SENSITIVITY OF MESENCHYMAL STROMAL CELLS TO A NEW IMIDAZOLE-BASED CATIONIC Pt(II) COMPLEX WITH HIGH *in vitro* ANTICANCER ACTIVITY

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INTRODUCTION

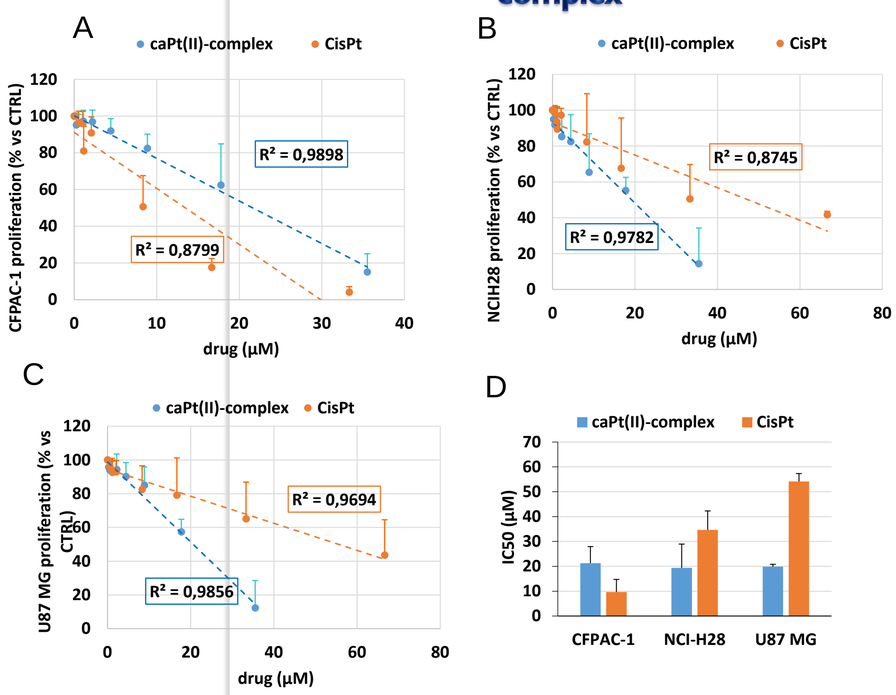
Platinum drugs endowed with a novel chemical structure could offer an alternative therapeutic strategy, allowing to enlarge the spectrum of activity and to overcome the many drawbacks of the well-known cisplatin (CisPt) and its derivatives **[1].** Our group synthetised a new caPt(II)-complex that showed a very effective cytotoxic effect on triple-negative breast cancer cells and on cell lines partially resistant to cisplatin **[2].** As previously reported Mesenchymal Stromal Cells (MSCs) from different tissues are able to uptake and then release drugs as free molecules and exosome associated drugs **[3,4]** suggesting new strategies to be apply in advanced cell therapy for treating cancer. The application of this strategy is in part depending on the solubility of the drug in medium and their stability during the drug loading procedure and may be that some anticancer molecules do not have these features. The same platinum-based drugs have very limited solubility in culture medium and also can suffer degradation process by pH modification and temperature. In this study, we compared the *in vitro* stability of CisPt and caPt(II)-complex and their *in vitro* activity against human tumour cell lines. The drug sensitivity of Mesenchymal Stromal Cells (MSCs) and their ability to uptake and release the drugs was also investigated.

MATERIALS & METHODS

AT-MSCs were isolated by enzymatic digestion with collagenase type I and expanded in StemMACS medium (Miltenyi Biotec, Germany) until passage 3. Primary cultures were analysed for their proliferation rate (Population doubling time), clonogenicity (CFU-F assay) and expression of the typical mesenchymal stem cell markers and multi differentiative ability towards mesodermal lineages. Drug stability was studied following incubation of CisPt and caPt(II)-complex at 37°C in complete cell culture medium both in the absence and in the presence of a monolayer of MSCs.

The activity of CisPt and caPt(II)-complex was tested on AT-MSCs and against malignant pleural mesothelioma cell line NCI-H28 **[5],** glioblastoma cell line U87 MG **[6]** and pancreatic adenocarcinoma cell line CFPAC-1 **[7]** (used as laboratory standard cancer cell line), by using a MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium) anti-proliferative assay in 96 multiwell plates **[8,9].** AT-MSCs were exposed to Cis-Pt (166.5 μM) or caPt (II)-complex (85 μM) for 24 hours and the amount of drugs incorporated and released by the cells was evaluated by the inductively coupled plasma mass spectrometry (ICP-MS). Data were expressed as average ± standard deviation (SD) and the differences evaluated according to Student's t-test performed. p values ≤ 0.05 were considered statistically significant. The linearity of response and the correlation were studied using regression analysis, by Excel 2013 software (Microsoft, Inc.)

