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Palladium-spermine complex (Pd_2Spm) triggers autophagy and caspase-independent cell death in triple-negative breast cancer cells

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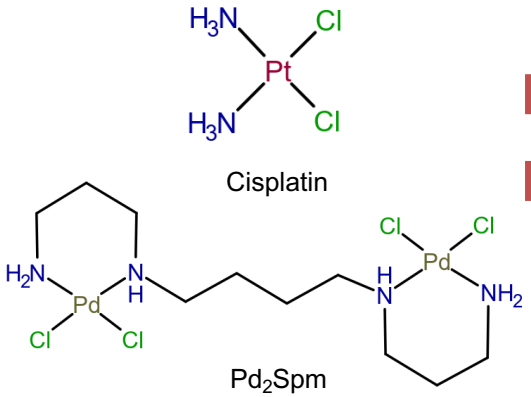
Palladium-spermine complex (Pd_2Spm) triggers autophagy and caspase-independent cell death in triple-negative breast cancer cells

Graphical Abstract



MDA-MB-231 breast cancer cell line

MDA-MB-231/R breast cancer cell line

Proliferation studies

Effects on proliferation in presence of **autophagy, apoptosis and necroptosis inhibitors**

Protein expression (WB) – autophagy and apoptosis markers

Phosphatidylserine externalization - marker of apoptosis

Abstract:

Triple-negative breast cancer (TNBC) is an aggressive breast carcinoma with a poor prognosis. Current treatment options with platinum-(Pt)-based chemotherapeutics are limited by toxicity/acquired resistance, which prompted the search for novel metal-based compounds. The dinuclear palladium(II)-spermine chelate (Pd_2Spm) has previously shown promising pharmacokinetics and *in vivo* antitumor effects. However, its impact towards chemotherapy-resistant TNBC is still to be addressed. This work developed a cell model of cisplatin resistance and compared the anticancer/antiproliferative effects of cisplatin (reference Pt-based drug) and Pd_2Spm in TNBC cells sensitive (MDA-MB-231) and resistant to cisplatin (MDA-MB-231/R). Pd_2Spm displayed a similar antiproliferative potency in MDA-MB-231 and MDA-MB-231/R cells, while cisplatin showed *ca.* 18-fold lower potency towards MDA-MB-231/R cells. When focusing on cell death, incubation of Pd_2Spm with either Necrostatin-1 (necroptosis inhibitor), Z-VAD (apoptosis inhibitor) or 3-Methyladenine (3-MA, autophagy inhibitor) showed that 3-MA can rescue Pd_2Spm-induced growth inhibition in MDA-MB-231 and MDA-MB-231/R cells. Furthermore, in MDA-MB-231 cells, Pd_2Spm triggered higher LC3-II levels and more profound Beclin-1 inhibition than cisplatin. Regarding apoptosis, Pd_2Spm did not induce the cleavage of caspase-3 and co-incubation with both Pd_2Spm and Z-VAD yielded only marginal effects in preventing the phosphatidylserine externalization compared to cisplatin. Thus, the present data provided more evidence on Pd_2Spm's cell death mechanisms, triggering a caspase-independent cell death with autophagy involvement. In addition, the potential of Pd_2Spm to overcome chemotherapy resistance is promising. Funding: PD/BD/135460/2017;UIDB/50006/2020;UIDB/00070/2020;UIDP/00070/2020.

Keywords:

breast cancer; Pd_2Spm; Pd(II)-based complexes; cisplatin

Introduction

- **Breast cancer** is the most **common cancer** and **leading type of cancer death** in women worldwide
- **Triple-negative breast cancer** (TNBC) is the **most aggressive subtype** of breast cancer
- **Current therapy with platinum(II)-based drugs** (cisplatin, carboplatin and oxaliplatin) **present limitation** (toxicity and development tumour resistance) that stimulate development of novel metal-based compounds
- **Pd_2Spm** [**di-nuclear palladium chelate with spermine** (biogenic polyamine)] with **promising pharmacokinetics and in vivo effects** in mice.
- **In vitro mode of action** of Pd_2Spm is herein studied

