High-Affinity Cu(I)-Chelator with Potential Anti-Tumorigenic Action - A Proof-of-Principle Experimental Study of Human H460 Tumors in the CAM Assay

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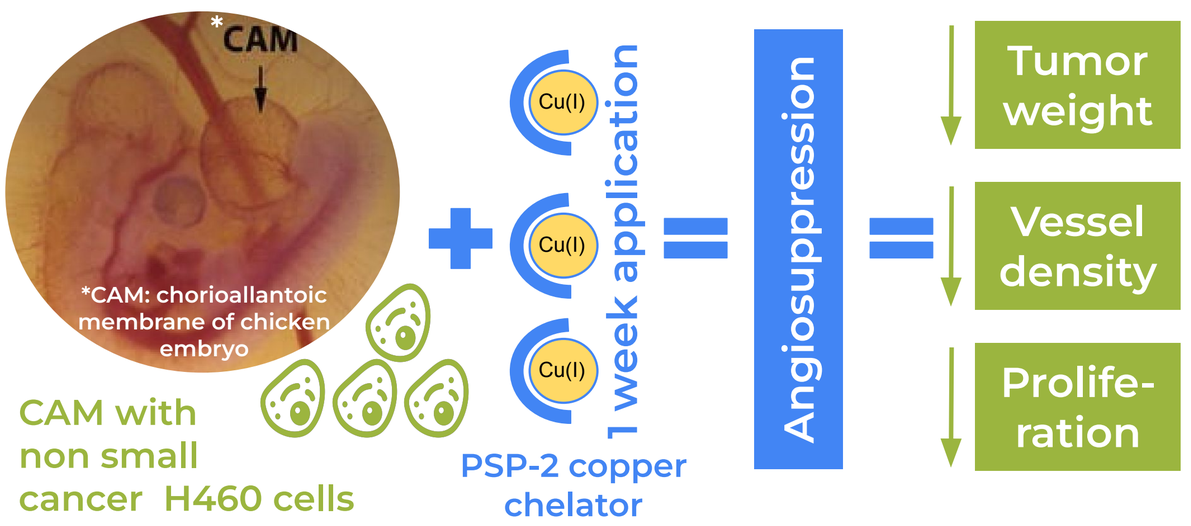
Introduction

Copper stimulates every process in angiogenesis from initiation to morphogenesis. In turn, angiogenesis activates and supports tumorigenesis.
Cancer patients exhibit elevated copper levels in tumors and in serum and this appear to be a hallmark of a broad range of malignant tumors, including colorectal, breast, gastrointestinal and lung cancers.
For this reason copper deprivation represents a promising approach in cancer therapy.
Our research team evaluated a new high-affinity Cu(I) chelator for its potential anti-angiogenic activity in ovo.

Methods

PSP-2 (1,2-bis (bis (dimethylphosphorothioylmethyl) phosphino) ethane) is a membrane-permeant high-affinity Cu(I) chelator.
In non-small-cell lung tumors, cancer patients exhibited approximately 50% higher copper concentrations compared to normal tissue.
We implanted non-small-cell lung tumor H460 cells in the chorioallantoic membrane assay of the chicken embryo. Then, we applied topically PSP-2 at low (30 mM) or high dose (60 mM) for 1 week and we did a comparison with the control group for tumor weight, vessel density and proliferation (ki67).



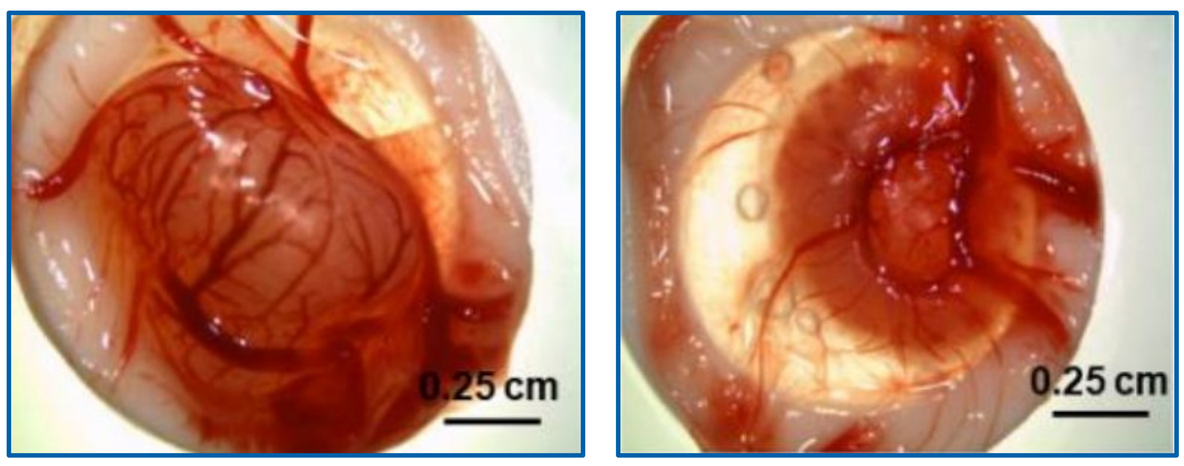


Figure 1
H460 tumors on the chorioallantoic membrane assay of the chicken embryo after 1 week. The control sample (left image) exhibits an increased volume compared to the low dose PSP-2 specimen (right image).

