

Treatment of Breast Cancer with Silver Antitumor Drugs Encapsulated In Biodegradable Polymeric Nanoparticles

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ABSTRACT

Several silver N-heterocyclic carbene complexes have been synthesized and tested for their anticancer activity. *In vitro* studies have shown these complexes to be active against ovarian, breast, melanoma, colon, renal, bladder, and prostate human cancer cell lines. An *in vivo* study on a silver carbene complex using an ovarian cancer (OVCAR-3) xenograft model in athymic nude mice has produced encouraging results. The administration of the silver complex subcutaneously into the tumor site resulted in necrosis of the tumors with no adverse effects to surrounding tissues or internal organs. Building on earlier results the silver complexes were encapsulated in nanoparticles in order to provide a platform for receptor targeting of specific cancer cells and to improve the stability of the complexes for systemic injection. We are currently investigating poly(lactic-co-glycolic acid) coupled with polyethylene glycol and utilizing folic acid receptors to provide this mode of delivery.

Keywords: silver, anticancer activity, nanoparticles, PLGA-mPEG, folic acid

1 INTRODUCTION

There remains an urgent need for cancer treatments that eradicate cancer cells effectively while minimizing toxic effects to normal cells. Although cisplatin remains the standard by which all metal based anticancer agents are measured there is an emphasis on research to produce alternative drugs with comparable or improved efficacy to cisplatin while reducing the toxicities associated with platinum-based drugs [1]. In addition, some cancer cells develop a resistance or are naturally resistant to cisplatin.

Several silver complexes with anticancer activity *in vitro* have recently been reported in the literature [2-5]. The advantage of silver is that its toxicity is quite low in comparison to platinum. In fact silver has been used since the 1880s as a broad spectrum antibiotic to treat burn wounds and as a prophylactic for the prevention eye infections in newborns. Based on this information, as well as the previous reports, and coupled with our experience in silver N-heterocyclic carbene complexes [6-9], we have begun to

investigate the anticancer activities of silver carbene complexes (SCCs) [10].

One concern with using silver complexes is that they degrade in the presence of chloride. This, coupled with the interaction with sulfur containing proteins present in the blood stream, make the systemic delivery of silver compounds challenging. One method to overcome this problem is using nanoparticles to encapsulate the silver complexes in order to prevent their degradation from or unwanted interaction with salts or proteins in the blood. Poly(D,L-lactic-co-glycolic acid) (PLGA) polymers are biodegradable, FDA-approved, and are extensively used for intravenous administration of therapeutic agents [11-16]. However, PLGA alone is known to be rapidly cleared by the reticulo-endothelial system (RES) [17]. This phenomena can be reduced by coupling PLGA with poly(ethylene glycol) (PEG). PEG is also used extensively with no known debilitating effects, is FDA-approved, and is widely known as a stealth agent [18-19]. PLGA-PEG nanoparticles alone are passively targeted to tumor cells. To increase this uptake, targeting moieties can be applied to the nanoparticles. One such moiety would be the use of folate. This utilizes the receptor-mediated endocytic delivery where the folate receptor is highly expressed in tumor cells but have a limited number on normal cells. Targeting should result in the minimal use of SCCs and produce nominal attacks upon healthy mammalian tissue. This further lowers the toxicity of SCCs relative to the platinum antitumor drugs.

2 IN VITRO STUDIES

Drawing from our library of SCCs, a select number of complexes were tested against a panel of cancer cell lines [20, 10]. An MTT assay was first run against ovarian, breast, and cervical cancer cell lines (OVCAR-3, MB-157 and HeLa, respectively). As a standard of comparison, the assay was performed on cisplatin, silver nitrate and silver acetate (Table 1). This table shows the IC₅₀ concentrations for the active compounds. The number reports the concentration that causes a 50% reduction in cell viability. As can be seen, the silver complexes are comparable to cisplatin in the ovarian and breast cancer cell lines. They had minimal effect on the cervical cell line. Phase contrast pictures were taken of the test cell line to obtain the morphology effect (Figs. 1 & 2). As the pictures show, the silver complexes had a significant effect on cell viability. They also indicate a high level of

effectiveness compared to cisplatin at the same concentrations. Live/dead cell assays were then performed to measure the efficacy of the silver complexes (Figs. 3 & 4). In Live/dead cell assays, the red fluorescence is indicative of live cells, green fluorescence specifies dead cells and

| | OVCAR-3 | MB157 | Hela |
|-------------------|---------|-------|------|
| Cisplatin | 12 | 25 | 25 |
| AgNO ₃ | 35 | 5 | 50 |
| AgOAc | 20 | 12 | NA |
| 1 | 35 | 8 | >200 |
| 2 | 30 | 20 | >200 |
| 3 | 20 | 10 | >200 |

