Chapter 7

TOPOISOMERASE II POISONING BY RUTHENIUM COORDINATION COMPLEXES: A STUDY OF STRUCTURE-ACTIVITY RELATIONSHIP.
INTRODUCTION

An earlier study by Jayaraju et. al. (1999) showed that a salicylaldoxime complex of cobalt (cobalt salicylaldoxime) poisons the activity of topo II. Molecular analysis implicated the oxime groups of the salicylaldoxime ligands as the topo II interacting moieties in the molecule. In the present study, we found that replacement of the cobalt atom with a ruthenium atom (RuSal), removed the topo II poisoning ability. This was surprising because, replacement of the central metal atom (which does not interact with topo II) completely abolished the biological activity of the molecule, while there was no alteration in the metal-ligand interaction or the chemical structure. To understand this effect, structural and conformational differences between the two molecules had to be analyzed, for which, minimal energy structural conformations of the molecules were generated through molecular modeling. Also, to examine whether other anticancer coordination complexes of ruthenium behave similarly with topo II, two coordination complexes of ruthenium have been tested for topo II antagonism. The first is RuIm, which has a metal atom flanked by two imidazole ligands. The ruthenium atom is also bonded to 4 chloride atoms. The second is RuInd, an indazole complex of ruthenium, which is similar to the imidazole complex, but with indazole ligands in place of imidazole. The synthesis and anticancer activity was first described by Keppler et. al. (1989). RuInd was found to be a more potent anticancer agent compared to RuIm. Both RuIm and RuInd possess significant antineoplastic activity against the Walker 256 carcinosarcoma, MAC 15A colon tumor, B16 melanoma and solid sarcoma 180 (Keppler et. al., 1990). These compounds
were more superior in their action against an autochthonous chemically-induced colorectal adenocarcinoma in rats compared to even 5-fluorouracil, which is an established cytostatic drug against human gastrointestinal carcinomas. Fruhauf and Zeller (1991) observed that RuInd brings about anti-tumor activity by interacting with DNA and inhibiting DNA synthesis. Though very effective, their clinical development was hindered due to extreme toxic effects on the body. Histological and blood-chemical investigations show major liver and kidney damage, hyperplasia and hyperkeratosis of gastric mucosa and anemia (Keppler et. al. 1990).

RESULTS

**Topoisomerase II antagonism by RuIm and RuInd:**

**Inhibition of DNA relaxation activity:**

Both metal complexes inhibit the supercoiled DNA relaxation activity of topo II. Ruind completely inhibits the DNA relaxation activity of topo II at a concentration of 250 μM, while RuIm inhibits the activity at a concentration of 300 μM. Figure 32 shows the dose dependent inhibition of topo II activity by these two drugs.

**Inhibition of the DNA stimulated ATPase activity of topoisomerase II:**

The ATPase assay shows that RuIm and Ruind significantly inhibit the DNA stimulated ATP hydrolysis activity of topo II. A comparison of ATPase inhibition by the two
complexes with \textbf{RuSal} is shown in \textbf{Figure 33}. These results correlate well with the inhibition of DNA relaxation activity by these complexes.

\textit{Formation of drug-induced, topoisomerase II mediated cleavage complex:}

Consequent to the relaxation and ATPase inhibition by \textbf{RuIm} and \textbf{RuInd}, the next step was to check for the ability of the two complexes to freeze topo II and cleaved DNA in a cleavage complex. The results of this assay show that RuInd was very potent in poisoning the enzyme activity by formation of a cleavage complex (at a concentration of 150 $\mu$M), as evidenced by the appearance of linear DNA in the assay gels (\textbf{Figure 34}). RuIm also forms the cleavage complex, but at a much higher concentration of 300 $\mu$M. As shown earlier in \textit{Chapter 3}, RuSal does not form the cleavage complex.

\textit{Anticancer activity assay:}

The anticancer activity of the two drugs was analyzed through $[^3\text{H}]$ thymidine incorporation assays on the two human cancer cell lines, colo-205 and ZR-75-1. The results of this assay agree with the previous reported findings that RuInd is a stronger anticancer agent compared to RuIm (\textbf{Figure 35}). The DNA intercalator was slightly more potent than RuInd, while RuSal showed the least effect on the cancer cell proliferation. The two drugs were more potent on the breast carcinoma (ZR-75-1) compared to the colon carcinoma (colo-205).
Molecular modeling analysis:

An earlier study on Cobalt salicylaldoxime (CoSAL) and its derivatives showed that in these molecules, the cobalt atom and the salicylaldoxime ligands are in the same plane, 180° to each other (Ph.D. thesis of D. Jayaraju). But in case of RuSal, the large atomic size of ruthenium may stearically hinder this planar conformation, which orients the ligands at an angle of −40° to each other. In RuIm and RuInd, the imidazole and indazole ligands are oriented in the same plane, giving the molecules a planar conformation, similar to that of CoSAL (Figure 36).

