Figure 1).

We evaluated the uptake of Re and Se into various malignant cells after exposure of the cells to 400 μM Re diseleno-ether for 48 h, and their efflux after a postexposure period of 48 h. We also examined the effects and tissue distributions of Re, Se, Mg and Zn after administration of the compound to mice.

Materials and Methods

Concentration of Rhenium in malignant cells. All cell lines used were of human origin from the American Tissue Culture Collection (ATCC) (LGC, Molsheim, France). Cells were maintained in exponential growth phase in 25 cm^2 flasks in RPMI-1640 medium supplemented with 5% heat-inactivated fetal calf serum, 2 μM glutamine, 100 U/ml penicillin-streptomycin and 2.5 μg/ml fungizone (Gibco–Invitrogen, Orsay, France) and were incubated at 37˚C in a humidified incubator with 5% CO_2/95% air.

While still in exponential growth phase, cells were exposed to 400 μM Re diseleno-ether for 48 h and the efflux of Re was also measured after a post-exposure period of 48 h. At the end of treatment or post-incubation, flask contents were trypsinized into single cells using trypsin/ethylene diamine tetra-acetic acic (Trypsin/EDTA; Gibco–Invitrogen, Orsay, France), neutralized with the same amount of RPMI medium and centrifuged for 2 min at 800 ×*g*. The cells were then placed in 5 ml of 0.075 M KCl at 37˚C for 30 min, centrifuged for 3 min at 800 × *g* for recovering the nucleus and then kept in 5% nitric acid until analysis.

Re concentrations were assayed in the nuclei by inductively-coupled plasma mass spectrometry [ICP-MS, from Perkin-Elmer (type Elan, BRC II, NF EN ISO 17294-1-2)] with a detection limit of 1 μg/ml.

Tissue distribution study

Animal husbandry. Athymic *nu/nu* mice (Balb/C nude), provided by Harlan, Gannat, France, were female, 5-6 weeks old, about 20 g each, and specific and opportunistic pathogen-free. They were acclimatized for at least seven days before the initiation of the designed study. A total of 60 mice were used.

Experimental design. The experiment was performed at Cellvax Laboratory, Maison Alfort, France. Animals were housed in individual polyethylene cages in a climate- and light-controlled environment. Lights were on between 7:00 AM to 7:00 PM; the temperature inside the animal facility was strictly maintained at 21±1˚C and relative humidity of 70% throughout the entire study period, and maintained in accordance with Cellvax approved standard operation procedures (SOP) with local Ethical Committee approval (Cometh Anses/ENVA/UPEC, Number 16). Animals were fed with commercially available rodent food (Safe, Les Tremblats, Augy, France). Water (sterilized water) was available *ad libitum*. Animals were numbered and given a unique animal identification ear notch mark. A Ph.D. and Veterinary Doctor at Cellvax company assumed the function of 'Ethical Manager' within this project.

Experimental groups. Three groups of 20 mice each were treated. Group 1 (negative control): Mice were treated with vehicle saline control. Group 2: Mice were treated per os (p.o.) by gavage with Re diseleno-ether at the dose of 10 mg/kg (corresponding to 14.9 μMol/kg) once-a-day from Monday to Friday, for a period of four weeks. Group 3: Mice were treated p.o. by gavage with Re diseleno-ether at the dose of 40 mg/kg (corresponding to 59.8 μMol/kg) once a day from Monday to Friday, for a period of four weeks.

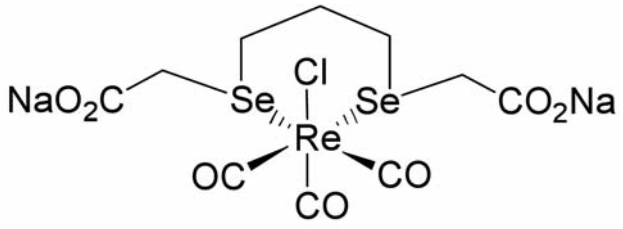

Figure 1. *Rhenium diseleno-ether structure*.

We aimed to study the antitumour activity of Re diseleno-ether on bone metastase. MDA-MB231 breast tumour cells were injected into a cardiac ventricle of the mice in groups 2 and 3. Unfortunately, the technique failed and deaths occurred in 13 mice during the first two weeks after their inoculation, with the formation of a cardiac and pulmonary extensive tumour at the autopsy. Therefore, the experiment was converted to a pharmacological study. The remaining mice (18 in group 1; 15 in group 2 and 14 in group 3) received treatment as scheduled from the day after the inoculation of malignant cells for the four week period, without any death in the last two weeks.