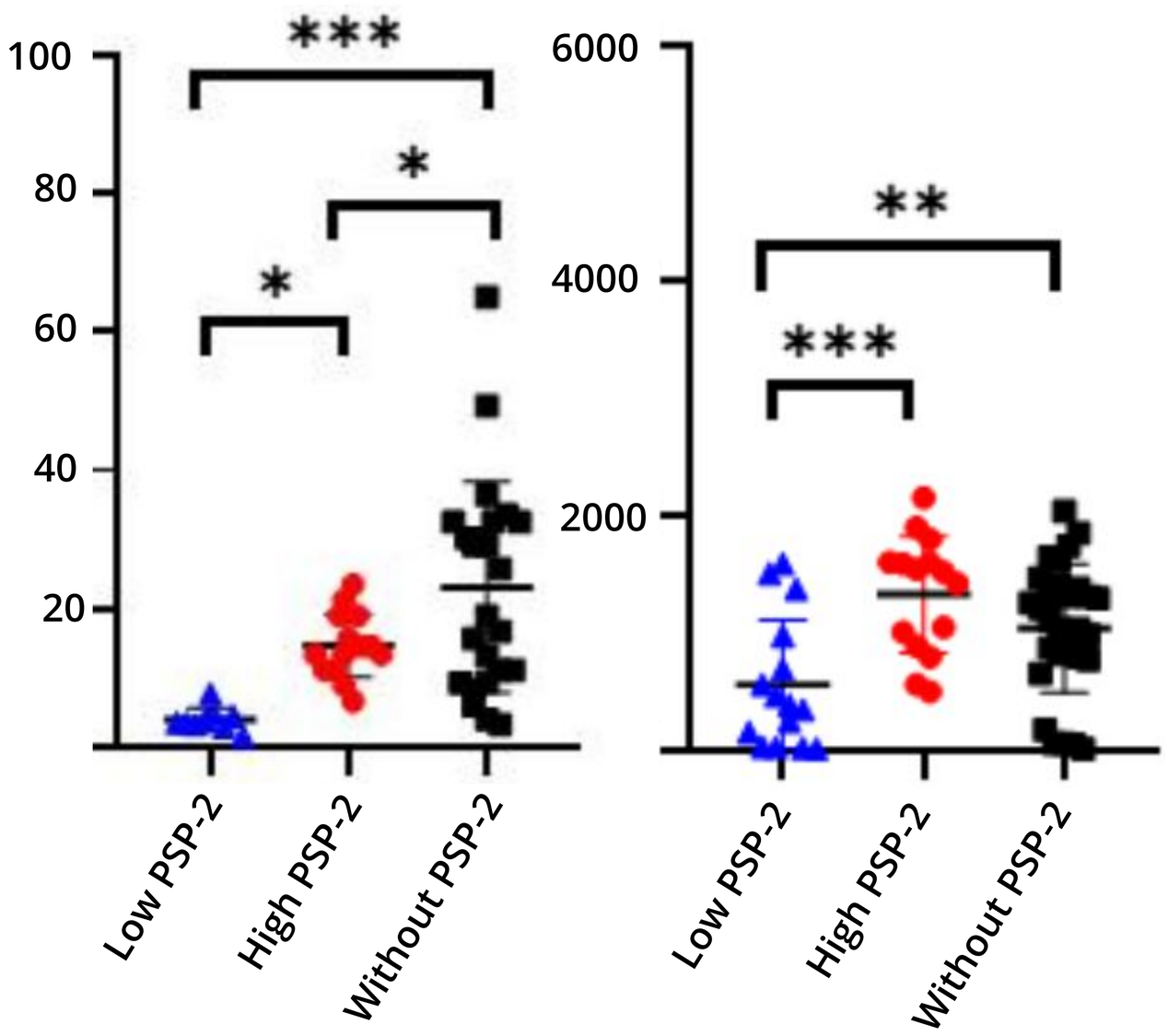


Figure 2
Vessel density (mm^{-2}).
$p<0.05$ (*), $p<0.001$ (***).

Figure 3
Ki67 positive cell count.
$p<0.01$ (**), $p<0.001$ (***).

Results

To assess the effect of PSP-2 on tumor growth, we compared the final weight of the tumors after fixation, resection, and deprivation of the residual tissue (**Figure 1**). H460 cells treated with PSP-2 had a significantly lower weight compared to controls ($p=0.0061$ for low dose PSP-2, respectively $p=0.0132$ for high dose PSP-2).
To assess the effect of PSP-2 on the of vessel we counted the number of the vessels within cross-section and normalized them to the cross-section area. Control group exhibited significantly higher vessel densities in comparison to the low dose and high dose PSP-2 specimes (**Figure 2**).
We next assessed the proliferation marker $ki67^{+}$ through the same process of vessel density (**Figure 3**). The control group and the high dose PSP-2 groups exhibited a significantly higher ki67+ cell count compared to the low dose PSP-2 group.

Conclusion

The findings obtained when applying PSP-2 with H460 tumor grafts on the chorioallantoic membrane assay of the chicken embryo point towards an **anti-cancer effect** of this high-affinity copper(I) chelator. Besides a reduction in tumor weight, **angiosuppression** previously observed in vitro was confirmed in this study in ovo.

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