Enhancement of Effects of Chitosan and Nanoparticles on Pancreatic Cancer Cells by Combination Treatments with the Anti-Cancer Drug Adriamycin

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ABSTRACT

Because of chitosan's roles in biomedical applications, this study investigated the effects of chitosan, chitosan in combination with silver or gold nanoparticles, and chitosan with nanoparticles and Adriamycin, Methotrexate or Cisplatin on human PANC-1 pancreatic cancer cells. Our results demonstrated chitosan, chitosan in combination with nanoparticles, and the three chemotherapeutic drugs exerted differential inhibitory effects on the survival/proliferation of PANC-1 cells: their inhibitory effects were greater when employed in combination with chitosan and nanoparticles. Treatment with chitosan, chitosan in combination with nanosilver particles, and chitosan in combination with Adriamycin and nanosilver particles exerted differential effects on the expression of AKT, p-AKT, ERK, and p-ERK proteins (important cell survival/proliferation signals) in PANC-1 cells. Thus, these results suggested that chitosan and nanoparticles may have chemotherapeutic potential in designing of new and improved treatments for this cancer.
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Keywords: Chitosan, silver and gold nanoparticles, pancreatic cancer cells, nanotoxicity, anti-cancer drugs

1 INTRODUCTION

Many nanoparticles are being exploited in numerous applications in diverse industries because of their unique properties [1]. In this context, it comes as no surprise that applications of nanoparticles in biomedical research and development are on the increase [2]. Apart from being employed as probes and in a variety of cell and tissue imaging, nanoparticles have also been gainfully exploited in the design of new agents employed in drug delivery and targeting in cancer chemotherapy [3-5].

Of the metallic nanoparticles frequently considered for possible applications, silver and gold nanoparticles have become increasingly popular due to their presumed inertness. For example, silver nanoparticles are increasingly employed in many consumer products [6] as they can be produced on a large and industrial scale [7]. Gold nanoparticles have become equally popular because of their ease of synthesis, chemical stability, and unique optical properties [8]. Thus, their apparently desirable qualities have prompted their novel applications in cancer nanobiotechnology.

As the deacetylated product of chitin and a linear polysaccharide, chitosan has been exploited in tissue engineering, wound healing, and drug delivery due to its presumed biocompatibility [2]. Thus, chitosan lends itself to cancer nanomedicine applications.

Being the fifth leading cause of cancer death worldwide, pancreatic cancer exhibits a prognosis that is devastatingly poor [9, 10]. Pancreatic cancer is extremely aggressive and spreads rapidly and extensively [9, 10]. Tragically, usually by the time it is first diagnosed, the cancer has already metastasized from the pancreas as there is no procedure or biomarker for early diagnosis of this cancer [9, 10]. Despite the current treatment of surgery, chemotherapy and radiotherapy or some combination of these, median survival from diagnosis is around 3 to 6 months and 5-year survival is < 5% [9, 10].

Our group has established a history of developing many neural and non-neural, normal as well as tumor cell types as *in vitro* models for systematic investigation of putative cytotoxicity of various nanomaterials, including metallic and non-metallic nanoparticles [1, 11-17]. The poor prognosis of pancreatic cancer and the lack of efficacious treatment for this disease have prompted us to initiate the current series of studies to determine the feasibility of combining chitosan and nanoparticles with well-established chemotherapeutic agents such as Adriamycin, Methotrexate or Cisplatin in the design of new combination chemotherapies for treating pancreatic cancer.

2 MATERIALS AND METHODS

2.1 Materials

Chitosan (from shrimp shells, minimum 75% deacetylated, and molecular weight 190-375 kDa), thiazolyl blue tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO), Adriamycin, Methotrexate, and Cisplatin were purchased from Sigma-Aldrich (St Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA, USA). Tetrachloroauric (III) acid ($HAuCl_4 \bullet 3H_2O$), trisodium citrate ($C_6H_5Na_3O_7 \bullet 2H_2O$) and silver nitrate ($AgNO_3$) were purchased from Fisher Scientific (Pittsburgh, PA, USA). The antibodies (monocloncal) employed were obtained from Cell Signaling Technology (Beverly, MA, USA). All chemicals were of analytical grade unless otherwise stated.

