Antibacterial, Antimalarial and Leishmanicidal Activities of Cu (II) and Nickel (II) Complexes of Diclofenac Sodium

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(Received on 10th July 2009, accepted in revised form 6th March 2010)

Summary: Metal complexes are famous for a wide array of chemotherapeutic effects. The current study was designed to synthesize and evaluate unexplored chemotherapeutic effects of Cu (II) and Nickel (II) complexes of the non-steroidal anti-inflammatory drug diclofenac. Nickel complex exhibited significant leishmanicidal activity against *Lieshmania major*, while the copper complex was found to possess low activity against the same pathogen. Both of the complexes revealed low antibacterial activities and were interestingly failed to produce any considerable antimalarial activity against *Plasmodium falciparum* 3D7. Selective leishmanicidal activities of Nickel (II) complex of diclofenac needs further improvement to be developed as potential new metal-based leishmanicidal agent.

Introduction

Diclofenac, a non-steroidal anti-inflammatory drug (NSAIDs), is one of the most frequently used medicines [1]. Diclofenac is used as one of the first-line treatments to treat and manage the pain and edema associated with rheumatoid arthritis, osteoarthritis, spondylitis, and many other inflammatory conditions. Other therapeutic applications include its use for the relief of pain associated with surgery, neoplastic diseases and dysmenorrheal. The anti-inflammatory effect of diclofenac and most of its other pharmacological effects are produced due to its inhibitory effect on the conversion of arachidonic acid to prostaglandins, which are primary mediators of inflammation. Diclofenac is a potent inhibitor of both Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2.), *in vitro* and *in vivo*, thereby decreasing the synthesis of prostaglandins, prostacyclin, and thromboxane products [2-4]. The antithrombotic effect is mediated mainly by COX-1, while anti-inflammatory effects are mediated through COX-2. Various metal ions like copper, cobalt, iron, nickel or manganese can take part in diverse functions and structures within biological systems [5].

Organo-metallic complexation of drugs is an interesting area of research to explore the effects of metal complexes on the behavior of drugs especially the NSAIDs [6-9]. It is known that several NSAIDs act *via* chelation or by inhibiting the activity of metallo-enzymes. The copper (II) complex of diclofenac has been found to have an anti-inflammatory activity far better than diclofenac [10, 11]. Other pharmacological activities of copper complexes, as antiarthritic, antiulcer, anticancer, antidiabetic, and antiepileptic drugs have also been reported [12-15]. Similarly other metals especially the binuclear complexes of diclofenac with nickel produces pronounced pharmacological effects as compared to diclofenac in free form [11, 16, 17]. In the current study our aim was to investigate the unexplored and novel pharmacological effects of the previously synthesized binuclear copper and nickel *i.e.* Cu (II) and Ni (II) complexes with diclofenac to find out new therapeutic windows.

Results and Discussion

Physicochemical and Spectral Characterization

Both the complexes Cu(II) and Ni(II) were synthesized as reported [6]. Briefly, melting point differences, formation of colored products, change in solubility, Infra Red spectral and Atomic Absorption analysis of the complexes were in close agreement with the literature data [6, 21].

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Pharmacological Activities

i. Antibacterial Activity

The antibacterial activities (Table-1,2) of complexes have been tested against *Escherchia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Shigella flexeneri*, and *Salmonella typhi*. Both of the compounds exhibited low antibacterial activities against *S. typhi*, and *B. subtilis*, however, no activity against *Escherchia coli*, *Staphylococcus aureus*, *Shigella flexenari*, *Pseudomonas aeroginosa* [ATCC 27853].

ii. Antimalarial Activity

Both the complexes were found almost inactive as compared to the standard drug. The IC_{50} values of Cu(II) and Ni(II) complexes against *Plasmodium falciparum* 3D7 were 14.55 ± 0.5 μg/mL and above 25 μg/mL, respectively. The IC_{50} value of Chloroquine was 0.025 μg/mL.

iii. Leishmanicidal Activity

Copper and nickel complexes of diclofenac were analyzed for *in-vitro* leishmanicidal activity against *Leishamania major* (Pakistani isolate). Nickel complex exhibited significant leishmanicidal activity (IC_{50}: 19.98 ± 0.28 μg/mL), while the copper complex was found low in leishmanicidal activity (IC_{50}: 104.11 ± 0.73 μg/mL). Both complexes were found less potent relative to the Amphotericin B (IC_{50}: 0.12 ± 0.2 μg/mL)

Discussion

Leishmaniasis is one of the renowned health problems throughout world. It constitutes a group of tropical infectious diseases that position as the second most important after malaria. Leishmaniasis affect 12 million people and threaten 350 million in 88 different countries around the world [22-24]. There are three major types of the disease: cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis. The organic pentavalent antimony compounds are currently available as treatment choice but produce severe side effects and are not effective against the fatal visceral leishmaniasis [22, 23]. Similarly malaria is a devastating infectious disease having a high mortality rate with over 40% of the world's population at risk. The most deadly form of malaria, caused by the protozoan *Plasmodium falciparum*, accounts for more than 2.5 million deaths annually [25-27]. Several transition metal complexes have been studied as possible drugs for leishmaniasis and malaria [25-28]. Results of *in vitro* leishmanicidal activity showed the promising behavior of nickel complex as a potential new leishmanicidal therapy. Structural modifications are needed for further improving the

Table-1: Antibacterial activity of Cu-Diclofenac complex.