Pharmacological study. At the end of the study, all mice were sacrified and tissue samples were drawn from liver, kidney, lung, spleen, blood, brain and bone. The weight of the samples was noted. They were then digested by fumic HNO_3 and assays of Re, Se, Mg and Zn were performed by ICP-MS.

Statistical analysis. Groups were compared 2×2 using the Wilcoxon rank test. For Re, tissue concentrations were compared between mice treated with Re diseleno-ether at the dose of 10 mg/kg/24 h *vs*. those treated at the dose of 40 mg/kg/24 h. For Se, Mg and Zn, tissue concentrations were compared between mice treated with Re diseleno-ether at the dose of 10 or 40 mg/kg/24 h and the controls. Results were considered significant when $p<0.05$.

Results

Uptake and efflux of Rhenium in cells exposed to Rhenium diseleno-ether. Preliminary studies (not presented) showed that MCF-7 cells were the most sensitive to exposure to Rhenium diseleno-ether, with an IC_{50} of 25 μM *versus* 280 μM for A549 and 350 μM for HeLa cells (for an exposure time of 72h). However, concentration of Re in the nucleus was less important in MCF-7-sensitive cells (0.08 μM /million cells) than in the other cell types (0.18 μM/ million cells in A549 and 0.97 μM/million cells in HeLa). These Re concentrations were also assayed after a post-exposure time of 48 h. There was no efflux of Re in MCF-7 cells after this wash-out period [not even at a

Table I. *Concentration of Rhenium (Re) in the nucleus of malignant cells following treatment with Rhenium diseleno ether.*

Cell line	Intra-nuclear concentration of Re (μM/10^6m of cells) after 48h exposure	Intra-nuclear concentration of Re (μM/10^6m of cells) after a post-incubation time of 48h
MCF-7 S	0.08	0.24
MCF-7 Mdr	0.15	0.09
MCF-7 R	0.25	0.12
A549 S	0.18	0.12
HeLa	0.97	0.19

MCF-7 are human breast cancer cells; MCF-7 S are sensitive cells, MCF-7 Mdr are multidrug-resistant cells and MCF-7 R are resistant cells; A549 cells derived from a human lung carcinoma and HeLa cells from a human cervix carcinoma.

Table II. *Tissue concentrations of Re after oral administration to mice of 10 or 40 mg/kg/24 h Re diseleno-ether, for 4 consecutive weeks, 5 days-a-week. Data are the mean±SEM.*

	μg/kg Wet tissue	
Tissue	10 mg/kg (n=15)	40 mg/kg (n=14)
Blood	150±139	303±116*
Liver	1314±624	3679±1409*
Kidney	798±490	1634±379*
Lung	204±193	271±85
Spleen	323±407	381±196
Brain	145±162	146±30
Bone	255±351	133±30
	μMol/kg Wet tissue	
Tissue	10 mg/kg (n=15)	40 mg/kg (n=14)
Blood	0.81±0.75	1.63±0.62*
Liver	7.06±3.35	19.78±7.58*
Kidney	4.29±2.63	8.78±2.04*
Lung	1.10±1.04	1.46±0.46
Spleen	1.74±2.19	2.05±1.05
Brain	0.78±0.87	0.78±0.16
Bone	1.37±1.89	0.72±0.16

*Statistically different (40 *vs*. 10 mg/kg/24 h).

greater concentration (0.24 μM/million cells)], while in A549 cells, the nucleus Re concentration decreased to 0.12 μM/10^3 cells and to 0.19/10^3 cells in HeLa (Table I). In MCF-7 Mdr and MCF-7 R, which are MCF-7 cells with an acquired resistance to cytotoxic agents, nucleus Re concentrations also respectively decreased from 0.15 and 0.25 μM/million cells to 0.09 and 0.12 μM/million cells, after the post-exposure period, indicating an efflux of Re out of the nucleus.

Rhenium concentrations in mouse organs. No Re was present in mice of the control group. An uptake of Re was observed in the tissues, with the main uptake in the liver and then in the kidney. There was a significant increase of Re concentration in the blood, the liver and the kidney in mice treated with Re diseleno-ether at the dose of 40 mg/kg/24h *versus* those treated with 10 mg/kg/24 h (Table II).

