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Improving the Efficacy of Cisplatin in Colon Cancer HT– 29 Cells via Combination Therapy with Selenium

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Abstract

In general, cis-platinum is ineffective in treating slow-growing colon cancer. A seleno-enzyme that neutralises reactive oxygen species is glutathione peroxidase (ROS). High ROS concentrations may affect the efficacy of cisplatinum, according to research. In HT-29 colon cancer cells, the impact of selenite supplementation during cis-platinum treatment was investigated. Agarose culture enables cells to form colonies, grow in three dimensions, and independently analyse mitosis, cell viability, and ROS production. Single cells were cultivated for seven days while suspended in agarose. At day 0, cultures were either left untreated with selenite or were pretreated with it. Cultures were either given cis-platinum alone or in combination with selenite on day 4. Cell viability and mitotic activity were assessed after 7 days. ROS degradation was measured using a glutathione peroxidase test.activity. Little dosages of selenite had no impact on mitosis or cell survival. More ROS breakdown and improved cis-platinum efficacy were seen in cultures that were treated with selenite and cis-platinum. If colonies are already present, cisplatinum alone proved less efficient than the combination of cis-platinum and selenite. Moreover, cells treated with the cisplatinum and selenite combination showed enhanced ROS breakdown, indicating a connection between ROS levels and cisplatinum efficacy.

Introduction

Selenium and cancer are still a contentious topic [1, 2]. Selenium may play a preventive function in cancer, according to certain studies that have been done on the topic, while others have come to the opposite conclusion [3–7]. In addition to these variations, clinical evidence shows that some tumours are more resistant to conventional treatments like cis-platin (cis-pt), necessitating the use of combination therapy or better therapeutic choices. Slow-growing colon cancer cell lines have higher propensities to develop resistance and are less responsive to cis-pt and other metal coordination treatments [8–10]. There is compelling evidence that selenium supplementation can lessen the nephrotoxicity of chemotherapy medications [11–14]. Likewise, selenium appears to have some bearing on reducing the emergence of resistant lines of cells [15–18]. Due to its function in the selenoenzyme glutathione peroxidase, selenium has been associated with a reduction in oxidative damage. By the oxidation of reduced glutathione, glutathione peroxidase (GPox) converts hydrogen peroxide into water. Glutathione reductase, an enzyme that depends on flavonoids, is in charge of producing new reduced glutathione by utilising NADPH as an electron donor. The availability of glutathione peroxidase and other selenium-dependent enzymes is predicted to increase with selenite supplementation [19,20]. The majority of research to date has concentrated on how to either selectively generate reactive oxygen species (ROS) in cancer cells or control ROS in normal cells to prevent harm [21–24]. In the data presented here, we hypothesise that reducing the level of ROS in cancer cells may enhance the effectiveness of cis-pt in the colon cancer cell line HT-29. We investigated the effects of selenite on the colon cancer cell line HT-29 after pre-treating it with sodium selenite and after co-treating it with cis-pt. Despite the fact that our work has shown that cis-pt can stop proliferation when given at plating, we were able to demonstrate that cis-pt is less effective when given after colonies have had time to form. Sadly, the circumstance that is most physiologically significant is the decline in effectiveness that is seen after colonies have established. Only once a malignant polyp has formed would chemotherapy be given. Our study shows that cis-pt and selenite combination therapy increases the number of dead cells found in colonies even when given after colonies have had a chance to form, potentially making cis-pt a viable option for colon cancer patients despite prior studies suggesting it is not a suitable first line treatment. The co-treatment of se with cis-pt is the clinically significant treatment regimen, despite the fact that we expected that pre-treatment with selenite would have this effect and that this was not observed in our investigation to the amount

that co-treatment was successful.

Discussion

Over monolayer culture, using agarose cell culture has a number of benefits. Up to two weeks' worth of experiments can be run. Single cells suspended in agarose can be plate-analyzed to determine their cytotoxicity (by the exclusion of trypan blue) and mitotic activity (number and size of cell clusters). This culture approach is also useful for figuring out how an enzyme works.For each experiment and for each treatment group (n=6), control (DMEM alone) and vehicle control (VC, DMSO) cultures were created. Using an Olympus IM inverted microscope, all cultures were examined for viability using the trypan blue exclusion assay and for mitotic activity by counting both individual cells and cell colonies (clusters of two or more cells). On day 4, cultures were evaluated,and day 7. The cultures were first centred at 4x and then counted at 10x for analysis on day 7. This was done to eliminate bias. A little more than 30% of the cell culture was examined. A modified Bonferroni test was used to account for overall error after a t-test was used to compare the proportion of single cells and cell colonies that were alive and dead between treatment groups.The IC 50 for cis-pt in the HT-29 cell line was established in order to calculate the ideal cis-pt concentrations for investigations. We calculated an IC50 of 70 mM using the XTT test in monolayer culture. The XTT assay can be used to show that cell growth is being inhibited, but it cannot determine cytotoxicity. Selenite (as Na2SeO4) was applied to HT-29 cells that were cultured in monolayers at increasing doses. Selenite has an LD50 of 1.6 mg/kg in rats despite not being considered a cytotoxic substance. At 29 gml, 80% fewer cells were found than in the control sample.

