



The Anti-angiogenic Effect Of The Platinum (II) Saccharinate Complex $trans-[Pt(sac)_2(PPh_3)_2]$ on Tube Formation And In Vivo CAM Assay



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PURPOSE

Metal based chemotherapeutic drugs including platinum complexes are promising agents to treat cancer with more efficacy compared to chemotherapeutic agents. This study was carried out to evaluate cytotoxic and anti-angiogenic effect of platinum (II) saccharinate complex $trans-[Pt(sac)_2(PPh_3)_2]$ (MP2 complex).

MATERIALS AND METHODS

Cytotoxicity assays

The cytotoxic effect of platinum (II) saccharinate complex on human umbilical vein endothelial cells (HUVECs) was examined with the ATP and SRB assay.

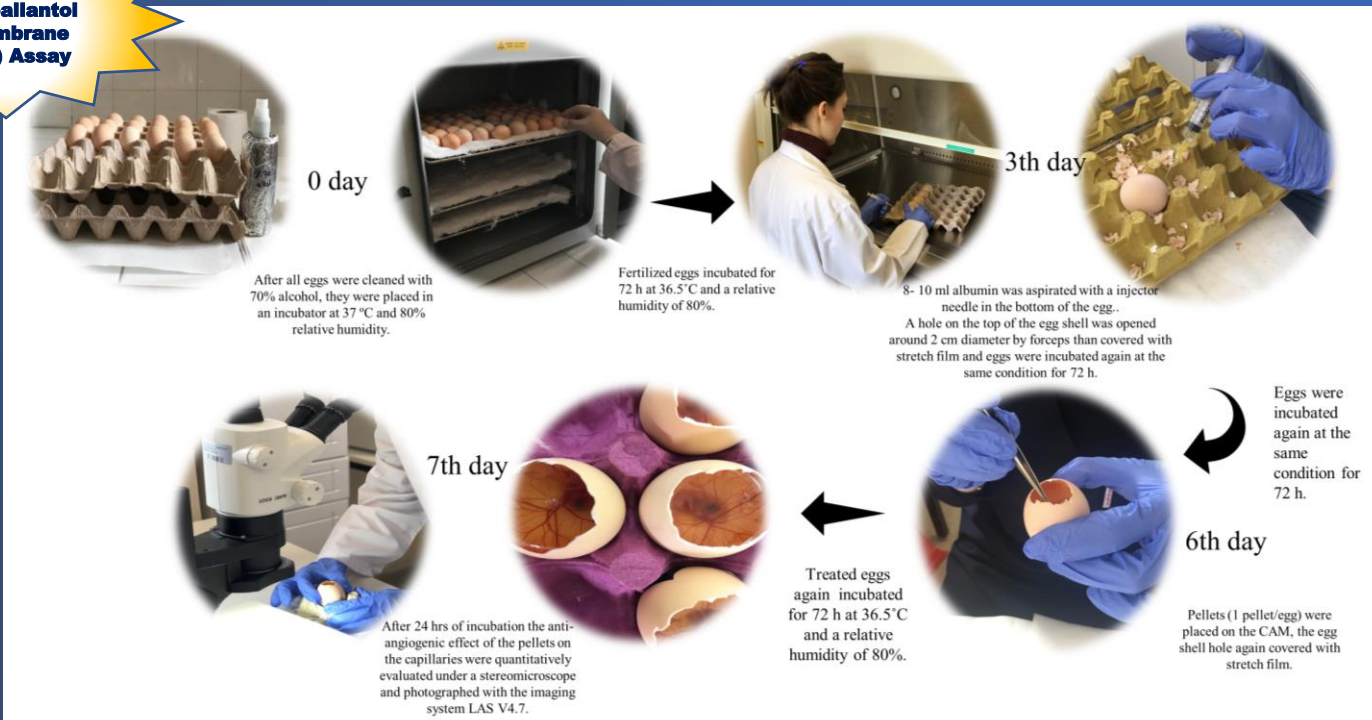
Tube formation assay

The anti-angiogenic activity of the platinum (II) saccharinate complex was evaluated by in vitro tube formation assay.

In vivo CAM assay

The anti-angiogenic activity of the platinum (II) saccharinate complex was evaluated by in vivo chick embryo chorioallantoic membrane (CAM) assay.