Selenium concentrations in mouse organs. Se was present in tissues of the control group as it is an essential element, with the highest concentrations being found in the liver and then in the kidney. There was a significant increase of the Se concentration in all tissues of mice treated with Re diseleno-ether at 10 mg/kg/24 h *versus* controls. There was a significant increase of Se in the liver at the dose of Re diseleno-ether of 40 mg/kg/24 h compared to 10 mg/kg/24 h (Table III).

Ratio of Se/Re concentrations in the liver. The ratio of Se/Re was 1.8 at the dose of 10 mg/kg/24 h Re diseleno-ether and 1.6 at the dose of 40 mg/kg/24 h. This is close to the theorical ratio of 2 considering that there are two atoms of Se and one atom of Re per molecule of Re diseleno-ether if the molecule is not metabolized. A four-fold increase in the administered dose of Re diseleno-ether (from 10 to 40 mg/kg/24 h) was followed by an increase of the liver Re concentration by 2.8-fold and of the liver Se concentration by 2.6-fold.

Ratio of Se/Re concentrations in the kidney. The Se/Re ratio was 1.6 at the dose of 10 mg/kg/24 h Re diseleno-ether and 1.0 at the dose of 40 mg/kg/24 h. A 4-fold increase in the administered dose of Re diseleno-ether (from 10 to 40 mg/kg/24 h) was followed by an increase of the kidney Re concentration by 2-fold and of the kidney Se concentration by 1.3-fold. The amount of Re and Se in the kidney was thus less increased than in the liver with increasing the dose of Re diseleno-ether.

Magnesium concentrations. No difference was noted in mice treated with 10 mg/kg/24h *vs*. controls in any tissue. A significant increase of the Mg concentration was observed in mice treated with 40 mg/kg/24 h *vs*. controls in the blood, the bone and the brain (Table IV).

Zinc concentrations. No difference was noted in the Zn concentrations, neither in mice treated with 10 mg/kg/24 h *vs*. controls in all tissues, nor in mice treated with 40 mg/kg/24 h (Table V).

Table III. *Tissue concentrations of Se after oral administration of 10 or 40 mg/kg/24 h Rhenium diseleno-ether, for 4 consecutive weeks, 5 days a week to mice . Data are the means±SEM.*

	µg/kg Wet tissue		
Tissue	Controls (n=18)	10 mg/kg (n=15)	40 mg/kg (n=14)
Blood	291±146	501±332**	479±216
Liver	2175±437	3194±799**	4639±1423*
Kidney	1465±291	2020±542**	2157±402
Lung	178±144	749±677**	472±204
Spleen	136±141	479±489**	223±127
Brain	292±55	858±822**	441±49
Bone	279±55	1081±1239**	482±67
	µMol/kg Wet tissue		
Tissue	Controls (n=18)	10 mg/kg (n=15)	40 mg/kg (n=14)
Blood	3.68±1.85	6.34±4.20**	6.06±2.73
Liver	27.53±5.53	40.43±10.11**	58.72±18.01*
Kidney	18.54±3.68	25.57±6.86**	27.30±5.09
Lung	2.25±1.82	9.48±8.57**	5.97±2.58
Spleen	1.72±1.78	6.06±6.19**	2.82±1.61
Brain	3.70±0.70	10.86±10.41**	5.58±0.62
Bone	3.53±0.70	13.68±15.68**	6.10±0.85

*Statistically different (40 *vs*. 10 mg/kg/24 h). **statistically different (10 mg/kg/24 h *vs*. controls).

Table IV. *Tissue concentrations of Magnesium after oral administration of 10 or 40 mg/kg/24 h Re diseleno-ether, for 4 consecutive weeks, 5 days a week to mice. Data are the mean±SEM.*

	µg/kg Wet tissue		
Tissue	Controls (n=18)	10 mg/kg (n=15)	40 mg/kg (n=14)
Blood	42±26	45±28	74±35***
Liver	323±71	271±75	399±62
Kidney	249±37	222±36	300±45
Lung	177±57	180±71	150±30
Spleen	313±97	339±146	431±143
Brain	982±289	1002±192	1556±167***
Bone	746±127	749±97	1160±139***
	µMol/kg Wet tissue		
Tissue	Controls (n=18)	10 mg/kg (n=15)	40 mg/kg (n=14)
Blood	1.75±1.08	1.88±1.17	3.08±1.46***
Liver	13.46±2.96	11.29±3.13	16.63±2.58
Kidney	10.38±1.54	9.25±1.50	12.50±1.88
Lung	7.38±2.38	7.50±2.96	6.25±1.25
Spleen	13.04±4.04	14.13±6.08	17.96±5.96
Brain	40.92±12.04	41.75±8.00	64.83±6.96***
Bone	31.08±5.29	31.21±4.04	48.33±5.79***