Table 1: IC₅₀ concentrations are reported in micromolar

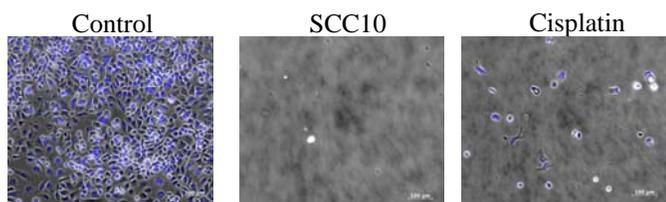


Figure 1: MTT assay of breast cancer cell line

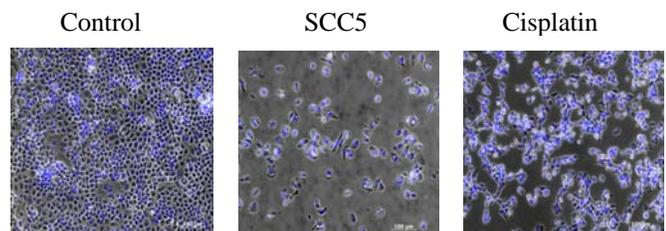


Figure 2: MTT assay of ovarian cancer cell line

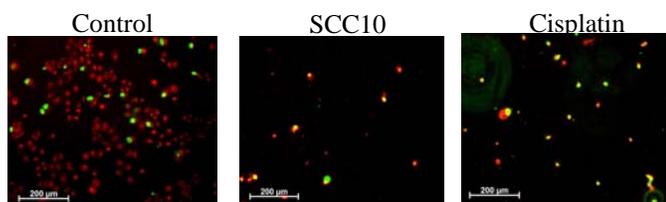


Figure 3: Live/Dead cell assay of breast cancer cell line

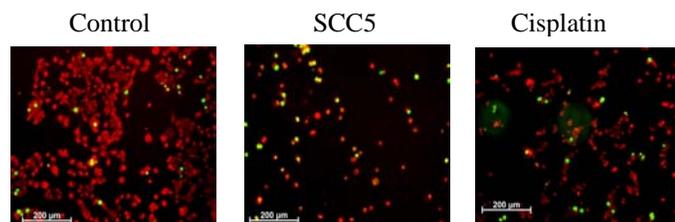


Figure 4: Live/Dead cell assay of ovarian cancer cell line

yellow indicates that the cells are dying. The viabilities of the OVCAR-3 cells were between 0-11% when exposed to the SCCs. The OVCAR-3 cells exposed to cisplatin resulted in 78% viability and control cells were 93% viable. When the MB-157 breast cancer cell lines were exposed to the SCCs and cisplatin, there was a 0-10% viability for all of them, with a 92% viability for the control cells. Because the SCCs were significantly better than cisplatin at lysing ovarian cancer cells and they completely eradicated the breast cancer cell line MB-157, we were encouraged to test the complexes on a broader panel of cancer cell lines.

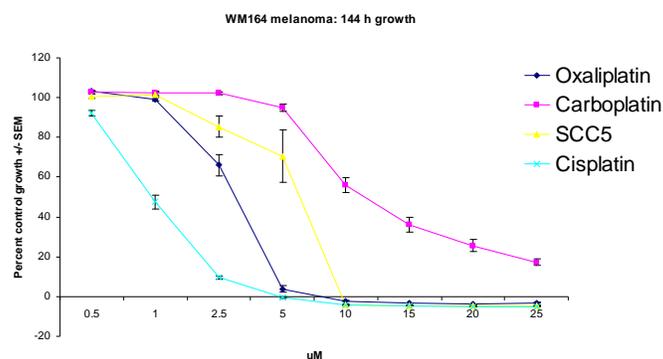


Figure 5: WM164 melanoma assay comparison

Therefore, antiproliferative effects were analyzed using the Sulforhodamine B (SRB) assay in WM164 (melanoma), WM9 (melanoma), A375 (melanoma), HT29 (colon), ACHN (renal), HT1376 (bladder), SKOV3 (ovarian) and PC3 (prostate) human cancer cell lines. Although cisplatin and oxaliplatin were the most effective agents across all cell lines they are also the most toxic to normal cells (Fig. 5). Alternatively, SCC5 and carboplatin are much less toxic, exhibited similar activity and have comparable effectiveness to both cisplatin and oxaliplatin. This indicates that SCC5 has the reactivity within a reasonable dosing range for a chemotherapeutic agent.

3 IN VIVO STUDIES

Since the *in vitro* studies showed very promising results with some cancers, a preliminary *in vivo* study was performed to determine the activity against an ovarian cancer xenograft model. Female athymic nude mice were injected with ten million OVCAR-3 cells subcutaneously above the shoulder. After six weeks, visible tumor growth was recorded and SCC5 was injected subcutaneously into the tumor site every third day for 10 days. At the end of the pre-determined time, the animals were sacrificed and the tumor, along with the organs, was inspected. According to pathological results, as well as visual, it was shown that SCC5 triggered major cell death of the tumors (Figure 6) [10].