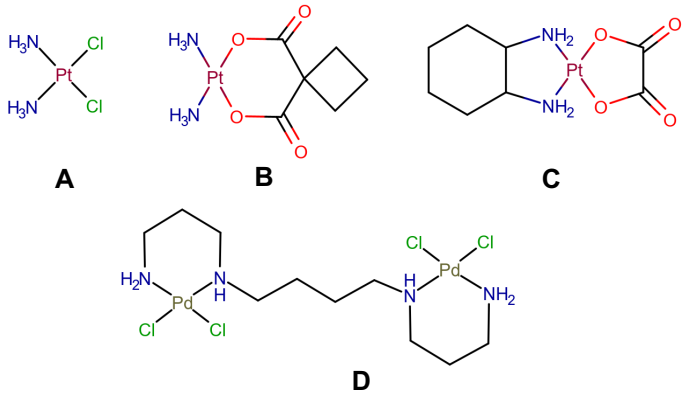


Figure 1. Structure of Platinum(II) and Palladium(II) compounds. **(A)** Cisplatin, (**B)** Carboplatin, **(C)** Oxaliplatin, (**D)** Pd_2Spm

Results

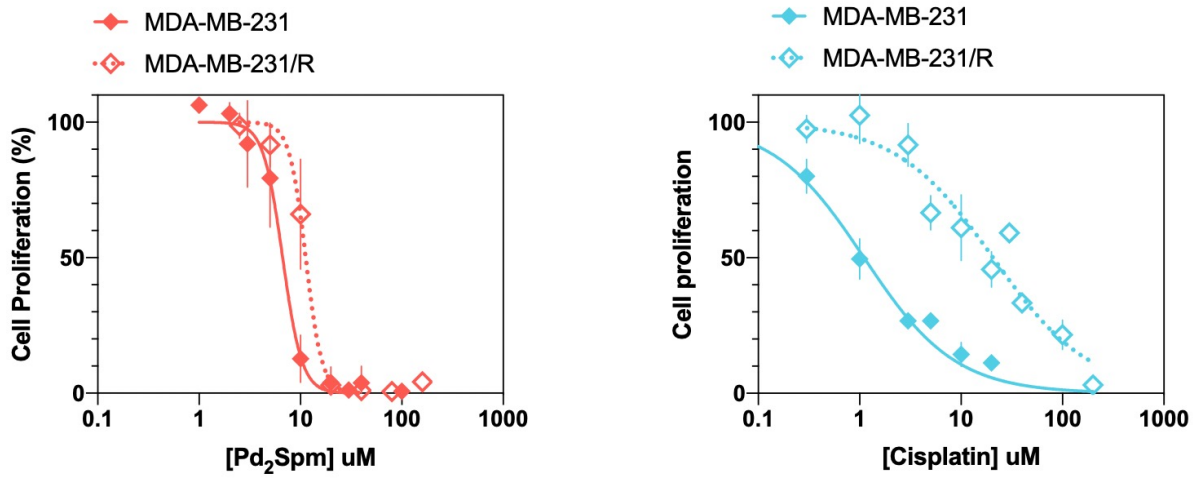


Figure 2. Dose-response curves of Pd2Spm and cisplatin in MDA-MB-231 cells (solid line) and MDA-MB-231/R (dotted line) at 72 h.