***Statistically different (40 mg/kg/24 h *vs*. controls).

Table V. *Tissue concentrations of Zinc after oral administration to mice of 10 or 40 mg/kg/24 h Rhenium diseleno-ether, for 4 consecutive weeks, 5 days a week. Data are the means±SEM. No statistical difference was observed between the groups.*

	µg/kg Wet tissue		
Tissue	Controls (n=18)	10 mg/kg (n=15)	40 mg/kg (n=14)
Blood	3956±1577	4125±1434	4517±1749
Liver	30400±4096	24489±3781	26579±4276
Kidney	18611±2099	18255±2268	18452±1948
Lung	14480±2772	17589±4825	12654±3345
Spleen	22921±4404	24230±5577	24506±4823
Brain	27428±4119	29429±2483	30851±1752
Bone	27791±3838	29682±6719	27023±1822
	µMol/kg Wet tissue		
Tissue	Controls (n=18)	10 mg/kg (n=15)	40 mg/kg (n=14)
Blood	60.86±24.26	63.46±22.06	69.49±26.91
Liver	467.69±63.02	376.75±58.17	408.91±65.78
Kidney	286.32±32.29	280.85±34.89	283.88±29.97
Lung	222.77±42.65	270.60±74.23	194.68±51.46
Spleen	352.63±67.75	372.77±85.80	377.02±74.20
Brain	421.97±63.37	452.75±38.20	474.63±26.95
Bone	427.55±59.05	456.65±103.27	415.74±28.03

Discussion

Cell uptake, distribution and metabolism of Re and Se. We demonstrated that exposure to Rhenium diseleno-ether induced a Re uptake in the nucleus. The cellular uptake and cytotoxic effects of Re have already been described by Choi *et al*. (2) using octahedral Re cluster complexes in HeLa cells. In our study, there was no efflux of Re after a post-exposure period of 48 h in MCF-7 cells. In contrast, there was an efflux in all other cell types. To avoid the consequences of efflux in these cells, it could be important to maintain a continuous exposure of the malignant cells to the Re metal; this was possible by an oral daily administration.

The Re diseleno-ether is amphiphilic, soluble in water, and thus easy to administer. It also possesses lipophilic properties that allow a great diffusion across cell membranes. This explains the important uptake of both Se and Re in tissues after oral administration of Re diseleno-ether in the animal experiment. This has never been demonstrated before.

Our results were similar to a distribution study (3) showing that the highest concentrations of Se were observed in the liver, then in the kidney and then by decreasing order in the spleen, the pancreas, the cardiac muscle, the brain and the skeletal muscles. In humans, the normal distribution of Se is 30% in the liver, 30% in the muscles, 15% in the kidneys, 10% in the plasma and 15% in the other parts of the organism (4).

The most important liver uptake may be a step before the transfer of metals in the blood and then in other tissues. It is known that after its uptake in the liver, Se will be incorporated in selenoproteins (5-7). In the tissues, there is no free Se, but only Se incorporated in selenoproteins, the active forms of Se. Approximately 60% of Se in plasma is incorporated in selenoprotein P, which contains 10 Se atoms per molecule as selenocysteine, and may serve as a transport protein for Se. In the tissues, the active selenoproteins are the four glutathione peroxidase enzymes (classical GPx1, gastrointestinal GPx2, plasma GPx3, phospholipid hydroperoxide GPx4), with a correlation between the plasma Se concentrations and the GSH-Px activities (8). In the human genome, 25 genes for selenoproteins have been identified (9). The elimination of Se occurs mainly through the kidneys with a tri-phasic half-life of elimination: 1 day, 8-9 days and 115-116 days. In the urine, Se exists as free Se or as methylated Se (3) .

Re-diseleno-ether is a candidate drug for delivering Re in the organism and for increasing the Se concentrations in the organs, where Re and Se will then exert specific biological effects.