Name of Bacteria	Pathogenesis	Zone of inhibition of sample (in mm)	Zone of inhibition of Imipenem as standard drug (in mm)
Escherichia coli	Urinary infection, wound infection, and gastroenteritis	—	30
Bacillus subtilis	Urinary infection, wound infection, ulceration, and septicemia	13	37
Shigella flexenari	Dysentery, and shigellosis	—	36
Staphylococcus aureus	Pneumonia, meningitis, and food poisoning	—	26
Pseudomonas aeruginosa	Respiratory infection, ear, and wound infection	—	32
Salmonella typhi	Enteric fever, food poisoning, bone infection, and septicemia	14	30

KEY: Conc. of sample: 1mg/mL, Conc. of Imipenem: 10 μg /disc, Size of well: 6mm

Table-2: Antibacterial activity of Ni-Diclofenac complex.

Name of Bacteria	Pathogenesis	Zone of inhibition of sample (mm)	Zone of inhibition of Imipenem as standard drug (in mm)
Escherichia coli	Urinary infection, wound infection, and gastroenteritis	—	30
Bacillus subtilis	Urinary infection, wound infection, ulceration, and septicemia	12	37
Shigella flexenari	Dysentery, and shigellosis	—	36
Staphylococcus Aureus	Pneumonia, meningitis, and food poisoning	—	26
Pseudomonas aeruginosa	Respiratory infection, ear, and wound infection	—	32
Salmonella typhi	Enteric fever, food poisoning, bone infection, and septicemia	15	30

KEY: Conc: of sample: 1mg/mL, Conc. of Imipenem:Higly 10 μg /disc, Size of well: 6m

Table-3: Antimalarial Activity of Ni and Cu complexes of Diclofenac.

Test organism		IC_{50} ± SD (µg/mL)
Plasmodium falciparum 3D7	Cu-Diclofenac Complex	14.55 ± 0.5
	Ni-Diclofenac complex	Above 25 µg/mL
	Standard Drug	0.025 ± 0.01

Table-4: Leishmanicidal activity Metal-Diclofenac complexes against *Leishamania major* (Pakistani isolate).

S. No.	Compound / Sample	IC_{50} (µg/mL)	Remarks
1	Amphotericin B	0.12 ± 0.2	Potent inhibitor
2	Ni-Diclofenac complex	19.98 ± 0.28	Significant activity
3	Cu-Diclofenac complex	104.11 ± 0.73	Low activity

efficacy and potency of the diclofenac-nickel complex. However, copper complex failed to exhibit significant leishmanicidal activity. The IC_{50} values of Ni and Cu complexes against Plasmodium falciparum 3D7 are almost 1000 times less potent than the standard compound. This behavior is good in the sense that these complexes are selectively effective against lieshmania parasite rather than malarial parasite thus lesser chances of resistance could develop against lieshmania parasite. Both of the complexes also exhibited low antibacterial activities against *S. typhi* and *B. subtilis*.

One interesting aspect of these complexes was that the biological activities of the metal complexes of diclofenac were better in comparison to diclofenac. This difference will be helpful in further exploring and designing therapeutically and biologically better analogues of the diclofenac in complexation with metals. According to scientific literature, a great deal is known about the role of copper complexation in enhancing the pharmacological profile of NSAID activity and reducing toxicity as compared to other metal complexes of diclofenac. Other pharmacological activities of copper complexes, and their potential as antiarthritic, antiulcer, anticancer, antidiabetic and antiepileptic drugs, have been reported [29]. Similarly copper complexes are known for *in vitro* DNA strand breakage which can be attributed to a wide spectrum of pharmacological activities such as antibacterial, antiprotozoal (including anti-malarial and leishmanicidal activities), antiviral, and antitumor activity [29]. However no considerable work has been done on antibacterial, antimalarial, and leishmanicidal activities of nickel complexes.

With the knowledge that many copper complexes possess anti-inflammatory activity and the finding that these copper complexes almost always have significantly stronger activity than their parent compounds, it has been hypothesized that the active form of many popular anti-inflammatory drugs are their copper chelates. The metal–ligand connection seems to play a specific role, which sustains the hypothesis advanced by Sorenson and co-workers [9]. That copper complexes are the vigorous form of these drugs. In these complexes, either the ligand simply behaves as a transporter that brings the metal ion to the target (despite of its consequent interactions with endogenous ligand) or the complex, by interfering with the inflammatory process, may act as a catalyst.