Chick Embryo Chorioallantoic Membrane (CAM) Assay



Score	Anti-angiogenic effect	Effects observed on CAM after treatment
< 0.5	Inactive	Normal embryo growth.
0.5-0.75	Weak	No capillary free area. Area with reduced density of capillaries around the pellet not larger than its own area.
> 0.75-1	Strong	Small capillary free area or area with significantly reduced density of capillaries. Effects not larger than double the size of the pellet.
> 1	Very strong	Capillary free area around the pellet at least double the size of the pellet.

Semi-quantitative scoring system of anti-angiogenic effects on chick embryo chorioallantoic membrane (CAM) after treatment.

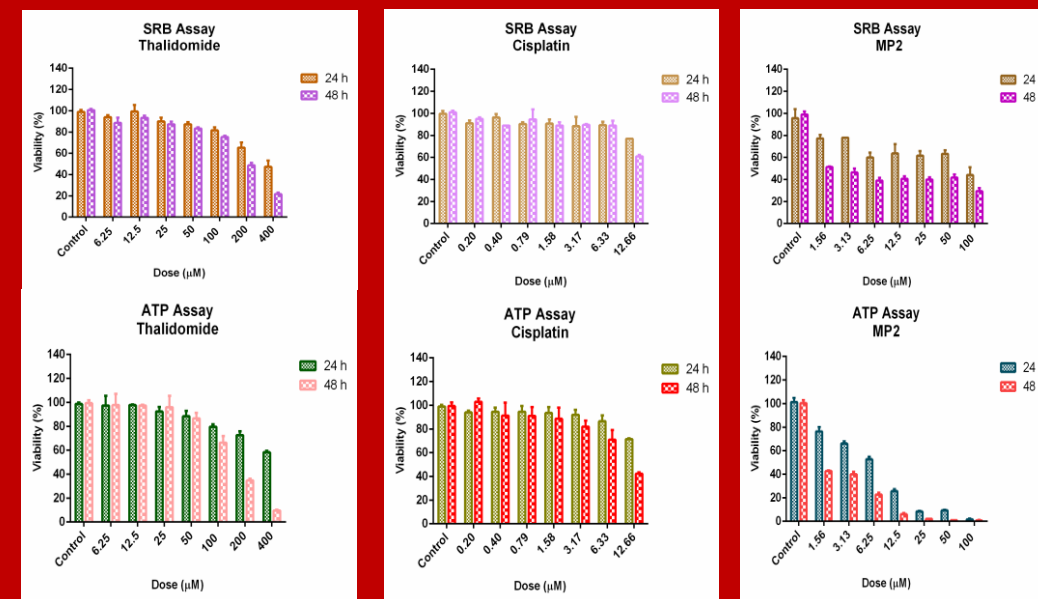
$$\text{Average score} = \frac{\text{Number of eggs (score 2)} \times 2 + \text{number of eggs (score 1)} \times 1}{\text{Total number of eggs (score 0, 1, 2)}} \quad \text{Formula used for scoring system.}$$

CONCLUSION

These results suggested that the platinum (II) saccharinate complex $trans-[Pt(sac)_2(PPh_3)_2]$ has showed significant anti-angiogenic activity at the tested concentration both in CAM and tube formation assay. Antiangiogenic treatment has a bright future ahead for cancer therapy. Angiogenesis is considered to be one of the hallmark of cancer. Therefore, pharmaceutical companies are in competition for developing new anti-angiogenic complexes/small molecules. Also, further in vivo experiments are needed for their clinical use as an anticancer drug in worthwhile due to the anti-angiogenic effects of novel platinum (II) saccharinate complex.