DISCUSSION

In order to investigate why RuSal does not poison topo II activity while CoSAL does, molecular modeling studies were carried out. The results of the modeling studies reveal that in CoSAL, planar conformation of the ligands along the horizontal axis of the molecule spatially orients the oxime groups to project out of the planar structure. This exposes the enzyme interacting oxime groups outside the plane of the molecule, thus facilitating a strong enzyme interaction. But in case of RuSal, the ruthenium atom induces a conformational change on the bidentate salicylaldoxime ligands and orients them at an angle of −40° to each other along the horizontal axis. This orientation masks the oxime
groups from the outside environment. As a result of this stearic interference by the ligand orientation, a proper interaction with the enzyme may be prevented. This could be the reason why RuSal partially inhibits the activity of topo II. Though the salicylaldoxime groups in CoSAL are also in a bidentate association with the metal atom, similar to RuSal, the cobalt atom is relatively smaller and may not induce any conformational stress on the ligands.

To investigate further if the abolition of topo II poisoning in RuSal is because of the spatial orientation of the ligands alone or possibly due to any other reason, two ruthenium coordination complexes which show excellent anticancer activity have been examined for topo II antagonism. The two complexes, RuIm and RuInd were selected due to two reasons. Firstly, they are similar to RuSal in being coordination complexes and possess two ligands on either side of the ruthenium atom. The second reason is that, the molecular modeling studies on their structures showed that the ligands are oriented in the same plane. 180° to each other, similar to CoSAL. Unlike RuSal, the ligands are monodentate, due to which they may not be under any conformational stress from the ruthenium atom, and are therefore in the same plane. Though these two molecules do not have the typical oxime interacting group, they have nitrogen atoms in the heterocyclic rings, at approximately the same positions as the oxime groups in CoSAL and RuSal. Hence, according to our reasoning, they would be able to antagonize topo II action at least to a small extent.

The topo II antagonism studies showed that these two compounds indeed poison the activity of topo II. The poisoning ability correlated well with their anticancer activity. This
shows that topoisomerase II antagonism may account for a significant amount of the anticancer activity attributed to these drugs.

Some studies have indicated putative structural and conformational requirements for topo II poisoning by drugs (MacDonald et. al., 1991, Capranico et. al., 1994, Zwelling et. al., 1992; Rene et. al., 1996). Though these conformations are not always a stringent requirement, such analysis on conformational requirements by structurally disparate topo II poisons will give an insight into the development of novel therapeutics directed against topo II. This is particularly important because, cancer cells regularly evolve mechanisms to resist the cytostatic action of anti-topo II drugs.

This case study about the effect of drug conformation on its molecular action merits a deeper investigation into the structure-activity relationship that drugs often possess. This would immensely help the cancer pharmacologist to develop rational drug molecules that attack a definite target.
FIGURE 32
Figure 33: The ATPase inhibition assay shows that RuInd strongly inhibits the ATP hydrolysis reaction of topo II compared to RuIm. Both show a dose dependent action. The effect of RuSal on the ATPasc action of topo II is also shown.
FIGURE 33

[Graph showing ATPase inhibition (%) versus drug concentration (μM) with different symbols for RuIND, RuIM, and RuSAL]
Figure 34: The cleavage assay on pBR322 DNA (lane 1) with topo II (lane 2) in the presence of 100 μM m-AMSA (lane 3) and 100, 150, 200, 250, 300 and 350 μM of RuInd (lanes 4 to 9) and the same concentrations of RuIm (lanes 10-15) shows that RuInd forms the cleavage complex at a concentration of 150 μM and RuIm at 300 μM.
**Figure 35:** The anticancer activity assay on the two cancer cell lines shows that Ru1nd is a very potent anticancer agent, which almost matches the anticancer effect of m-AMSA. Ru1m also showed a significant effect on the proliferation of the cancer cells. RuSal was the least effective. As observed in the earlier studies, the ZR-75-1 cells (B) were less responsive to the anti-proliferative action compared to the colo-205 cells (A).
FIGURE 35

A

B

PROLIFERATION (%) vs. DRUG (μM)

- m-AMSA
- Rulnd
- Rulm
- RuSal

PROLIFERATION (%) vs. DRUG (μM)

- Rulnd
- Rulm
- RuSal
- m-AMSA
Figure 36: A comparison between the molecular models of CoSAL, RuSal, RuInd and RuIm shows that in CoSAL, RuInd and RuIm, the ligands attached to the metal atom are in the same plane with each other, while in RuSal, the ligands are oriented at an angle of $-40^\circ$ to each other along the horizontal plane of the molecule. The central metal atom in all the molecules is shown in green. In RuIm and RuInd, chlorides are shown in yellow and ring nitrogens are shown in white. In CoSAL and RuSal, the N-OH oxygens and the metal coordinating oxygens are shown in red. In CoSAL, the OH groups (of N-OH) project out of the plane of the molecule, while in RuSal, the OH groups are in the same plane as the respective salicylaldoxime ligands.