Selective uptake of Re and Se by the tumor cells. Further experiments will need to verify that there is a more selective uptake of Re and Se by tumor cells than by healthy cells. However, there are already some arguments in favour of this hypothesis. As shown by Drake, activated oncogenes prime cells for Se-induced pro-oxidative apoptosis, thereby providing the needed margin for killing cancer cells while leaving normal, healthy cells unharmed (10). Various Re compounds synthesized by Ho *et al*. (11) have a similar activity against malignant cells in culture (MOLT-4) with an IC_{50} ranging from 1 to 24 μM, *vs*. 18 μM for cisplatin, with an apparent lack of toxicity for human fibroblasts in culture. New imidoselenocarbamate derivatives have also proved their efficacy against cancer cells (12). They have been evaluated in MCF-7 cancer cells and one non-malignant mammary gland-derived cell line (MCF-10A). The selectivity index could be determined by the comparison of the cytotoxic effects in MCF-7 and MCF-10A cells and the ratio between the cytotoxic parameters found in MCF-10A and MCF-7 was greater than six (13). Se seems to have a remarkable specificity for cancer cells resistant to conventional chemotherapy (14). Se uptake depends on extracellular reduction, and the extracellular environment is a key factor specific to selenite cytotoxicity. The extracellular reduction is mediated by cysteine, and the efficacy is determined by the uptake of cysteine by the x(c)(-) antiporter and secretion of cysteine by multi-drug resistance proteins, both of which are frequently over-expressed by resistant cancer cells.

Biological effects of Re. The main biological effect of Re should be the formation of adducts with proteins or with the DNA. The formation of stable covalent adducts with proteins was shown with lysozyme, with a binding of Re to His15 *via* the replacement of one coordinated water molecule (15), and confirmed by X-ray crystallography (16). Re can bind to DNA adenine through the N1, N6 positions (17), or to guanine through the N7 position (18, 19). The coordinated purine ligands are oriented around the tricarbonyl core with two types of structures elucidated by X-ray crystallography, an HH (head-to-head) and HT (head-to-tail) conformed for each of the guanines (20, 21). Re can induce Re/nucleotide 1:1 or Re/nucleotide 1:2 adducts. In contrast to cisplatin, binding of Re with one or two bases is reversible with less stable adducts than with cisplatin (22, 23). The cytotoxicity of Re (I) folate conjugates in folate receptor (FR)-overexpressing cancer cells was also explained by interactions occurring between the Re (I) complex moiety and DNA (24).

Re may also have other properties and it is due to its protective effect on the erythrocyte membrane that Re clusters have been proposed to counteract the cisplatin-induced anemia action on the oxidative system (25, 26).

Biological effects of Se. Se may function as an intracellular messenger to regulate signaling pathways, the activity of kinases and NF-kappa B, and immune functions, modulating glutathione and reactive oxygen species levels.

Role of Se on the oxidative system. The role of Se as an antioxidant is well-known, attributed to selenoproteins, but it may also have an oxidant effect (27-29). This dual effect may depend on its concentration (30). High doses of Se generate oxygen radicals and lead to apoptotic cell death by directly oxidizing critical thiol-containing cellular substrates (10). Selenate (Se^{+6}) and selenite (Se^{+4}) can be metabolically reduced. However, selenoethers, such as selenomethionine and Se-methylselenocysteine, even though they are not oxidizing agents may be converted to methylselenol (CH3Se-) that can be oxidized to methylseleninic acid or may react with O_2 to produce superoxide and reactive oxygen species (ROS). As an oxidant, Se can be used to kill cancer cells. The inorganic Se compounds, selenate and selenite, due to their pro-oxidant characters, could possess anticancer activity. However, these Se compounds are highly toxic compared to organic Se forms.

Role of Se on signaling pathways. Se compounds, like methylimidoselenocarbamates are described as multi-kinase inhibitors (31). The most effective compound, quinoline imidoselenocarbamate, inhibits the PI3K/Akt/mTOR pathway (32), which is persistently activated and contributes to malignant progression in various cancers. The up-regulation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway is prevalent in many cancers. This phenomenon makes PI3K and Akt fruitful targets for cancer therapy since

they are mediators of cell survival signaling. Se has been shown to decrease Akt phosphorylation at Thr308 and Ser473 in prostate cancer cells (33). An inhibitory effect on activation of Akt by selenite has also been observed in colorectal cancer cells (34). A suppression of the activity of mTORC1 by a Se treatment was observed in HT-29 colon cancer cells, through both Akt-independent and -dependent pathways (35). An inhibition of mTORC2 has also been observed with selenocarbamate (36-38) as well as with selenocyanate (39). Additionally, Se plays an important role by regulating the expression of pro-inflammatory genes in immune cells. Supplementation of Se to Se-deficient macrophages leads to a significant decrease in the LPS-induced expression of two important pro-inflammatory genes, cyclooxygenase-2 (*COX-2*) and tumor necrosis factor-alpha (*TNF-alpha*) *via* the inhibition of MAP kinase pathways (40). The suppression of COX-2 protein expression by selenomethionine was also observed in human colon cancer cell lines (41).