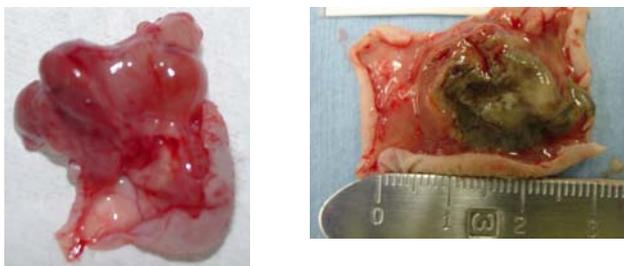


Figure 6: a) normal OVCAR-3 tumor mass (left); b) necrotic OVCAR-3 tumor mass (right)

Another interesting result was that all of the internal organs were healthy and the pathology reports indicated no ill effects.

4 Nanoparticle Studies

With such promising results from the *in vitro* and *in vivo* studies, we decided to pursue loading the SCCs into nanoparticles. We formulated a library of SCCs to have different properties that would be desired in formulating the nanoparticles. The partition coefficients of various SCCs were determined and the data used to select complexes that would be ideal in encapsulation. SCC10 is a lyophilic complex that showed its effectiveness *in vitro* and it was presumed to be an ideal candidate for the PLGA-PEG-fol nanoparticles (Fig. 7). The nanoparticles were fabricated by using a water-in oil-in water (w/o/w) emulsion, centrifuged down and lyophilized.

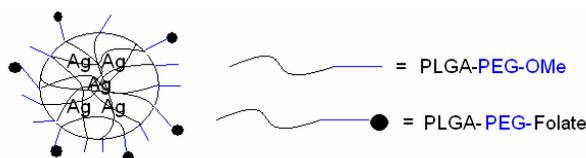


Figure 7: Cartoon of PLGA-PEG-fol nanoparticles

SEM images were taken of the nanoparticles and it was verified that they averaged between 1-3 microns (Fig. 8). Based off of a 10% loading, it was determined that the average load was 7.7% using absorbance. A 6-week release study was performed, which showed an initial 19% burst release with a steady release for 2 weeks (Fig. 9).

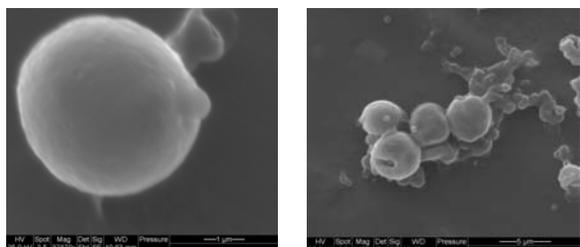


Figure 8: SEM pictures of SCC10 loaded nanoparticles

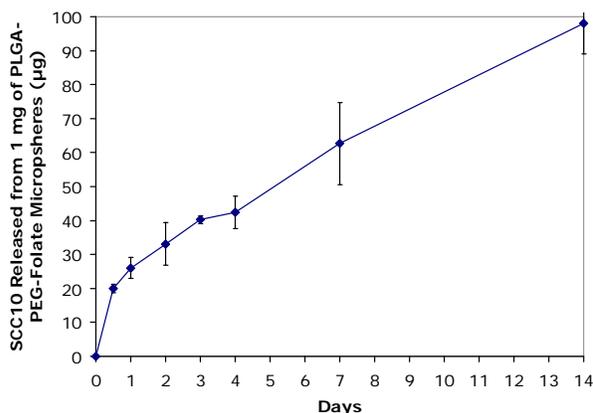


Figure 9: Release study of SCC10 loaded nanoparticles

After it was determined that silver was loaded into the nanoparticles, an initial *in vitro* study was performed using the MB157 breast cancer cell line. A live/dead cell assay was used, with an additional test done on blank nanoparticles. As can be seen in Figure 10, the SCC10 loaded nanoparticles were just as effective against the breast cancer cell line as shown in previous studies. Notably the blank nanoparticles do not have an effect against the cells.

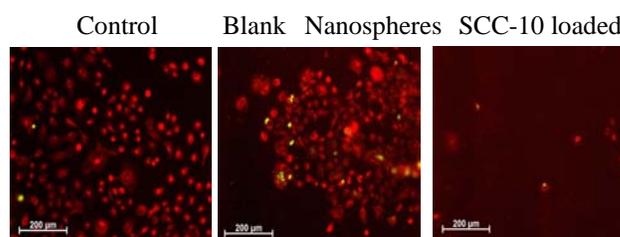


Figure 10: Live/dead cell assay on breast cancer cells

6 CONCLUSIONS

Our SCCs provide effective inhibition of cancer cell growth against various cell lines. Preliminary *in vivo* studies show that they are effective at eradicating the tumor once it is there as well as not showing any major toxic effects. To eliminate the challenge of silver precipitating out in chlorinated solutions, SCCs have been successfully encapsulated into nanoparticles. The targeted nanoparticles will deliver the silver complexes to the desired location so that the tumors may be compromised. The preliminary *in vitro* studies show promise and optimization of SCC loading into nanoparticles are being explored as well as the beginning of *in vivo* studies in conjunction with the Cleveland Clinic.

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