According to the findings of the monolayer investigations, selenite may have cytotoxic or cytostatic properties at greater concentrations.We carried out a dose response in agarose culture to establish the lowest concentration of selenite needed to notice an impact but not high enough to cause cell death. As values estimated in monolayer are frequently not indicative of those seen in agarose culture, this step was essential. Treatment of cells continuously for 7 days with selenite concentrations of 0.75, 1.5, 3, 6, 11, 23, and 45 gml completed the dosage response of the HT-29 cells to selenite (data not shown). Based on these findings, we concluded that the initial selenite concentration was high enough at all doses examined to induce cell death. At lower doses, we conducted the experiment once more. Results for the lower dose response to selenite, including 0.05, 0.09, 0.18, and 0.33 gml. This information allowed us to establish the greatest dose tested at which selenite alone did not result in appreciable cell death. Our research into ROS breakdown showed that a dose of 0.05 gml was adequate to boost selenite enzyme function over control, even though it was possible to increase the selenite dose to 0.33 gml and yet not contribute to cell death. Based on these findings, we continued with 0.33 gml selenite in the combination trials because it was sufficient to boost GPox activity without causing cell death.The HT-29 cells' dosage response to cis-pt in agarose culture was then finished. Similarly, because the cells were grown in the three-dimensional, physiological agarose culture paradigm, it was important to complete the dosage response rather than compare to the literature. The vehicle control and concentrations of 6, 12, and 24 gml cis-pt were compared.For every concentration examined, there was a statistically significant rise in cell death when compared to the control. Cell counting and the trypan blue exclusion experiment both allowed us to confirm that all cis-pt doses examined stopped proliferation. Comparing the quantity of untreated single cells that are still alive to the increased fewer treated cultures had treated single dead cells and fewer colonies formed as a result. Figure 3 demonstrates that most cells become colonies when left untreated, with very few of those colonies having dead cells.

Conclusions

The findings of this study demonstrate that cis-pt, when given prior to colony formation, was effective at reducing mitotic activity and cell survival at all doses examined. Most significantly, our data demonstrate that cis-pt cytoxicity was reduced when colonies could develop prior to treatment. Moreover, we were able to link the reduced cytoxicity to a reduced ROS breakdown. We verified through dose response tests that ROS breakdown continued to occur at the appropriate selenite doses (0.05 to 0.33 gml), where there was no cytotoxcity. This information implies that even though the ROS breakdown was still happening, the selenite concentrations employed were not causing cell death. Because selenium supplementation would increase the ROS pool, it is crucial to observe the dosage response.There are a number of possible explanations for the observed effects of the co–treatment ranging from increases in various selenoprotein levels to increased availability of cis–pt that may have been bound to glutathione. One of the more common or well studied mechanistic possibilities is change in the level of selenoenzyme thioredoxin reductase 1, which when treated with cis–pt appears to increase insertion of an essential selenocysteine residue [26]. Another explanation is that low dose selenium could be inhibiting repair of the DNA strand breaks induced by cis–pt, similar to what has been observed with resveratrol [27,28].

More generally, research has shown that uncoupling protein 2

expression, which would also alter ROS levels, relates to cis–pt cytoxicity [24]. The mechanism by which selenite and cis–pt co–treatment increase cell death after colony formation is unknown and further studies are required to elucidate said mechanism, however, the above mentioned possibilities representplausible starting points.and will consequently call for increased ROS breakdown. We were certain that the cell death was unrelated to the elevated ROS pool because the amount of selenium at which the cells were treated did not significantly contribute to cell death. Based on our findings, we draw the conclusion that the co-treatment of selenite and cis-pt, which if the concentration is high enough, will cause cell death alone, is what caused the increase in observed cell death. The probable connection between cis-pt effectiveness and ROS breakdown stands out when taken into account with the cis-pt selenite combo findings. The data clearly demonstrating that cytotoxicity is increased when the selenite cis-pt treatment was administered, even after colonies were formed, support this observation.The observed effects of the co-treatment could have been caused by a variety of selenoprotein levels rising or by an increase in the availability of cis-pt, which may have been bound to glutathione.

Changes in the concentration of the selenoenzyme thioredoxin reductase 1, which when exposed to cis-pt appears to promote insertion of an important selenocysteine residue, are one of the most frequent or well-studied mechanistic hypotheses [26]. Another hypothesis is that low dose selenium, like resveratrol, may be preventing the repair of DNA strand breaks brought on by cis-pt [27,28]. More generally, studies have demonstrated a connection between cis-pt cytoxicity and uncoupling protein 2 expression, which would also change ROS levels [24]. The method of co-treatment with selenite and cis-pt The mechanism underlying the rise in cell mortality following colony formation is unknown, and more research is needed to clarify it. Nevertheless, the aforementioned hypotheses offer plausible places to start.Our results unequivocally demonstrate that the combination therapy was more successful at eradicating cells from established colonies. This discovery is crucial since existing colony creation is more closely related to the clinically relevant malignancy that is already present. Also, we were able to demonstrate that pretreatment could raise the proportion of colonies with dead cells, but only if selenite treatment persisted along with cis-pt treatment after colony formation.These findings imply that pretreatment is not required because cells that were pretreated with selenite and then treated with cis-pt did not succumb to cis-pt as quickly as cells that were just given combination therapy on days 4–7. These findings point to a potential therapeutic role for co-treatment. While often not a first, cis-pt If combined with selenite, it may be a first-line treatment for colon cancer. Our findings emphasises the significance of concluding such investigations, which are required to fully translate this potential medicine to the bedside.

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