Synthesis, Characterization and Analgesic Activity of Cadmium(II) Complex of Tolfenamic Acid

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ABSTRACT: Tolfenamic acid is a member of fenamate group of non-steroidal anti-inflammatory drugs (NSAID). It is generally used to treat migraine. In recent studies, metal complexes of traditional drugs like, manganese(II) complex of tolfenamic acid and mefenamic acid; cobalt(II) and zinc(II) complexes of tolfenamic acid are reported to give better biological activities than their parent drugs. In this study, tolfenamic acid was complexed with a divalent metal, cadmium(II) to form the drug-metal complex. The peripheral and central analgesic activities of this complex were tested to find out the impact of complexation on the pharmacological activity of the drug. Cadmium nitrate tetrahydrate was used for the complexation reaction and Fourier transform infrared (FT-IR) spectroscopic technique was used to identify the drug-metal complexation. Writhing method and tail flick technique were used to test peripheral and central analgesic activities, respectively in Swiss albino mice model. Comparison of the data obtained for positive and negative controls with the drug metal complex revealed a statistically significant increase in analgesic activities. Extensive experimentation is underway for further development in order to achieve better activity and assess toxicity profiles.

Key words: Tolfenamic acid, Writhing, Drug-metal complex, Analgesic activity, Cadmium.

INTRODUCTION

Tolfenamic acid or 2-[(3-Chloro-2-methyl-phenyl) amino]benzoic acid is an analgesic agent, belonging to the non-steroidal anti-inflammatory (NSAID) class of drugs (Figure 1). Tolfenamic acid is a derivative of fenamic acid and has chemical structure similar to mefenamic acid, flufenamic acid and other fenamate NSAIDs. It has been discovered by the scientists of Medica Pharmaceutical Company in Finland.[1,2]

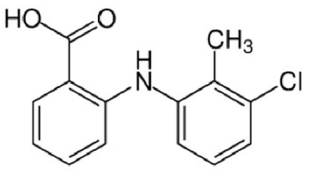

Figure 1. Structure of tolfenamic acid.

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Although being part of NSAID, fenamate-derived drugs are used generally in the treatment of migraine attacks. Migraine is a recurrent headache attack with moderate or severe intensity, which occurs in one side of the headache and is pulsating in nature. Migraine attack is caused by vasodilatation of blood vessels in the scalp, following serotonin induced vasoconstriction.[3,4] Prostaglandins (PGE) have been reported to escalate this condition of vasodilation.[5]

Fenamate NSAIDs have been shown to reduce prostaglandin production and alleviate migraine attacks.[4,6,7] Tolfenamic acid, along with other fenamate NSAIDs treat migraine condition by inhibiting biosynthesis of prostaglandin and also by blocking prostaglandin receptors.[1,7]

Metal complexes of drug have gained popularity as a new class of therapeutics. Metal centers, being positively charged, are favored to bind to negatively charged biomolecules. Proteins and nucleic acids

offer excellent ligands for binding to metal ion. Pharmaceutical use of metal complexes therefore has excellent potential. Broad arrays of medicinal applications of metal complexes have been investigated, and several recent reviews have summarized the advances in these fields.[8-11] Metal complexes of fenamate drugs such as, manganese(II) complexes of mefenamic acid ($[Mn(mef)_2(H_2O)_2]$) and tolfenamic acid ($[Mn(tolf)_2(phen)(H_2O)]$) have been reported to show better free radical scavenging activity and soybean lipoxygenase inhibitory activity than their un-complexed counterpart[12,13], whereas the zinc(II) complex of mefenamic acid ($[Zn(mef)_2]$) has demonstrated improved anti-inflammatory effect.[13] Cobalt(II) and zinc(II) complexes of tolfenamic acid have also shown better peripheral analgesic activity than the parent drug.[14] Moreover, cadmium(II) complex of meclofenamic acid ($[Cd(meclo)_2(H_2O)_2]$) has been reported to give better antiproliferative activity than free meclofenamic acid and metallodrug cis-platin.[15]

In the current study, tolfenamic acid was complexed with cadmium to form cadmium(II) tolfenamic acid complex, and the peripheral and central analgesic activities of this complex were tested on mice model.

MATERIALS AND METHODS

General experimental procedures. Tolfenamic acid (10 g) was collected from Beacon Pharmaceuticals Limited. Cadmium nitrate tetrahydrate, sodium hydroxide, Tween 80, and ethanol were collected from Merck, Germany. The FT-IR spectra of tolfenamic acid and final cadmium(II) complex of tolfenamic acid were acquired on IR Prestige-21 of Shimadzu Corporation at Department of Chemistry, University of Dhaka.

Synthesis of complex. In this study, cadmium(II)-tolfenamic acid complex was prepared by following the published method.[16] Tolfenamic acid (81 mg) was dissolved in ethanol and ethanolic solution of sodium hydroxide (16 mg) was added to it to reduce its acidity. Cadmium nitrate tetrahydrate (51.8 mg) was dissolved in ethanol in a separate container. The alkaline solution of tolfenamic acid was then added to the cadmium nitrate solution. About 2 mL of distilled water was added to the above reaction mixture. The resulting mixture was mechanically stirred for 30 minutes and then kept in a refrigerator for 7 days. After refrigeration, white crystalline product of $[Cd(tolf)_2 \bullet H_2O]_2$ was collected (52.38 mg) from the mixture. The obtained product was found to be stable in air and light under ambient conditions. The drug-metal complex was the only solid pure product of this reaction and other reactants were soluble in solvent.

Characterization of the parent drug and complex compound. FT-IR spectra of tolfenamic acid alone and its complex with cadmium(II) were acquired to confirm the presence of coordination bond. The FT-IR spectra were recorded in the range 4000-400 cm^{-1} in solid phase and KBr was used to form disk.