2.2 Cell Culture

Human pancreatic cancer PANC-1 cells, obtained from ATCC (Manassas, VA, USA), were cultured in an incubator at 37˚ C and 5 % (v/v) CO_2 in RPMI 1640 medium, supplemented with 10% (v/v) FBS.

2.3 Preparation of Nanosilver/Chitosan and Nanogold/Chitosan Scaffolds and Cell viability assay

These procedures were essentially the same as those previously published [18].

2.4 Western Blot Analysis

Expression of proteins of interests was determined by Western blot analysis employing the chemiluminescence technique as described previously [12, 15].

2.5 Statistical analysis of data

Experiments were performed at least three times with a minimum of 6 replicates for each set, and all data were recorded as mean ± standard deviation (shown in figures). Data analysis was carried out by one-way analysis of variance, followed by post-hoc Student-Newman-Keuls test for multiple comparisons using KaleidaGraph version 4 (Synergy Software, Reading, PA, USA). Significance level was set at $p<0.05$.

3 RESULTS AND DISCUSSION

Exposure of PANC-1 pancreatic cancer cells to 1.5% chitosan alone for 72 hours (hrs) lowered their survival by ~50% (Figures 1-3). Exposure of these cells for 72 hrs to Adriamycin (Figure 1), Methotrexate (Figure 2) or Cisplatin (Figure 3) alone induced a dose-related decreases in their survival at 0.0001 to 1.3 µM, Adriamycin being the most effective while Cisplatin being the least effective. Combing 1.5 % chitosan with the drugs enhanced their inhibitory effects on survival of the PANC-1 cell.

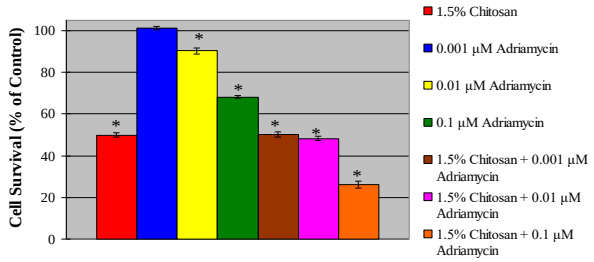

Figure 1. Effects of 1.5% chitosan, different concentrations of Adriamycin, and 1.5% chitosan in combination with different concentrations of Adriamycin on survival of PANC-1 cells for 72 hours.

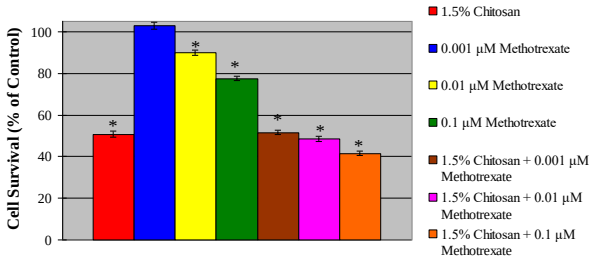

Figure 2. Effects of 1.5% chitosan, different concentrations of Methotrexate, and 1.5% chitosan in combination with different concentrations of Methotrexate on survival of PANC-1 cells for 72 hours.

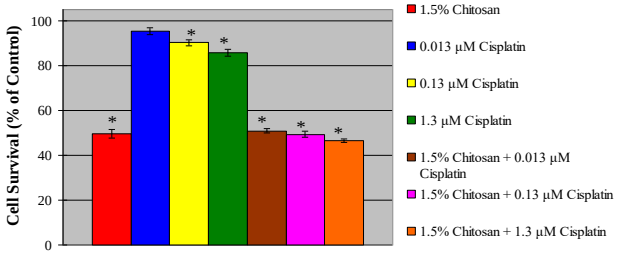

Figure 3. Effects of 1.5% chitosan, different concentrations of Cisplatin, and 1.5% chitosan in combination with different concentrations of Cisplatin on survival of PANC-1 cells for 72 hours.

As Adriamycin was the most effective among the three drugs examined (Figures 1-3), we chose it for further study. We examined the effects of chitosan alone, chitosan in combination with nanoparticles and/or 0.1 µM Adriamycin on survival of PANC-1 cells in culture for up to 14 days (Figure 4). After 14 days, chitosan with nanosilver and 0.1 µM Adriamycin was the most effective treatment combination in lowering PANC-1 cell survival: there were

almost no live cells left after this combination treatment (Figure 4). Chitosan with nanosilver was the second most effective combination treatment in lowering the survival of these cancer cells (Figure 4). Chitosan alone was the least effective treatment in lowering the PANC-1 cell survival even though the effect was still significant (Figure 4). On the other hand, treatment with chitosan and nanosilver particles showed more inhibitory effect than treatment with chitosan and nanogold nanoparticles, indicating that nanosilver particles were more cytotoxic than nanogold particles to PANC-1 cells (Figure 4).

