Abstract

Title: Small hydroxy-9,10-anthraquinones and their complexes as effective anticancer drugs
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Cancer is a leading cause of death the world over and is currently on the rise. To counter the growing menace, chemotherapy, radiotherapy in combination with surgery are used as modes of treatment. Anthracyclines are chemotherapeutic drugs used against leukemia, lymphoma, cancer of the breast, the bladder, endometrium, the lungs, the ovaries, stomach, thyroid and in certain sarcomas. They impart cytotoxicity in multiple pathways of which that involving the presence and/or generation of reactive oxygen species (ROS) is significant. They are good DNA binding agents capable of intercalating DNA and deforming its three-dimensional structure. Drugs of this family also inhibit replication of DNA by interfering with the activity of DNA topoisomerase, pivotal in damaging cells and leading to their death. However, a major limitation of anthracyclines is their severe and chronic toxicity, significant being cardiotoxicity. Hydroxy-9,10-anthraquinones that form the core of anthracyclines are reduced to semiquinone by electrons being assisted by several enzymes. This results in a sequence of reactions culminating in the formation of ROS. ROS produced have beneficial activity owing to its role in cell killing. At the same time, they are responsible for toxic side effects, cardiotoxicity as mentioned already being of a serious concern. Another major problem with anthracylines is their high cost. Through this study, an attempt was made to see if hydroxy-9, 10-anthraquinones, the core of anthracyclines, or their modified forms that are less costly could become effective substitutes of anthracyclines. This would then benefit many economically challenged communities. Anthraquinone being the functional unit in anthracyclines or in their analogues, therefore like in case of anthracyclines, the hydroxy-9,10-anthraquinones would also be cardiotoxic, unless of course ROS is regulated. Hence, modification of anthracyclines or their simpler analogues is important and one way to modify them would be to form metal complexes. Studies have earlier shown complexes of this category of drugs are able to modulate formation of semiquinone which in turn regulate generation of ROS.

In the research carried out, simpler analogues of anthracyclines like alizarin and carminic acid were chosen. Mn^{II} complexes of alizarin and carminic acid and Co^{II}/Co^{III} complexes of alizarin were prepared and characterized to investigate their ability to either modulate formation of semiquinone or realize their ability to bind to DNA against that known for standard anthracyclines or hydroxy-9, 10-anthraquinones. In the absence of single crystals, that are rare for the hydroxy-9, 10-anthraquinone ligands owing to planarity, structures were elucidated by computational techniques. Both complexes and parent molecules were investigated for their ability to affect nucleic acid bases or DNA in a free radical pathway. Experiments were also performed on both types of cell lines (carcinoma and normal) using hydroxy-9, 10-anthraquinones and their complexes.

Interaction of complexes with calf thymus DNA under varying conditions of ionic strength, pH and temperature reveal complexes address aspects of limitations of hydroxy-9,10-anthraquinones if compared with anthracyclines. For example, an increased affinity of the complex towards DNA and the fact that its binding constant values do not decrease with either an increase in pH or decrease in ionic strength of the medium are positive aspects of complex formation. This is unlike simpler analogues of anthracyclines where a comparatively lower binding constant value under different conditions was reported earlier, a fact that would seriously affect drug action on cancer patients if one were to use simpler analogues. Since fluctuation of pH in body fluids of cancer patients is expected owing to use of various drugs,

the findings on DNA binding for complexes were an important aspect of this investigation. An attempt was also made to realize the influence of pKa values of simpler analogues (alizarin and purpurin) on the evaluation of contributions of different forms of compounds used towards overall binding to DNA that has substantial medicinal applications particularly if the complex interferes with cancer cells through binding with DNA.

Another important aspect of the study was to use the prepared Mn^{II} and Co^{II} complexes of hydroxy-9, 10-anthraquinones to generate reactive species electrochemically by either one-electron or two electron reduction under aerated or de-aerated conditions to find out what happens when similar species are generated on anthracyclines that are used as drugs following reduction by any electron donating group present in a biological electron-transport chain. Behavior of generated reduced species in solution, their stability etc., were studied but more importantly what was followed is interaction of such electrochemically generated species with nucleic acid bases or calf thymus DNA to have an idea of the extent of damage such one-electron reduced species of quinone (semiquinone) or two-electron reduced species (quinone-dianion) might have on biological targets. Such electrochemical reduction experiments were achieved by maintaining a glassy carbon electrode at a constant potential (the reduction potential of the compound under investigation), maintaining either nucleic acid bases or calf thymus DNA in the immediate vicinity of such generation of reduced species. Such studies help to throw light on the mechanism by which compounds affect DNA. It was seen hydroxy-9,10-anthraquinones were generally more effective in causing damage to nucleic acid bases or to DNA in the free radical pathway and that under above mentioned conditions complexes were at a disadvantage. However, interesting aspect is that if all factors are considered together, complexes were more effective than the hydroxy-9,10-anthraquinones, on different carcinoma cell lines. Hence, despite being at a disadvantage in the free radical pathway, owing to decreased semiquinone formation, confirmed by decreased semiquinone generation in transfer of electrons from NADH to molecular oxygen in NADH dehydrogenase assay, complexes performed better. This means complexes have other attributes that make up for a loss in activity in the free radical pathway. Hence, with an expected check on cardiotoxicity and that complexes do not ultimately compromise on drug efficacy, observed through experiments on HeLa, Hep G2, SIHA and PC3 (carcinoma cells) and WI 38 lung fibroblast normal cells, it may be said metal complexes of hydroxy-9,10-anthraquinones may be considered viable alternatives of anthracyclines as anticancer agents.

Another important aspect of the study was the interaction of chosen compounds and their complexes with ROS present within cells. This was studied on different cell lines using the H_2DCFDA assay. It was found complexes exhibit increased ROS within a particular cell line in comparison to hydroxy-9, 10-anthraquinones that would be utilised to kill cells i.e. show greater cytotoxic activity in comparison to hydroxy-9, 10-anthraquinones. In a way, the study on "ROS present within cells" helped in explaining findings of the MTT assay. Performance on several cancer cells and on normal WI 38 lung fibroblast cells revealed complexes were more effective on carcinoma cells than on normal cells, an important finding with regard to complex formation of hydroxy-9, 10-anthraquinones.

Last, but certainly not least, the aspect of cost would also be addressed through this study since complexes are far less costly than anthracycline drugs that are in use.

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