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Ferrocifen derivatives that induce senescence in cancer cells: selected examples

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ABSTRACT

Platinum coordination complexes represent an important class of anti-tumor agents. Due to recognized drawbacks, research into other types of metallodrugs has been diversified with the aim of finding new chemical entities with alternative mechanisms of action to overcome classical chemoresistance. P5 and DP1, two closely related ferrocenyl complexes bearing a similar ferrocenyl-ene-phenyl motif and displaying marked differences in their conformations and oxidation state versatility, were assayed in cancer cell models characterized by various sensitivities to pro-apoptotic stimuli. P5 and DP1 exert growth inhibitory effects between 0.5 and 10 μM against glioma and melanoma cells including pluripotent stem-like cells. These effects are due, at least partly, to senescence induction with typical SA-β-galactosidase staining and senescence-associated secretory phenotype (SASP) as measured by the secretion of IL-1α, IL-1β, IL-6, IL-8 and TNF-α. Regulation of these cytokines' secretion may be related to AP-1 and other transcription factors unrelated to senescence. An in vivo graft of B16F10 cells after in vitro pre-incubation with DP1 or P5 led to increased survival in mice. In conclusion, P5 and DP1 ferrocenyl complexes induce senescence in various cancer cell models associated with distinct sensitivity to pro-apoptotic stimuli.

1. Introduction

Organometallic complexes of arsenic played an important role, particularly at the start of the twentieth century, in the treatment of incurable illnesses of the time [1]. An iconic example includes Paul Ehrlich's development of Salvarsan®, one of the first key treatments for syphilis, a disease from that period that could be compared to AIDS today. In the course of developing this molecule, Ehrlich introduced the concept of chemotherapy, which is thus historically associated with an organometallic entity that is an entity containing a direct metal–carbon or similar bond [1]. During the 1960s, the discovery by Rosenberg [2] of the antitumoral properties of the coordination complex cisplatin, soon to be followed by carboplatin and oxaliplatin, proved to be an innovation in anti-cancer metallodrugs. At the moment, these complexes of platinum, either alone or in combination, are used in more than 70% of treatments. However, due to resistance problems and serious, particularly renal, secondary effects, and a fairly narrow range of application, research into other types of metal complexes has developed. This research has progressed, in particular, from an exploration of the coordination complexes of Ru with NAMI-A and KP1019 to an examination of the organometallic species of this metal [3–6].

The covalent nature of transition metals, resulting in strong metal–carbon bonds, and the space-filling nature and versatility of their oxidation states compared to those of the principal groups, have supported the development of new organometallic chemistry for medical applications. Among the usable organometallic complexes, iron complexes are distinctive due to the abundance of this metal in the body and the particular nature of ferrocene, which is a compact and stable aromatic metallocene with unique redox properties. In fact, this entity has been widely used with varied success for biomedical purposes [7]. Among these compounds, ferroquine (FQ) was synthesized in 1997 [8] and reached phase II clinical trials, and the ferrocifen family was developed by some of us starting in 1996 [9–11].

The current study characterizes the antitumor activity of two of these ferrocifen derivatives, P5 and DP1 (Fig. 1Aa and Ab). P5 is 1,1-di(4-hydroxyphenyl)-2-ferrocenyl-but-1-ene [12], DP1