Experimental

Materials

All chemicals, solvents and metal salts used were of the analytical grade, obtained from Merck chemicals (Germany). They were used as such without further purification.

Instruments

The analysis of carbon, hydrogen and nitrogen were carried out by Euro Vactor Elemental analyzer, Euro Model EA 3000 Italy. Infrared absorption spectra were recorded on Fourier transform IR (FT-IR) spectrophotometer of Shimadzu (Japan), mini press KBr (Potassium bromide) disc was used to prepare transparent disc of samples. The major and important peaks are reported in cm^{-1}. Atomic absorption spectrometer A Analyst 700 Perkin Elmmer USA, has been used for the quantitative estimation of metal contents in complexes. Melting points were determined on Reichert Thermovar of F. G. Bode Co. Austria by taking crystals of samples on the cover slip. Digital PH/ MV meter of Model Nova-210 C, Nova scientific Co., Ltd., Korea was used to adjust the pH of the drugs with sodium hydroxide.

All the experimental work *i.e.* synthesis of complexes including melting point and solubility determination were carried out in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gomal University D. I. Khan. IR spectra were obtained in Ferozsons Labs (pvt) Ltd, Nowshera

N.W.F.P. Atomic Absorption and elemental analysis data were taken in PCSIR Labs Peshawar. *In vitro* biological activities of the complexes were determined in H. E. J. Research Institute of Chemistry, University of Karachi, Karachi.

Test Organisms

Bacterial strains were *Escherchia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Shigella flexeneri (*clinical *isolate), Staphylococcus aureus*, ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *and Salmonella typhi* ATCC 19430, were used for antibacterial activity. *Plasmodium falciparum* 3D7, *Lieshmania major* (DESTO) (Pakistan isolate) were used for antimalarial and lieshmanicidal activities, respectively. The parasites were cultured in RPMI-1640 medium (Sigma, USA) supplemented with 10% fetal bovine serum (Sigma, USA), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 26 °C. Imipenem (for antibacterial activity), Chloroquine (for anti-malarial activity) and Amphotericin B (for lieshmanicidal activity) were used as standard drugs.

Methods

The Cu and Ni complexes of diclofenac sodium were prepared according to the reported procedure [6]. Briefly, 30 mL of 0.1 mol/100 mL aqueous solution of the metal ion were added to 60 mL of 0.1 mol/100 mL aqueous solution of the ligand (CL/CM=2), maintaining the pH of the mixture in the range from 5.5 to 6.5, by the addition of small aliquots of sodium hydroxide solution. The mixture was stirred for about 2 h, at room temperature, and then the precipitate was filtered off, washed with water and dried in vacuum, over silica gel, for at least 48 h. The analysis of all the solid compounds agreed with the literature and empirical formula $M(D)_2(H_2O)_x$ [where M and D, are the metal and the diclofenate ions, respectively and x = 1 for Ni (II) and X = 2 for Cu (II)].

Determination of Pharmacological Activities

Antibacterial Activity

The complexes were checked against various organisms for their antibacterial activity by Agar well diffusion method [19].

In-Vitro Antimalarial Activity

The antimalarial activities of compounds were conducted using the tritiated ^{3}H-hypoxanthine incorporation assay [20, 21]. Parasite growth was estimated by [^{3}H]-hypoxanthine incorporation. Concentrations of compounds inhibiting 50% of the parasite growth (IC_{50}) were determined graphically by plotting concentration versus percent inhibition. All tests were performed at least in triplicate. Chloroquine was used as the positive control.

In-Vitro Lieshmanicidal Activity

Lieshmania major (DESTO) promastigotes were grown at 22-25 °C in RPMI-1640 (Sigma) containing 10% heat-inactivated (56 °C for 30 min) fetal bovine serum. Promastigote culture in the logarithmic phase of growth was maintained and the final concentration of parasites was adjusted to 2 × 106 parasites mL^{-1}. The test compound (1 mg) was dissolved in 50 μL DMSO, and then the volume was made up to 1 mL with the addition of complete media. About 20 μL of test compound dilution was added in first well, which contained 180 μL of media, then serially diluted. A total of 100 μL of parasite suspension was added into each well of the 96-well plates and incubated at 21-22 °C for 72 h, in the presence and absence of amphotericin B (as a positive and negative controls, respectively). The experiments were carried out in triplicate and the numbers of surviving parasites were counted in Neubauer chamber. The 50% inhibitory concentrations (IC_{50}) were determined by a Windows based EZ-Fit 5 Perrella Scientific Software.

Statistical Analysis

All experiments were performed in triplicates and results were expressed as mean ± SD. Statistical analyses were performed using GraphPad and EZ-Fit 5 Perrella Scientific Software.

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