Figure 1: *In vitro* anticancer activity of Cis-Pt and caPt (II)-complex



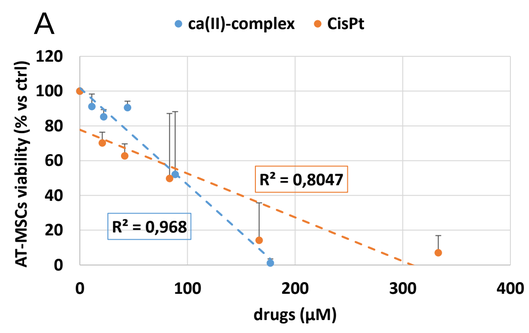
The activity of CisPt and caPt(II)-complex was evaluated *in vitro* against three human cancer cell lines, (pancreatic carcinoma CFPAC-1, glioblastoma U87 and mesothelioma NCI-H28) showed a dose-response kinetics of inhibition with a significant coefficient of correlation (R^2).

(A). Against CFPAC-1 proliferation, CisPt was significantly ($p<0.01$) more active (IC50 = 9.64 ± 5.10 μM) than caPt(II)-complex (IC50= 21.25 ± 6.68 μM).

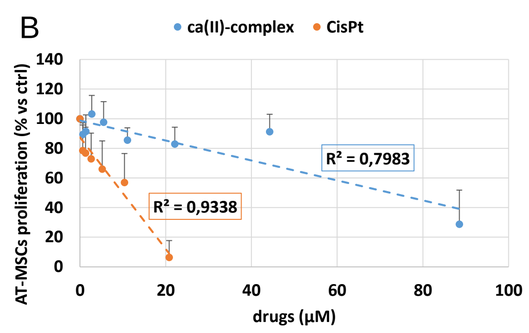
(B,C). A significant higher activity ($p<0.05$) of caPt(II)-complex was seen both against NCI-H28 mesothelioma cells (IC50 = 19.37 ± 9.57 μM versus 34.66 ± 7.65 μM for cisPt) and against U87 MG (19.85 ± 0.97 μM versus 54.14 ± 3.19 μM for cisPt). (D). IC50 values (μM) expressed as histogram.

Figure 2: Mesenchymal stromal cells sensitivity to Cis-Pt and caPt (II)-complex

In vitro cytotoxicity test



In vitro antiproliferation test



C

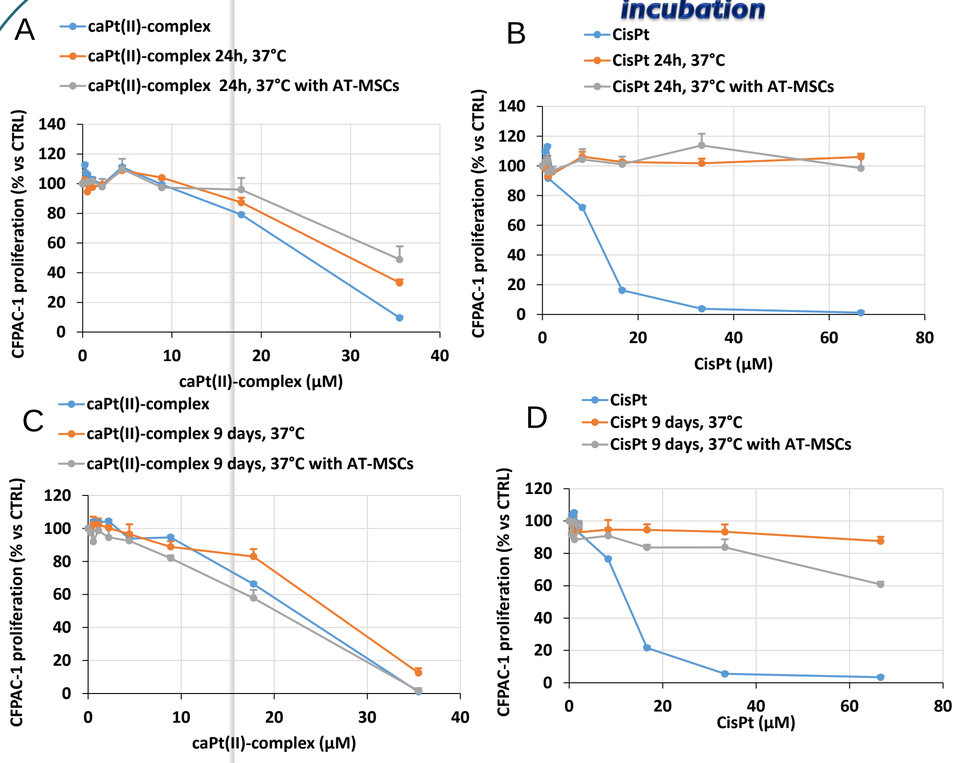
R&M	IC50 (μM)	
mean ± SD	caPt(II)-complex	CisPt
AT-MSCs	91.11 ±29.47	82.22 ± 54.25

D

R&M	IC50 (μM)	
mean ± SD	caPt(II)-complex	CisPt
AT-MSCs	69.9 ± 5.58	11.41 ±5.28

The two drugs were tested both for their cytotoxic **(A)** and antiproliferative activity **(B)** on AT-MSCs. As evidenced by the linear regression analysis, in the 24 hours cytotoxicity assay the MSCs showed to have a similar sensitivity both to caPt(II)-complex and to CisPt **(C).** On the contrary, in the antiproliferation test the IC50 for the two drugs were significantly ($p< 0.05$) different **(D).**