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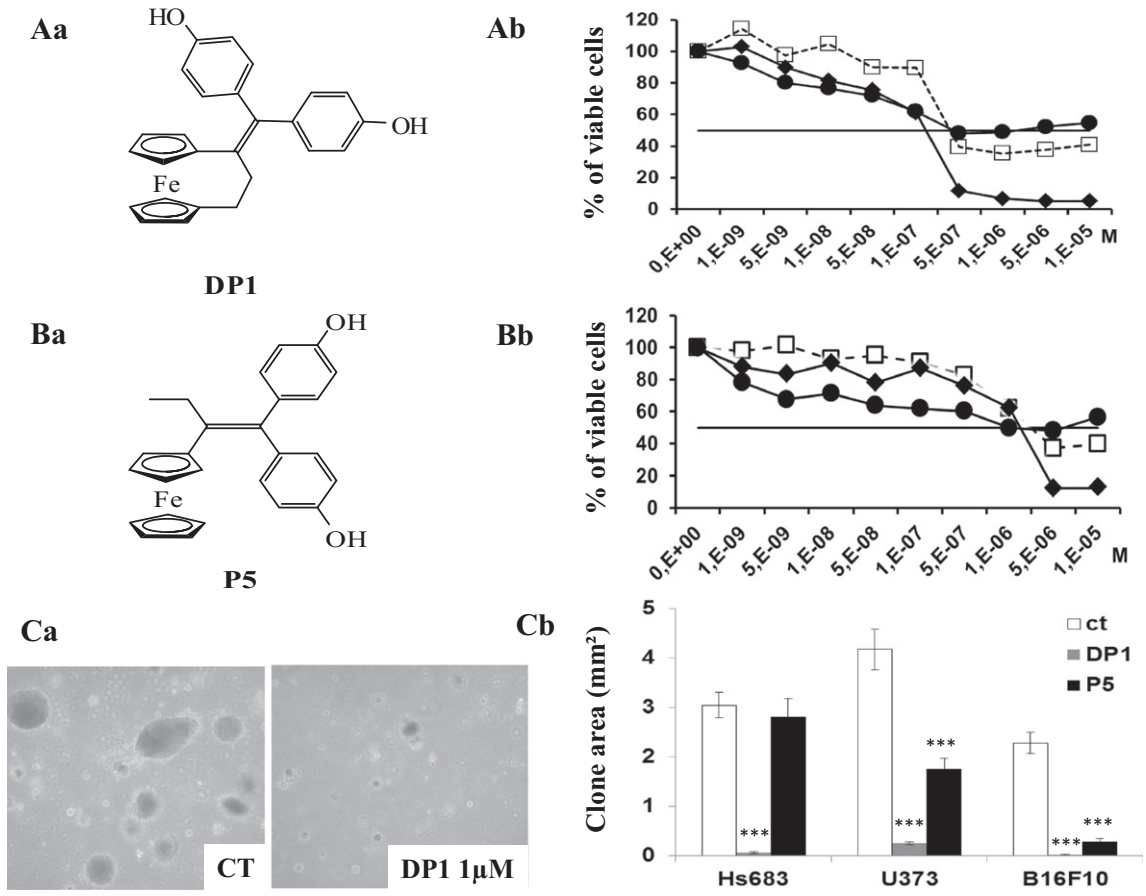

Fig. 1. Ferrocenes inhibit the global growth of cancer cells. Ab: Dose–response curves of three cancer cell models treated with DP1 obtained by the colorimetric MTT assay after 72 h of treatment (Aa). White squares: Hs683 human glioma cells; black dots: U373 human glioma cells; black diamonds: B16F10 mouse melanoma cells. Bb: MTT dose–response curves of the cellular models treated with P5 (Ba). C: Ca: Illustrations of Hs683 clones that developed after 4 weeks in the control versus DP1-treated conditions. Cb: Quantification of the area covered by the clones per field in the 3 cell line models after 4 weeks of culture in the absence or presence of DP1 or P5 at 1 μM. Data are presented as the mean ± SEM of a minimum of 10 fields and a maximum of 20 fields per experimental condition.

is 1-(di-4-hydroxyphenyl-methylidenyl)-[3]ferrocenophane [13], and both are prepared from a McMurry coupling reaction between propanoylferrocene or [3]ferrocenophanone, respectively, with dihydroxybenzophenone. While P5 could lead to the formation of quinone methide due to an electronic delocalization from the iron to the phenol group [14], the electron could stay in the iron environment in the case of DP1, due to the ansa C3 bridge constraint.

Nevertheless, this study highlights that these two compounds induce similar cellular and molecular phenotypic changes related to senescence induction in various cancer cell models. Forcing cancer cells to enter into senescence may represent an interesting therapy to stabilize cancer patients as discussed below [15].

2. Materials and methods

2.1. Cell lines and compounds

Human glioma Hs683 (ATCC code HTB-138) and U373 (ECACC code 08061901) cells and mouse melanoma B16F10 (ATCC code CRL-6475) cells were cultivated in RPMI culture medium supplemented with 10% of heat inactivated fetal bovine serum and antibiotics as previously described [16].

DP1 [13] and P5 [12] were synthesized in our laboratory as previously described.

2.2. MTT colorimetric assay

MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assays were conducted once in sextuplicate over a 72 h period of treatment as previously described [16] except for the recovery MTT assay. For this assay, the cells were treated in 25 cm^2 flasks with 10 μM of DP1 or P5 for 72 h or left untreated (control). Treated or control cells were then seeded in 96 well plates and allowed to grow for 1, 2, 3, 4, 6 and 8 days without any additional treatment. The absorbance of the control cells measured on day 1 was arbitrarily normalized to 100%.

2.3. Clonogenic assay

Soft agar clonogenic assays were performed as previously described [16]. A minimum of 10 pictures per experimental condition were quantified, measuring the area covered by the clones after 4 weeks of culture. Each experiment was conducted once in triplicate.

2.4. Senescence induction measurements

Senescence induction was evaluated through senescence associated (SA) β-galactosidase staining as previously described and according to the manufacturer's instructions (Sigma-Aldrich, Diegem, Belgium)