Results

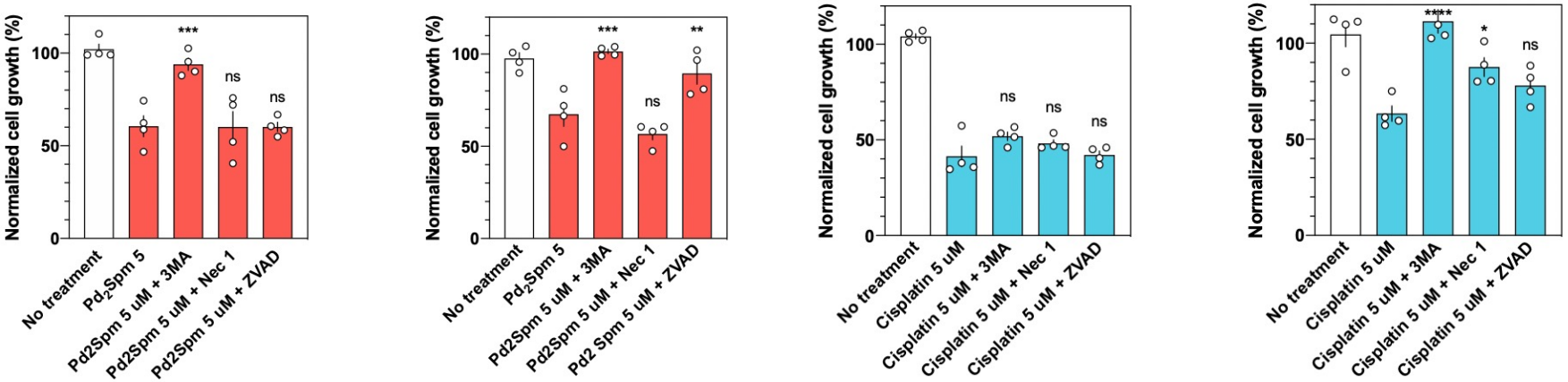


Figure 2. Effect of simultaneous incubation of Pd2Spm or cisplatin with 3-methyladenine 5 mM (3MA), or Necrostatin-1 50 μM (Nec-1) or Z-VAD 50 μM on cell proliferation.

Results

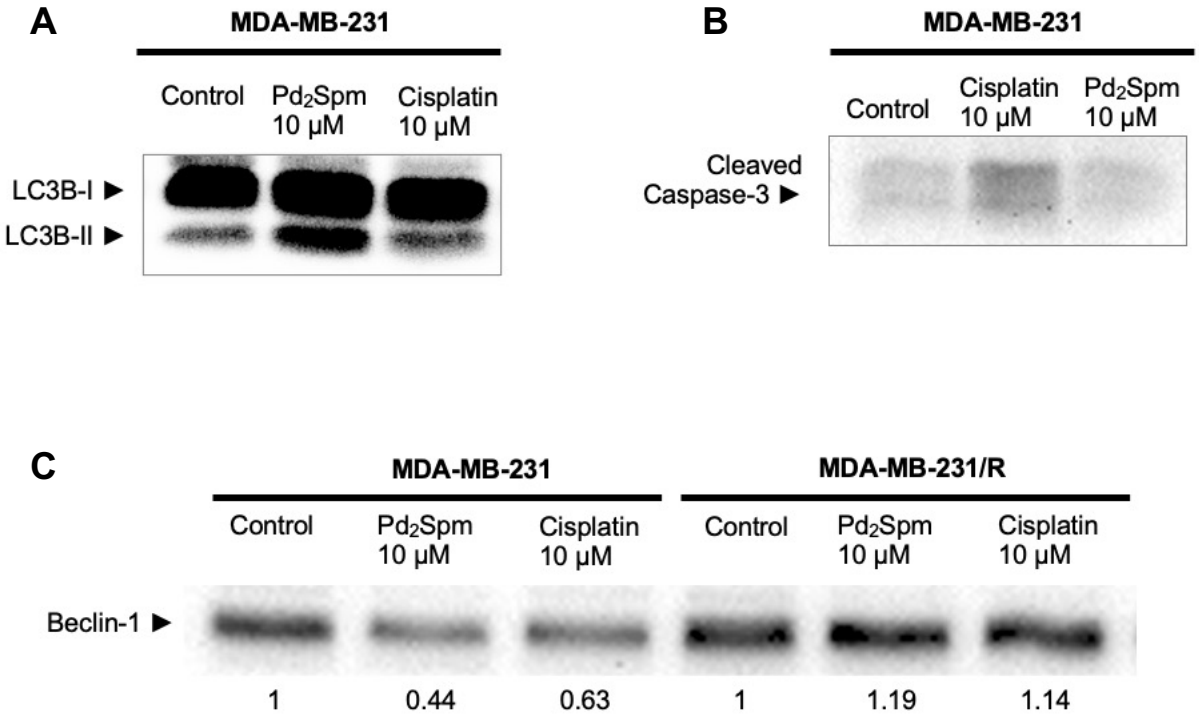


Figure 3. Protein expression of LC3B-I/II (A), cleaved caspase-3 (B) and Beclin-1 (C) in cells incubated with cisplatin or Pd_2Spm

