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Uptake-release by MSCs of a cationic platinum(II) complex active *in vitro* on human malignant cancer cell lines

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ABSTRACT

In this study, the *in vitro* stability of cisplatin (CisPt) and cationic platinum(II)-complex (caPt(II)-complex) and their *in vitro* activity (antiproliferative and anti-angiogenic properties) were investigated against three aggressive human tumor cell lines. caPt(II)-complex shown a high stability until 9 days of treatment and displayed a significant and higher activity than CisPt against both NCI-H28 mesothelioma (19.37 ± 9.57 μM *versus* 34.66 ± 7.65 μM for CisPt) and U87 MG glioblastoma (19.85 ± 0.97 μM *versus* 54.14 ± 3.19 for CisPt). Mesenchymal Stromal Cells (AT-MSCs) showed a significant different sensitivity (IC_{50} = 71.9 ± 15.1 μM for caPt(II)-complex and 8.7 ± 4.5 μM for CisPt) to the antiproliferative activity of caPt(II)-complex and CisPt. The ability of MSCs to uptake both the drugs in a similar amount of 2.49 pM /cell, suggested a possible development of new therapies based on cell mediated drug delivery.

1. Introduction

In the last decades the field of cancer therapeutic treatment has been marked by important advancements thanks to the introduction of new surgical procedures and new chemotherapeutic drugs. Unfortunately, the 4th most common cause of cancer-related death is due to three different tumors still lacking for a proper response: malignant pleural mesothelioma (MPM), [1] glioblastoma multiforme (GBM) [2] and pancreatic adenocarcinoma (PAC) [3]. These types of solid tumor are in fact difficult to defeat because they spread aggressively and resulted highly resistant to conventional chemotherapeutic agents other than the fact that they exhibited a high propensity to recur.

MPM is a malignant neoplasm of the pleura related to asbestos exposure. It tends to grow over the serosal surface and finally encases the lung, causing death by asphyxiation. Currently the standard first-line treatment is a platinum-based doublet containing a third-generation antifolate like pemetrexed (PMX) but any second-line treatments for MPM has been approved yet [4–6].

GBM is a highly malignant and aggressive primary brain tumor. Despite of an arsenal of therapeutic interventions, the prognosis of glioblastoma remains very poor. Cisplatin based therapy is one of the most important chemotherapy treatments for GBM and it is repurposed as the second line against GBM albeit its efficacy is limited by drug resistance and undesirable side effects including neurotoxicity that limits its efficacy [7,8].

PAC has recently emerged as one of the most aggressive tumors with a death rate that has remained relatively stable in the last ten years, thus providing a limited progress in this field. In the treatment of

Abbreviations: MPM, malignant pleural mesothelioma; GBM, glioblastoma multiforme; PAC, pancreatic adenocarcinoma; GCB, gemcitabine; CisPt, cisplatin; MSCs, mesenchymal stromal cells; caPt(II)-complex, cationic platinum(II) complex; CFPAC-$_1$, pancreatic carcinoma cell lines; U87, glioblastoma; NCI-H28, mesothelioma

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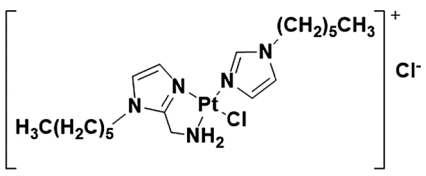
pancreatic cancer, platinum derivatives are frequently used in combination schedules with gemcitabine (GCB) in several phase II trials [9,10].

These tumors remain some of the most lethal thus evoking the need for new therapeutic treatments in order to improve patient survival and quality of life. To date, the use of platinum drugs for the treatment of these pathologies has shown minimal success mainly due to a failure in the delivery to the tumor site so that high doses of the platinum chemotherapeutic agent is required for a positive response to the therapy. Over the last thirty years, the research field of platinum anticancer drugs has experienced an extensive development with the recognition of the potential anticancer activity of platinum compounds whose novel structural properties developed for active platinum drugs allowed to enlarge the spectrum of activity, to overcome cellular resistance and to lower toxicity. In this regard, violating the apparently demanded neutrality, many cationic monofunctional platinum-based anticancer agents were synthetized and characterized [11]. This focusing is because, in comparison with the bifunctional cisplatin, monofunctional compounds display a distinct mechanism of action and a different antitumor profile taking into consideration their ability to effectively bind to DNA and to inhibit transcription both *in vitro* and *in vivo*. They are often characterized by the general formula cis-$[Pt(NH_3)_2(L)Cl]^+$, where L is an *N*-heterocycle and the choice for L is usually for a small *N*-heterocyclic amine ligand with a molecular weight less than 200, such as pyridine (for pyriplatin) or phenanthridine (for phenanthriplatin). Indeed, the choice of the *N*-heterocycle ligand has been suggested as the main responsible for determining the anticancer activity spectrum and the relative mechanism of monofunctional platinum complexes [12]. Upon the above premises, many cationic platinum complexes have been identified with different biological behavior as compared to cisplatin but endowed with equivalent or higher potential to kill cancerous cells [13].