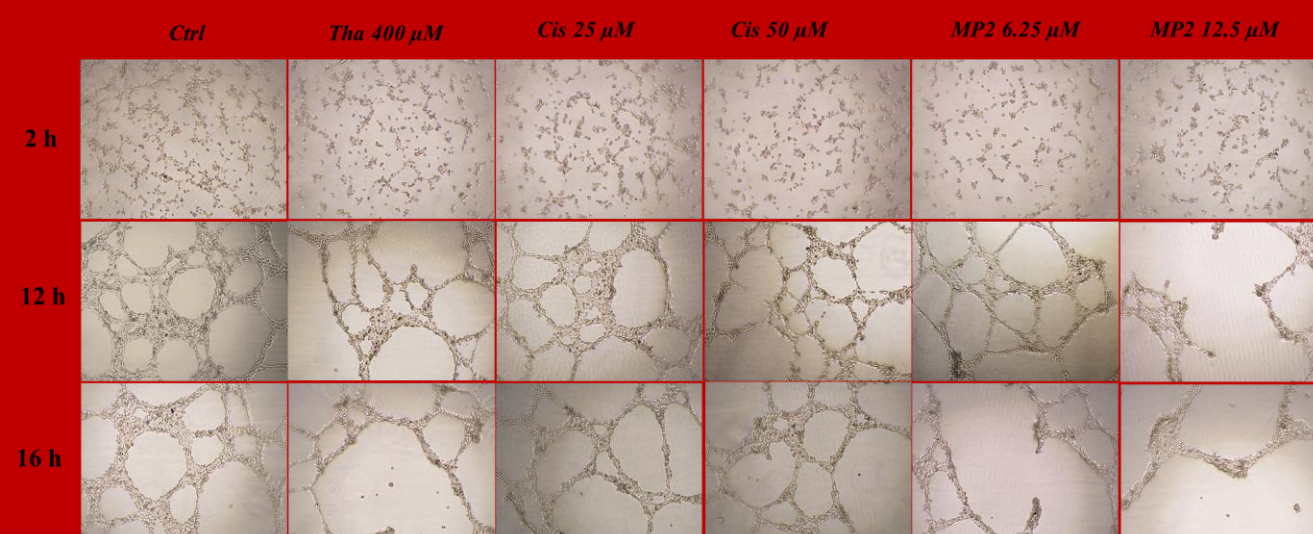
RESULTS

Cytotoxic effect of platinum (II) saccharinate complex (MP2) by SRB and ATP assay.



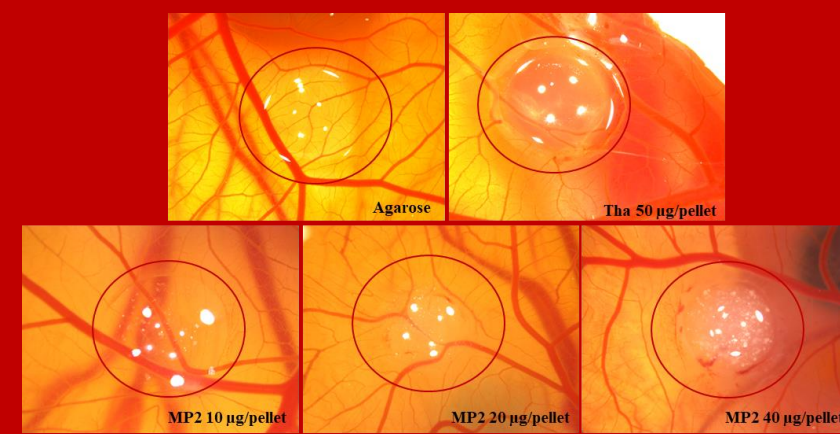
According to the SRB and ATP assay, cell viability was inhibited in a dose-dependent manner at tested concentrations (1.56-100µM) of platinum (II) saccharinate complex $trans-[Pt(sac)_2(PPh_3)_2]$ (MP2) after 24-48 h. Especially, 12.5 µM and higher concentrations MP2 complex was showed significant decrease on cell viability. Moreover, MP2 complex exhibited higher cytotoxic effect compared to thalidomide and cisplatin.

Tube formation assay on HUVECs.



Assesment of platinum (II) saccharinate complex (MP2 6.25-12.5 µM), positive control thalidomide (tha 400 µM) and cisplatin (cis 25-50µM) at 2-12-16 h treatment by tube formation assay on HUVECs. MP2 complex showed strongly reducing effect on tube structure at all tested times compared to vehicle and positive controls.

Anti-angiogenic effect of platinum (II) saccharinate complex (MP2) by in vivo CAM assay.



According to the microscopic evaluations the platinum (II) saccharinate complex $trans-[Pt(sac)_2(PPh_3)_2]$ (MP2), strong anti-angiogenic effect was shown at the concentration of 40 µg/pellet compared to the positive control (±)-thalidomide. At the 20 µg/pellet concentration of platinum (II) saccharinate complex (20 µg/pellet) was shown strong antiangiogenic effect but in low concentration of platinum (II) saccharinate complex (10 µg/pellet) was shown weak effect.

Sample	Concentration (µg/pellet)	Average score	Anti-angiogenic effect	Irritation/Tox (%)
The platinum (II) saccharinate complex (4 mg/ml)	40	1.375	Very strong	-
The platinum (II) saccharinate complex (2 mg/ml)	20	0,97	Strong	-
The platinum (II) saccharinate complex (1 mg/ml)	10	0,66	Weak	-
(±)-Thalidomide (Positive control)	50	0.82	Strong	-
Agar (Blanc)	% 2.5, w/v	0.2	Inactive	-