Results

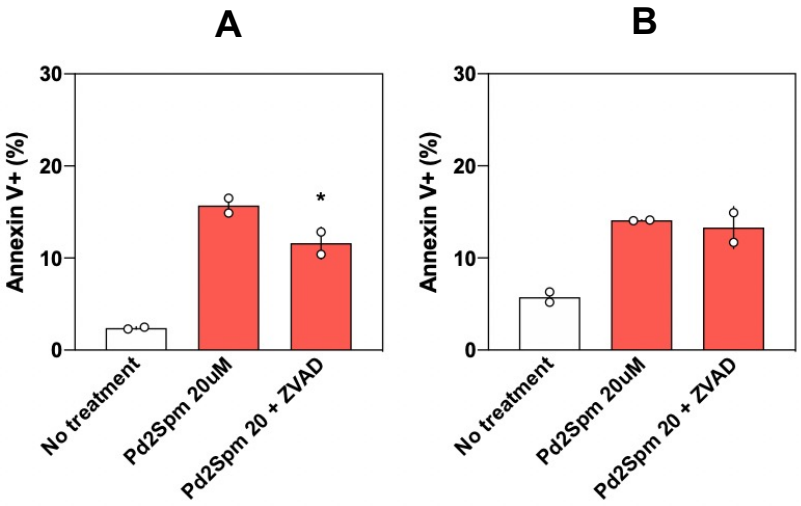


Figure 4. Effect of Pd_2Spm treatment in presence and absence of Z-VAD on the phosphatidylserine externalization in MDA-MB-231 (A) and MDA-MB-231/R (B) cells

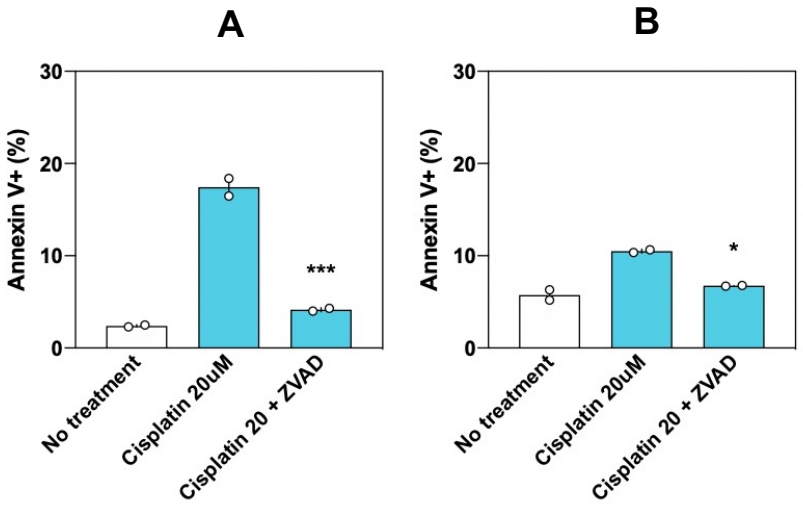


Figure 5. Effect of Cisplatin treatment in presence and absence of Z-VAD on the phosphatidylserine externalization in MDA-MB-231 (A) and MDA-MB-231/R (B) cells

Conclusions

In this work we have shown that

1. Pd_2Spm shows **equivalent efficacy towards resistant breast cancer cells** (as compared to loss of cisplatin's effect in resistant breast cancer cells)
2. Incubation of Pd_2Spm with **3-MA (autophagy inhibitor) rescues cells from Pd_2Spm effect** (i.e. pharmacological inhibition of autophagy rescue cells treated with Pd_2Spm)
3. **Pd_2Spm** seems **not to trigger apoptotic response** in contrast to cisplatin that caused cleavage of caspase-3
4. **Pd_2Spm** triggered conversion of **LC3B-I/II to greater extend than cisplatin** in MDA-MB-231 cells **implying higher activity of autophagy**

Altogether, Pd_2Spm may become promising Pd(II) candidate for TNBC treatment, particularly in cells that acquired resistance to cisplatin.

Acknowledgments

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