Recently, our research group has synthesized a series of cationic bulky triamine platinum compounds of general formula [Pt(N-N′)N′Cl] X^- where *N-N′* is an aminomethylimidazole ligand [14] and the *N′* an imidazole ring, both bearing the same alkyl group at the *N1* position. Notably, when the alkyl group is a long linear carbon chain (C6) as in complex **2c**, the cationic platinum (II) compound showed a very effective and potent cytotoxic effect in triple-negative breast cancer cells and in cell lines partially resistant to cisplatin [15]. Its cytotoxic capability closed to its completely different pharmacodynamic and cellular uptake behavior than cisplatin, makes it as a valid candidate for evaluating cytotoxicity in these three tumor cell lines (Scheme 1).

As previously reported mesenchymal stromal cells (MSCs) from different tissues are able to uptake drugs (eg.: Paclitaxel, Gemcitabine, Doxorubicin et al.) [16–20] and release them both as free molecule and exosome associated drugs [21]. The drug is released *in vitro* in amounts effective against cancer cells and also *in vivo* the drug loaded MSCs may be used as a physiological tool for drug delivery by injecting them both *in situ* and by systemic way [22]. These characteristics of MSCs suggest new strategies to apply in advanced cell therapy, in particular for treating cancer. Their potential application is in part depending on the solubility of the drug in medium and their stability during the drug loading procedure. The same platinum-based drugs have very limited solubility in culture medium and also can suffer degradation process by pH modification and temperature [23]. The present study evaluated these biological characteristics of platinum based drugs by comparing a new cationic platinum(II) complex (caPt (II)-complex) to cisplatin (CisPt). Our results evidenced that caPt (II)-complex has a very high stability when incubated at 37 °C in cell culture medium and that its *in vitro* activity against glioblastoma and mesothelioma was significantly higher than that exerted by CisPt. We also demonstrated that caPt (II)-complex can be easy incorporate and release by MSCs without loss of its anticancer activity.



Scheme 1. Cationic platinum(II)-complex (caPt(II)-complex).

2. Materials & methods

2.1. Drugs and tumor cell lines

Rates of Cisplatin (CisPt) and caPt (II)-complex were prepared at the concentration of 4 mg/ml in dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA). Working solutions were freshly prepared according to the experimental design by serial dilutions in complete culture medium. The chemotherapeutic drug Paclitaxel (PTX, kindly provided by Freseneius-Kabi, Italy; stock solution for infusion 6 mg/ml), was used as standard drug for antiproliferation assays. The *in vitro* activity of CisPt, caPt (II)-complex and PTX was tested against three tumor cell lines: malignant pleural mesothelioma (MPM) cell line NCI-H28 [24], glioblastoma cell line U87 MG [25] and pancreatic adenocarcinoma cell line CFPAC-1 [26], used as laboratory standard cancer cell line. Cells lines were maintained by 1:5 weekly dilution in Roswell Park Memorial Institute medium (RPMI) medium (NCIH28), Dulbecco's Modified Eagle (DMEM LG) medium (U87 MG) and Iscove's Modified Dulbecco's Medium (IMDM) medium (CFPAC-1), supplemented by Foetal bovine serum (FBS) 10%. All reagents for culture were provided by Euroclone, UK.

2.2. Adipose tissue mesenchymal stromal cells (AT-MSCs)

As starting biological material were used lipoaspirate samples of adult donors after signed informed consent of no objection for the use for research of surgical tissues (otherwise destined for destruction) in accordance with the Declaration of Helsinki. The approval for their use was obtained from the Institutional Ethical Committee of Milan University (n.59/15, C.E. UNIMI, 09.1115). The Adipose Tissue Mesenchymal stromal cells (AT-MSCs) were isolated from 20 ml of lipoaspirate processed as previously described by using Lipogems device (Lipogems® International Spa) [27] and 2 ml for a sample of lipogems (LG) were processed for MSC isolation as previously described [28]. Briefly, the sample was disaggregated by enzymatic digestion with 200 U/ml of collagenase tipe I (Life technologies, USA), then was centrifuged (1000xg, 15 min) and the floating fraction was separated from the cellular pellet. Cellular pellet was plated on 25cm^2 flask (Euroclone, UK) and expanded in StemMACS medium (Miltenyi Biotec, Germany) until passage 3. Primary cultures were analyzed for their proliferation rate (Population doubling time), clonogenicity (CFU-F assay), expression of the typical mesenchymal stem cell markers and multi-differentiative ability towards mesodermal lineages (osteogenic, adipogenic and chondrogenic differentiation).

2.3. Drug sensitivity of tumor cells and AT-MSCs to Cisplatin (CisPt), caPt (II)-complex and PTX

The effect of CisPt and caPt (II)-complex against cell proliferation has been studied in 96 multiwell plates (Sarstedt, Germany). Briefly, 1:2 serial dilutions of pure drug (from 0.15 to 20 μg/ml) were prepared in 100 μl of culture medium/well and then to each well were added 2000 tumor cells. As standard internal positive control, the sensitivity of tumor cells to paclitaxel (PTX) were determined by adding the drugs at increasing two folds concentrations from 0.78 to 50 ng/ml. After 7 days of culture (anti-proliferative assay) at 37 °C and 5% CO_2, cell growth was evaluated by MTT assay (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium) as previously described [29,30]. Against AT-MSCs, also cytotoxicity assay (24 h at 37 °C, 5% CO_2) was performed at