Se compounds may also activate p53 and p38 pathways (42, 43) and inhibit PSA expression in the androgen-responsive LNCaP prostate cancer cell (44).

A large number of potential Se-responsive genes with diverse biological functions such as inhibition of cell invasion, DNA repair, and stimulation of transforming growth factor beta signaling have been identified. A genome-wide analysis could be useful to select patients that may benefit from a Se compound (45). An up-regulation of pro-apoptotic genes, and a down-regulation of cell growth-regulatory genes was observed in a model of mammary adenocarcinomas induced by 7,12-dimethylbenz[*a*] anthracene (DMBA), in the rat, after treatment by a selenocyanate compound (46).

Role of Se on NF-κB. One other major role of Se is its ability to inhibit NF-κB activation and up-regulate IκBα normal half-life (47). All inflammatory diseases may activate NF-κB by producing ROS and the main marker of inflammation in patients is C Reactive Protein (CRP). Se decreases the synthesis of CRP while inhibiting the binding of NF-κB to gene promoters, attenuating cytokine releases (48).

The inhibition of NF-κB by Se may be efficient in decreasing osteoclast activation in patients with bone metastases. Pro-inflammatory mediators such as interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), cyclooxygenase-2 and inducible nitric oxide synthase (iNOS), expressed by osteoblasts when stimulated in co-culture with human breast cancer cells (MDA-MB-231), are pivotal to osteoclast activation and metastasis. These genes are regulated by NF-κB. Supplementation of osteoblasts with Se reduced the activation of NF-κB leading to a decrease in IL-6, MCP-1, COX-2 and iNOS in response to MDA-MB-231 conditioned medium (49).

Role of Se on immune system. Finally, Se compounds may play a role in the immunity of cancer patients, through redox signaling (50). Se can stimulate antibody formation, the activity of helper T cells, cytotoxic T cells and Natural Killer Cells (51-55) and induce metalloproteinase-dependent L-selectin shedding from monocytes (56). Se may also interfere with immunity *via* selenoprotein action on macrophages (57). However, an excess of Se could in contrast inhibit the activity of natural and lymphokine-activated killer cells (58, 59).

Interactions between Re, Se and metals. We noted an increase in Mg concentration, but only in mice treated with 40 mg/kg of Rhenium diseleno-ether compound *vs*. control in the blood, the bone and the brain. No change was observed in Zn concentration. However, known interactions exist between Se and Zn finger proteins. Reducible Se compounds interfere with the DNA-binding capacity of a Zn finger protein essential for nucleotide excision repair with a release of Zn from the Zn finger motif. Zinc finger motifs are highly reactive towards oxidizing Se compounds. The oxidation of thiol groups of metallothionein by Se may induce a release of Zn providing it for essential reactions (60).

Effects of Re on malignant cells. As a result of their action on DNA, Re compounds can induce cytotoxic effects on malignant cells, with mode of action depending on the ligand. For example, Re (I) thymidine complexes were tested against A549 lung carcinoma cell line (61); moderate toxicity was observed for conjugates carrying the Re moiety at position C5' or N3 with an IC_{50}=124-160 μM. No toxicity was observed for complexes modified at C2' or C5. A complex with a dodecylene spacer at C5' exhibited remarkable toxicity, more potent than cisplatin, with an IC_{50} value of 6.0 μM. With oximine Re (I) compounds, chlorido complexes were more efficient than bromido compounds in inducing apoptotic cell death of cancer cells (62). Long-chain Re glucosamine conjugates were found to be non-toxic with doses as high as 1 mM (63). Very recently, Leonidova *et al*. synthesized organometallic Re (I) compounds with specific phototoxic effects towards malignant cells (64). The apoptotic effects with Re (IV) compounds have been observed by Martinez-Lillo J *et al*. (65).

Effects of Se on malignant cells. Se compounds may induce cell deaths. Sanmartin *et al*. reviewed different processes of cell death induced by Se compounds (36). The mechanisms of Se-induced apoptosis are associated with the chemical forms of Se. Modulation of mitochondrial functions has been reported to play a key role in regulation of apoptosis and also to be a target for Se compounds.