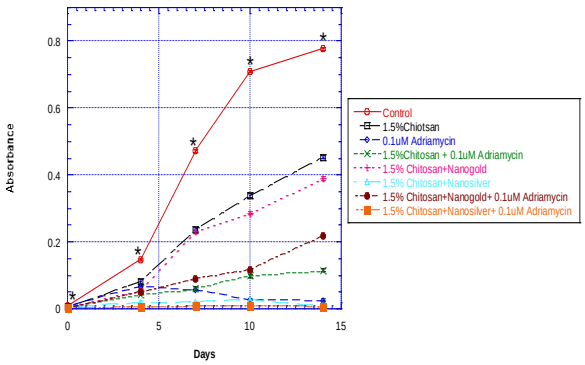

Figure 4. 2: Effects of 1.5% chitosan and 1.5% chitosan in combination with nanoparticles and/or 0.1 μM Adriamycin on survival of PANC-1 cells.

As the molecular mechanisms underlying the effects of the combination drug treatments on PANC-1 cells were unknown, we determined their expression of cell survival signaling proteins of the AKT and ERK pathways. We found that treatment of PANC-1 cells with chitosan alone induced increased expression of p-AKT in the cells, but their increases in p-AKT expression were more marked when treated with chitosan combined with nanosilver particles or treated with chitosan combined with both nanosilver particles and 0.1 μM Adriamycin: however, the total AKT in PANC-1 cells remained unchanged after any of the treatments (data not shown). The effects of the treatments on ERK signaling in PANC-1 cells differed from those on AKT signaling. The combined treatment of chitosan with nanosilver particles induced decreased expression in total ERK in PANC-1 cells: their p-ERK expression was decreased when treated with chitosan in combination with 0.1 μM Adriamycin. Treatment of PANC-1 cells with chitosan alone and chitosan in combination with nanosilver particles and 0.1 μM Adriamycin resulted in an increase in their p-ERK expression (data not shown). However, treatment of these cells with chitosan in combination with nanosilver particles did not induce any changes in their p-ERK expression (data not shown). Thus, because the combination treatments employed induced dissimilar effects on expression of the AKT and ERK cell survival pathways in PANC-1 cells, such effects alone could not account for the decreased survival induced in these cells by the combination treatments. This conclusion naturally prompted us to consider other molecular and cell death mechanisms that could possibly mediate the effects on survival of PANC-1 cells induced by such combination treatments. Clearly, this is a fruitful area for further investigation.

4 CONCLUSIONS

As far as we are aware, ours is the first study to report on the effects of chitosan and nanosilver and nanogold particles, with and without the combination treatment with chemotherapeutic agents such as Adriamycin, Methotrexate, and Cisplatin on PANC-1 pancreatic cancer cells. This study investigated our hypothesis that the anti-cancer property of chitosan is enhanced if it is employed in combination treatment with nanosilver or nanogold particles and/or anti-cancer drugs. Consistent with our hypothesis, our results demonstrated that chitosan and the nanoparticles exerted some anti-survival/proliferative effects on PANC-1 pancreatic cancer cells and the presence of the chemotherapeutic drugs tested markedly enhanced the anti-survival/proliferative effects of chitosan and the nanoparticles on PANC-1 pancreatic cancer cells. Because the combination treatments employed induced dissimilar effects on expression of the AKT and ERK cell survival pathways in PANC-1 pancreatic cancer cells, such effects alone could not account for the decreased survival induced in these cells by the combination treatments. Consequently, other molecular mechanisms may be involved and these await further elucidation. Nonetheless, our findings may have pathophysiological implications in the cytotoxicity of chitosan in combination treatments with silver and gold nanoparticles with and without established anticancer drugs. As such they may also point to the use of these agents in the design of new and/or improved treatments for pancreatic cancer.

5 ACKNOWLEDGMENTS

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