Figure 3: Stability of Cis-Pt and caPt (II)-complex at 37°C of incubation



F

	IC50 (μM)
CisPt fresh	11.29 ± 0.51
9 days, 37°C	> 66.60
9 days, 37°C with AT-MSCs	> 66.60
caPt(II)-complex fresh	22.58 ± 1.29
9 days, 37°C	19.51 ± 0.92
9 days, 37°C with AT-MSCs	24.53 ± 0.79

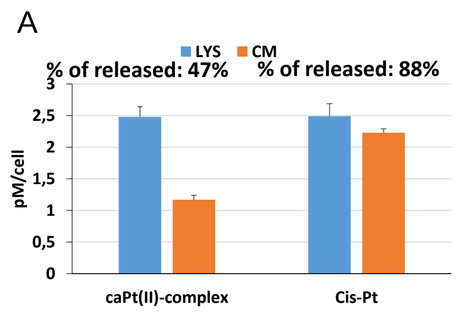
Both CisPt and caPt(II)-complex were studied for their stability following the incubation at 37°C in the complete cell culture medium both in the absence and in the presence of a monolayer of AT-MSCs. The stability has been evaluated by determining the dose response inhibiting kinetics against CFPAC-1 proliferation.

(A,C): caPt(II)-complex maintained its ability to inhibit cancer cell proliferation after 24 hours and 9 days of incubation, with a dose response kinetics similar to that of the fresh drug both if incubated in the absence and presence of MSCs monolayer.

(B,D): CisPt completely lost its anticancer activity after 24 hours (and obviously, after 9 days) in the both two incubation conditions.

(E,F): By comparing the IC50 values of caPt(II)-complex no significant differences ($p<0.05$) were observed between the fresh and the 9 days incubated drug.

Figure 4: Uptake Release of CisPt and caPt(II)-complex by AT-MSCs



B

pM/cell	caPt(II)-complex	Cis-Pt
LYS	2.48 ± 0.16	2.49 ± 0.2
CM	1.17 ± 0.07	2.23 ± 0.06
% of released	47	88

To evaluate the amount of the two drugs both in conditioned media (CM) and cell lysates, a IC-MS analysis was performed, that demonstrated that MSCs were able to incorporate both the drugs. As reported in figure (**A,B**) the CisPt found in the lysate was in amount of 2.48 ± 0.16 pM/cell and that of caPt (II)-complex of 2.49 ± 0.2 pM/cell. To verify their release from the MSCs, the presence of the two drugs were also determined in the CM. The amount resulted of 1.17 ± 0.07 pM/cell for caPt(II)-complex and of 2.23 ± 0.06 pM/cell for CisPt respectively. This indicated that at 24 hs of subculture the drug loaded MSCs released about 47% of the caPt(II)- complex and 88 % of the CisPt.

DISCUSSION AND CONCLUSIONS

Our results evidenced that caPt (II)-complex exerts a remarkable anticancer activity *in vitro* against the three cancer cells lines studied. While the activity against pancreatic carcinoma cells exerted by caPt(II)-complex is lower than that exerted by cisPt (21.25 ± 6.68 vs 9.64 ± 5.10 μM) a significant ($p<0.05$) higher activity of this new molecule was evidenced both against human mesothelioma NCIH28 (19.37 ± 9.57 vs 34.66 ± 7.65 μM) and a human glioblastoma cell line U87 MG (19.85 ± 0.97 vs 54.14 ± 3.19 μM).

Concerning the stability, our findings evidenced that caPt(II)-complex is a very stable compound if compared to CisPt. The treatment of the two drugs at 37°C demonstrated that whereas CisPt lost all its anticancer activity after only 24 hours of treatment, caPt(II)-complex maintain 100 % of its activity after a long time of incubation (**figure 3**). The cancer activity is maintained also if caPt(II)-complex is incubated in the presence of a monolayer of MSCs confirming that the molecule did not suffer modification due to cell metabolism and significant variation of pH of the culture medium produced by the cell growth (**figure 3 A,C**).

The analytical data on lysates confirmed a significant incorporation of both the molecule that was very similar (caPt(II)-complex 2.49 ± 0.2 pM/cell and CisPt 2.48 ± 0.16 pM/cell) while the amount of drug released in the conditioned medium was of 47 % for caPt(II)-complex and near to 90 % for CisPt. (**see figure 4A**).

In conclusion, the high stability of caPt(II)-complex together with its significant anticancer activity against mesothelioma and glioblastoma makes this new platinum derivative a very interesting molecule able to improve cancer chemotherapy. Furthermore, the low sensitivity of AT-MSCs to the antiproliferative action exerted by caPt(II)-complex together with their ability to uptake and release the drug suggest further investigation in order to optimize the drug loading procedure and verify the possibility to set up a system based on cell mediated delivery of caPt(II)-complex.

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