Imidoselenocarbamates have been compared with imidothiocarbamate derivatives (containing sulfur instead of Se) (12). The nature of the heteroatom (Se better than S) has

a marked influence on the anti-proliferative activity of the compounds. The active role of Se has also been demonstrated in a study performed by Moreno *et al*. (66) that replaced sulfur by Se in many quinazoline and pyrido [2,3-d] pyrimidine derivatives. Se compounds showed a greater activity against tumor cell lines.

Selenite is cytotoxic in low-to-moderate concentrations, with a remarkable specificity for cancer cells resistant to conventional chemotherapy (14). However, very low doses of Se may produce adverse effects, as shown in LNCaP prostate cancer cells (69).

Synergistic effects have been shown between methylselenic acid and taxanes with greater than additive apoptosis effect on DU145 and PC-3 HRPCa cells (67). A synergistic effect of doxorubicin and selenium has also been observed in apoptosis induction in MCF-7 breast cancer cells. It was shown that doxorubicin and selenium cooperatively activate Fas signaling by targeting key regulatory steps (68).

Se may protect the cells. Se may induce death in cancer cells, but in other cases protects the cells, by reducing oxidative stress. It was shown that Se could reduce hypoxia-induced apoptosis in a neuroblastoma cell line (70). In healthy cells (leucocytes), selenomethionine may prevent the toxicity of bleomycin, decreasing bleomycin-induced strand breaks and favoring the repair of DNA damage (71). Simple salts of Se (Na_2SeO_4) behave as nucleophilic targets for the electrophilic alkylating agents, thereby preventing DNA damage from alkylating agents (72).

Se is implicated in metal detoxification. Se compounds may prevent metal-mediated radical and non-radical oxidative DNA damage (73, 74). Metal binding with Se-containing compounds is the primary mechanism of Se antioxidant activity, and selenoproteins play an important role in protection against metal toxicity (75). For example, equimolar Hg and Se bind to selenoprotein P (Sel P) to form a complex and more than 1,000 units of (Hg-Se) may bind to Sel P based on the fact that there are 10 selenocysteinyl residues per Sel P (76). On the other hand, selenite-induced cytotoxicity and apoptosis in human carcinoma cells can be inhibited with copper ($CuSO_4$) as an antioxidant (77). In contrast, simultaneous treatment with selenite and cadmium (Cd) nitrate increases the Cd cellular uptake, and may thus increase the Cd toxicity (78). Due to the multiple interactions between Se and metals, the interactions between Se and platinum (Pt) or gallium (Ga) have to be considered in order to interpret the results in the case of a combined therapy with Re diseleno-ether and Pt or Ga compounds (79).

Antitumour activity of Re compounds in animal models. Several cluster Re compounds have been studied by A. Shtemenko, N. Shtemenko, P. Collery *et al*. Re compounds with GABA ligands (80), Rhenium di-adamantate (81) and dichlorotetra-μ-isobutyratodirhenium (III) (82) were injected subcutaneously, as liposomes, in rats bearing tumours. While they have little effect on tumor growth, they statistically potentiate cisplatin activity.

Anti-tumour activity of Se compounds in animals. In our study, we used a dose of 10 mg/kg Re diseleno-ether, containing 2.4 mg/kg Se or 2.4 ppm Se, or a dose of 40 mg/kg Re diseleno-ether, containing 9.5 mg/kg Se or 9.5 ppm Se. An oral administration of doses ranging from 3 ppm to 40 ppm Se, as inorganic (83) or organic Se, mainly selenocyanate compounds showed an antitumour activity without significant toxicity, in a model of orthotopic PC3 tumors in the prostates of male nude mice, with a parallel decrease in angiogenesis (83) or in the prevention of chemically-induced tumors (84-86). The strongest effect has been observed with imidoselenocarbamate compounds, *via* intraperitoneally injections; a quinoline imidoseleno-carbamate was found to suppress prostate tumor growth by almost 80% in a mouse xenograft model, without causing toxicity (32).

Se compounds may also be used to potentiate anticancer drugs, like irinotecan, paclitaxel or tamoxifen (67, 87, 88). The prevention of the cisplatin (CDDP) toxicity by Se (sodium selenite) has been noted, without affecting its efficacy on the growth of a human yolk sac tumour grown in nude mice (89). The *in vivo* synergic interaction with anticancer drugs seems to be highly dependent on the method of administration of Se.

Clinical trials with Re compounds. We did not find any clinical trial with stable, non-radioactive Re compounds in the literature.

