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ORIGINAL ARTICLE

A detailed *in vitro* study of naproxen metal complexes in quest of new therapeutic possibilities

Md. Sharif Hasan *, **Narhari Das**

Department of Clinical Pharmacy & Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh

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KEYWORDS

Naproxen;
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Abstract *Objective:* The present study was designed to investigate the *in vitro* anti-inflammatory, antimicrobial, cytotoxic and antioxidant effects of Naproxen metal complexes.
Methodology: The anti-inflammatory activity was evaluated by HRBC membrane stabilization method while antimicrobial activity by disk diffusion method. The cytotoxicity was evaluated by brine shrimp lethality bioassay and compared with vincristine sulfate. Antioxidant potential was evaluated in terms of DPPH radical scavenging potential, ABTS scavenging potential, reducing power assay, superoxide dismutase assay and total antioxidant capacity by specific standard procedures.
Results: The Naproxen metal chelates showed significant anti-inflammatory effects in dose dependent manner. Naproxen standard showed maximum inhibition occurred 73.21% at the dose of 2000 μg/ml. Among Naproxen metal chelates, Naproxen silver complex showed potent antimicrobial activity against most of the tested microorganisms while Naproxen zinc complex showed better activity against gram positive strains than gram negative. In brine shrimp lethality bioassay, varying degree of lethality to Naproxen metal chelates was observed showing Naproxen iron complex surprisingly very potent cytotoxic activity compared to vincristine sulfate where other metal complexes displayed reduced cytotoxicity than parent Naproxen while Naproxen exhibited the lowest antioxidant assay among all the metal complexes compared to the standard ascorbic acid.
Conclusion: The present study demonstrated that Naproxen and its complexes possess *in vitro* anti-inflammatory activity while silver, zinc and iron complexes possess higher antimicrobial and cytotoxic properties than the parent ligand and possess very mild antioxidant activity.

1. Introduction

Recent findings on the chemical and biochemical activity of metal complexes play an essential role in agriculture, pharmaceutical and industrial chemistry.[1] In therapeutics, the use of metal complexes with traditional drugs as therapeutic agents for treatment of different diseases has been extensively

* Corresponding author.
E-mail addresses: sha0843@yahoo.com (M.S. Hasan), narhari10@gmail.com (N. Das).
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studied.[2–6] As they generally possess different mechanisms of activity from the organic compounds, the development of metal complexes provides an alternative route of novel drug delivery system.[7] Very recent works on metal complexes have proved that binding of a drug to metalloelement enhances its activity and in many cases the complex possesses even such activity that the parent compound does not have.[8] Thus we have motivated to study metal binding properties of Naproxen derivatives with different transition metal ions and analyzed its different biological properties for the sake of getting any new possibilities of using Naproxen metal complexes for different therapeutic purposes.

Our whole study was designed for understanding the most potential therapeutic activities achieved by formation of metal complexation in the most important biological fields such as inflammation, cytotoxicity, antimicrobial efficacy or efficiency and anti-oxidation reactions. We studied anti-inflammatory activity as there are many modes of anti-inflammatory actions of different transition metals when complexes with organic ligands.[9] Anti-inflammatory activity is measured in HRBC or erythrocyte membrane which is analogous to the lysosomal membrane and its stabilization implies that the synthetic compound may stabilize lysosomal membranes.[10] Stabilization of human red blood cell membrane (HRBC) by hypotonicity induced membrane lysis can be taken as an *in vitro* measurement of anti-inflammatory activity of the drugs. Cytotoxic properties are studied for the complexes which are considered as valuable tool for the screening of anti-tumor or anti-neoplastic agents under controlled *in vitro* conditions. We used brine shrimp lethality bioassay for cytotoxic studies as a preliminary screening technique to find out the potential toxicity profiles.

Multi-Drug Resistant (MDR) microorganisms are the great risk of health hazards in many countries for the today's world. There is no doubt that metal complexes are highly well known for their anti-microbial activities for both resistant and non-resistant species. It is also clearly observed from many studies that anti-microbial activities of metal complexes sometimes possess enhanced activity than their conventional organic drugs itself because metal complexation may lead to varying degree of synergistic effect for either metal ion or ligand or for both.[11–13] Thus, we used a vast range spectrum of microorganisms for determining the anti-microbial properties of synthesized compounds by disk diffusion method. Antioxidant activities are related to those compounds which are capable of protecting a biological system against the potential harmful effects of oxidative processes, therefore, making it important in medicine for the prevention and treatment of free radical pathologies.[9] It has received increased attention in the last years from nutritionists and medical researchers due to their potential chemical and molecular mechanisms in oxidative stress, DNA damage, protein modification, and enzyme activity with emphasis on the chemical and cell-free biological system.[14–17]

To the best of our knowledge and available literature on the subject no detailed research works have been done on all of these properties of Naproxen metal complexes. In the present study we report Naproxen metal complexes with there *in vitro* anti-inflammatory, cytotoxic, anti-microbial and antioxidant properties.

2. Material and methods

2.1. General procedure for synthesis of transition metal complexes of Naproxen

Equimolar metal salts dissolved in water were added to the sodium salt of Naproxen so that the ratios *n*(metal):*n*(ligand) of monovalent, divalent and trivalent ions used were 1:1, 1:2 and 1:3 respectively in each case and immediate precipitation was occurred. Then the solid complexes were isolated by filtration, washed with the corresponding solvent (water) and finally dried at room temperature.[18] The synthesized samples were freely soluble in different coordination solvents such as DMF, DMSO, THF and moderately soluble in chloroform, CCl_4. However, they were insoluble in water, ethanol, and acetone.

2.2. Chemicals and reagents

All chemicals were obtained commercially and were of analytical grade. Sodium phosphate was collected from the Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Bangladesh. All the solutions, reagents and buffers were prepared with distilled water. Vincristine sulfate, used as a standard drug in cytotoxicity assay was collected from the Techno Drugs Limited, Bangladesh. Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich, India. Sodium Chloride Crystal GR from Merck Ltd, Mumbai, India, was used to prepare seawater in brine shrimp lethality bioassay. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, and gallic acid were purchased from Sigma Chemical Co. (St. Louis, USA). Naproxen was used as a standard drug.

2.3. In vitro anti inflammatory activity

2.3.1. Preparation of red blood cells

Human blood was collected from a donor not consuming any NSAIDS drugs for past two weeks. The blood was subjected to centrifugation and the supernatant part was carefully pipetted out with sterile pipettes. The packed cells were resuspended with equal volume of normal physiological saline (pH 7.4) and centrifuged again. The process was repeated five times until the supernatants were clear. A 10% HRBC suspension was then prepared with normal physiological saline and used immediately.[19]

2.3.2. Membrane stabilizing activity assay

4.5 ml of reaction mixture consisting of 2 ml hypotonic saline (0.25% w/v NaCl), 1 ml of sodium phosphate buffer (0.15 M, pH 7.4) and 1 ml of metal chelates was dissolved in normal physiological saline. Then 0.5 ml of 10% HRBC was also added. Two controls were used, one with 1.0 ml of isotonic saline instead of metal chelates, and the second control with 0.5 ml of isotonic saline instead of red blood cells. The mixture was incubated at 56 °C for 30 min. The tubes were cooled under running water for 20 min and the mixture was centrifuged at 3000 rpm. The supernatants were separated and the absorbance of the supernatants was noted at

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