Abstract

Title: Synthesis of metal complexes of 5-nitroimidazoles to realize theirbiological potential

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5-nitroimidazoles are effective drugs for different parasites and pathogenic microbes. They are also potential radiosensitizers in the treatment of cancer. Efficacy of 5-nitroimidazoles depends mainly on the ease of formation of the nitro-radical anion ($R\text{-}NO_2^{\cdot-}$). The compounds are reduced in the presence of the enzyme pyruvate ferredoxin oxidoreductase (PFOR); reduction of the nitro group helps themto enter cells by passive diffusion creating a favourable concentration gradient. Anti-microbial efficacy of 5-nitroimidazoles depend on the reduced species which following reduction (at the nitro group) bind to DNA disrupting or breaking strands that are able to cause cell death. As radiosensitizers, they interact with radicals formed on DNA, following the latter's interaction with the products of the radiolysis of water, forming $R\text{-}NO_2^{\cdot-}$ that thereafter enhance strand unwinding or strand breaks.

However, like many known drugs, 5-nitroimidazoles also suffer from adverse drug reactions, neurotoxicity and drug resistance following their prolonged use. Too much generation of reactive intermediates like $R\text{-}NO_2^{\cdot-}$ is responsible. In spite of all controversies, the positive impact of 5-nitroimidazoles tends to outweigh their negative aspects. However, for a safe use of this family of drugs, a more logical approach would be to generate the correct amount of reactive intermediates. Among several approaches, one is, to prepare metal complexes of suchdrugs that enhance their efficacy and address issues related to toxic side effects.

As a part of this work, Ornidazole [1-chloro-3-(2-methyl-5-nitro-1H-imidazole-1-yl)propan-2-ol], an important member of the family of 5-nitroimidazoles was chosen. Cu^{II} and Zn^{II} complexes of it were prepared and characterized through physicochemical experiments in solution and different spectroscopic methods of analysis in solid state. Structures of Cu^{II} and Zn^{II} complexes were determined from single crystal X-ray diffraction and powder X- ray diffraction respectively. DNA binding experiments were performed using calf thymus DNA as the target. A comparison was made between Ornidazole and the prepared complexes by employing physicochemical and biological approaches.

Preparation of complexes decreases formation of $R\text{-}NO_2^{\cdot-}$. This was realized by performing an enzyme assay using xanthine oxidase, a model nitro-reductase. While neurotoxic side effects should decrease following complex formation owing to decreased $R\text{-}NO_2^{\cdot-}$ generation, it would lead to a compromise on therapeutic efficacy in the free radical pathway. We tried to find out aspects related to biological activity of the prepared complexes to see if that is affected in anyway, owing to decreased free radical ($R\text{-}NO_2^{\cdot-}$) generation. For this purpose, several bacterial strains and *Entamoeba histolytica* (HM1:IMS Strain) were chosen as biological targets. Experiments reveal, not only complexes compete with Ornidazole, in fact, under longer exposure times, complexes perform better than Ornidazole. Efficacy of complexes are probably due to their ability to bind to DNA better than Ornidazole which can

be understood from DNA binding studies performed to evaluate interaction of Ornidazole and its complexes with calf-thymus DNA using cyclic voltammetry.

Attempts were made to look at aspects of interaction between R-NO_2^- and other reduction products of Ornidazole and its complexes with nucleic acid bases and calf thymus DNA to realize and correlate what might be happening when such molecules either on their own or complexed to metal ions are enzymatically reduced within a biological target cell. Reduction products of Ornidazole and its complexes were generated by reducing them electrochemically i. e. holding compounds at their respective reduction potentials, determined earlier with the help of cyclic voltammetry. Purine/pyrimidine bases and calf thymus DNA were maintained in the immediate vicinity of reduced species of each compound. Reactions of generated reduction products with purine or pyrimidine bases were followed using HPLC while the amount of calf thymus DNA not modified was determined by treating DNA with ethidium bromide and recording its fluorescence. The study revealed the damage and/or modification caused to different targets by reduced species that were formed either on Ornidazole or on its prepared complexes. The damage caused to purine and/or pyrimidine bases was subsequently correlated with that observed on calf thymus DNA. The study reveals complexes were better in causing modification to nucleic acid bases and DNA when compared to Ornidazole under identical experimental conditions. This supports the fact why there is better performance by complexes on *Entamoeba histolytica* or on several bacterial targets related to bio-film formation etc. as compared to Ornidazole alone.

Experiments were also carried out to compare performance of complexes as radiosensitizers and/or hypoxic cytotoxins with that of Ornidazole. Nucleic acid bases (thymine, cytosine and adenine)or calf thymus DNA, considered as targets, were irradiated with ^{60}Co γ rays, either in the absence or in the presence of Ornidazole and its complexes. Radiation-induced damage of nucleic acid bases were followed by HPLC while modification of calf thymus DNA was followed by the ethidium bromide fluorescence technique. Studies indicate complexes have better radiosensitizing properties than Ornidazole on a chosen biological target.

Overall, the study indicates that the modified forms of 5-nitroimidazoles achieved through complex formation are better in biological activity than the molecules chosen in this research, be it on bacterial cells or on amoeba or as radiosensitizers on model biological targets. Results reveal an expectation, although not verified medically, that the complexes (modified forms of 5-nitroimidazoles) might be less neurotoxic.

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