Clinical trials with Se compounds. The low adverse effect level of Se has been estimated in humans by Yang *et al*. (90) to be 1540±653 μg/24h and the no-adverse effect level 819±126 μg/24h. However, the observations in a polluted environment with a chronic exposure to inorganic Se cannot justify that organic compounds will have the same toxicity profile.

Intakes of 400 μg/day and plasma selenium of 1000 ng/ml (Dietary Reference Intakes, Academy Press, New York, 2000, p. 384) have been established as the no observed adverse effect level (NOAEL), for Se as an antioxidant; however, this cannot be a definitive rule for new compounds aiming to treat cancer patients.

In fact, a dose of 200 μg/day Se failed to demonstrate significant effects as reported in a meta-analysis of clinical trials concerning the prevention of cancer by Se (91). In these trials, the usual dose of Se was 200 μg/24 h Se, mainly as selenised yeast tablets. However, at a dose of 200 μg/24 h

Se, an increase in plasma Se concentration has been observed in several studies. After an oral administration of 200 μg Se methionine in 18 patients for 6 months, plasma Se concentrations increased from 78±8 μg/l to 97±8.4 μg/l (92). Following Se supplementation in a daily dose of 200 μg, in patients with ovarian cancer undergoing chemotherapy (93), Se concentration in serum significantly increased after 2 and 3 months of supplementation, as compared to the values after 1 month. A significant increase in the activity of GSH-P(x) in erythrocytes after 3 months of supplementation was also noted. In an other study, 36 women aged between 18 and 23 years received (daily for 32 weeks), 200 μg Se as Se-enriched yeast (selenomethionine, SeMet), or brewer's yeast mixed with selenate, or no added Se (placebo) in a double-blind trial (94). Plasma Se plateaued at 190 ng/ml for the SeMet at week 16, and the selenate group reached an earlier plateau of 110 ng Se/ml at week 7. Trimethylselenonium was detected in basal urines, as a minor component, in supplemented subjects.

High doses of Se have been administered in men with biopsy-proven prostate cancer (95). They received either 1,600 or 3,200 μg/day of selenized yeast, for almost 12 months. Eight subjects were treated with 1,600 μg/day and 16 with 3,200 μg/day. The mean plasma Se levels achieved with supplementation were 492.2 ng/ml (SD=188.3) and 639.7 ng/ml (SD=490.7) for the 1,600 and 3,200 μg/day doses, respectively. No obvious selenium-related toxicities were observed.

In two randomised studies, with a small number of patients treated by radiotherapy, one for advanced head and neck cancer (96), and one for cervical and uterine cancer (97), the patients receiving a dose of 500 μg sodium selenite on days of radiotherapy and 300 μg sodium selenite on days without radiotherapy were compared to those irradiated without any Se substitution. Weekly patient analysis for the Se group showed a significant reduction of dysphagia (head and neck cancer) and in the number of episodes and severity of RT-induced diarrhea (cervical and uterine cancer).

There is still a great need for phase I clinical trials with Re and/or Se compounds in patients with advanced cancer, with the aim to determine the most effective dose to be administered, especially of organic compounds that can be given orally. This is our objective with our Re diseleno-ether compound, following completion of the required pre-clinical studies.

Conclusion

We propose a new compound, Re diseleno-ether, combining one atom of Re and two atoms of Se as a new anticancer agent. We demonstrated that both the uptake of Re in the nucleus of malignant cells and its efflux depends on cell type. We verified that following oral administration in mice there was a dose-dependent uptake of both Re and Se in the main tissues of the organism, and mainly in the liver. There is some evidence that Re and Se have potential biological interesting properties, on the formation of adducts with nucleosides for Re, and on the oxidative system, the Pi3 kinase/Akt and MTOR pathway, the regulation of NF-κB and on the activation of T lymphocytes and NK cells, for Se. Beneficial therapeutic effects may be expected in cancer patients, thanks to the antitumor properties of both Re and Se, but also due to the effects of Se against inflammation, and as an enhancer of immunity. However, the doses as well as the method of treatment shoud be carefully managed, due to the dual role of Se, as pro-oxidant or anti-oxidant. Too low doses could have an opposite effect and stimulate cancer growth. On the other hand, in the case of a combined treatment with cytotoxic agents, Re diseleno-ether could act as a detoxyfying agent and prevent DNA damage caused by alkylating agents or, in contrast, synergistically improve their efficacy. Pre-clinical and toxicological data in animals will be the next step to define the therapeutic index of this promising new compound.

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