Experimental animal. Twenty Swiss-albino mice of either sex (25-30g) were obtained from the Animal Unit, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. They were housed at room temperature in Animal House, State University of Bangladesh, with sufficient ventilation. They were kept in this environment for 7 days prior to the experimentation. Animals were orally fed with mice food poultice and water ad-libitum.

As cadmium is a toxic element and carcinogen, the amount of cadmium, used in test sample was maintained below the level defined as toxic level.[17] In addition, mice used in the experiment were observed for 7 days after the experimentation, for any signs of abnormality. In this period, no mouse displayed any abnormal behaviors and showed mortality. All the biological experiments were conducted at the Department of Pharmacy, State University of Bangladesh by following the Helsinki declaration-2000.

Biological evaluation

Peripheral analgesic activity. Peripheral analgesic activity was evaluated by acetic acid induced writhing method.[18] In this experiment, acetic acid was administered intra-peritoneally to the

experimental mice, to create pain sensation. Mice contracted body at regular interval due to pain. This contraction or squirm is called 'writhing'. The number of writhings was counted and taken as an indication of pain sensation. In this method, any substance that has got analgesic activity, is supposed to lessen the number of writhing of animals in a given time frame and number of writhing is then compared with that of a predetermined control group. The writhing inhibition of sample was taken and compared with that of positive and negative controls. Tolfenamic acid and 0.9% NaCl solution in distilled water were used as positive and negative controls, respectively. Positive control and drug-metal complex were given to the mice at dose 25 mg/kg of body weight. The degree of analgesia or the percentage of inhibition of writhing was calculated using the following formula:

$$\% \text{ Inhibition of writhing} = \frac{\text{Mean of negative control group} - \text{Mean of test group}}{\text{Mean of negative control group}} \times 100$$

Central analgesic activity. The evaluation of central analgesic activity was carried out by tail immersion variant of the tail flick method.[19] The change in sensitivity of test animal due to central analgesic activity of drugs is measured in this method. A constant heat stress was applied to mice tails by immersing it in a hot water bath. This immersion of mice tails in hot water bath acted as pain stimulus. When the stimulus exceeded the threshold, the mice show a quick withdrawal of their tails from the water. Time taken by the mice to withdraw their tails is termed as tail immersion time. Compounds having central analgesic activity elongate this responding time. In this experiment, morphine was used as the positive control and 0.9% NaCl solution in distilled water was used as the negative control. The percentage elongation of time was calculated according to the following formula:

$$\% \text{ Elongation of reaction time} = \frac{\text{Mean reaction time of test group} - \text{Mean reaction time of negative control group}}{\text{Mean reaction time of negative control group}} \times 100$$

Statistical analysis. The statistical analyses were done by using the Statistical Package for Social Science version (SPSS) 22.0 software, and the statistical differences between groups were analyzed by one-way analysis of variance ANOVA followed by Tukey test. The results were represented as means ± SEM and differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Fourier-transform infrared spectroscopy. The infrared spectrum of Cd(II) complex with tolfenamic acid was compared along with that of tolfenamic acid (Figure 2). The tentative assignment of the different vibrational modes indicated the reaction of tolfenamic acid with Cd(II). The prepared compound was identified and confirmed as a complex of cadmium(II) and tolfenamic acid by similarity in presence and positions of peaks in their IR spectra. In the spectrum of tolfenamic acid, the O-H stretching mode of carboxyl group was observed in the 3150-2450 cm^{-1} region, and the peaks corresponding to carbonyl group was observed at 1660 cm^{-1}, the stretching vibration of secondary amine at 3340 cm^{-1}, the bending vibration of secondary amine at 1501 cm^{-1} and the peak of CH_3 was observed at 1435 cm^{-1}. However, in the infrared spectrum of the complex, the O-H stretching mode of carboxyl group was absent. The peaks of carbonyl group, stretching and bending vibrations of secondary amine were observed at 1613 cm^{-1}, 3327 cm^{-1} and 1498 cm^{-1}, respectively.

In the complex, the peak for CH_3 group was observed at 1460 cm^{-1}. In addition, the peaks at 515 cm^{-1} and 3313 cm^{-1} were observed in the spectrum of the complex which were absent in the spectrum of tolfenamic acid. These additional peaks correspond to metal-ligand bonds of Cd(II)-tolfenamato and Cd(II)-

OH_2 groups. The carboxyl group is assumed to form the coordination bond with Cd(II), as this group contains the most acidic hydrogen of tolfenamic acid. During complexation reaction, deprotonation occurs in the carboxyl group instead of the secondary amino group, a weaker donor group compared to carboxylic group. The softening of the C-O bonds of the carbonyl group indicated the formation of another coordination bond between Cd(II) and carbonyl group. The IR spectra of tolfenamic acid and the Cd(II)-tolfenamato complex also demonstrated the presence of the peaks of secondary amine group. The presence of additional peak at about 515 cm^{-1}, indicated the formation of a coordination bond between Cd(II) cation and tolfenamato anion. The coordinated H_2O was also identified in the spectrum of the Cd(II)-tolfenamato complex at about 3313 cm^{-1}. The complex can be suggested as a binuclear complex of Cd(II), as it is commonly observed for the reaction between other divalent metal ions and tolfenamic acid; with Co^{2+} (Cobalt)[14,20], Cu^{2+} (Copper)[21], Zn^{2+} (Zinc)[14] and Ni^{2+} (Nickel).[22] Therefore, the molecular formula of the complex is presumed to be $[Cd(tolf)_2 \bullet H_2O]_2$ as shown in figure 3.

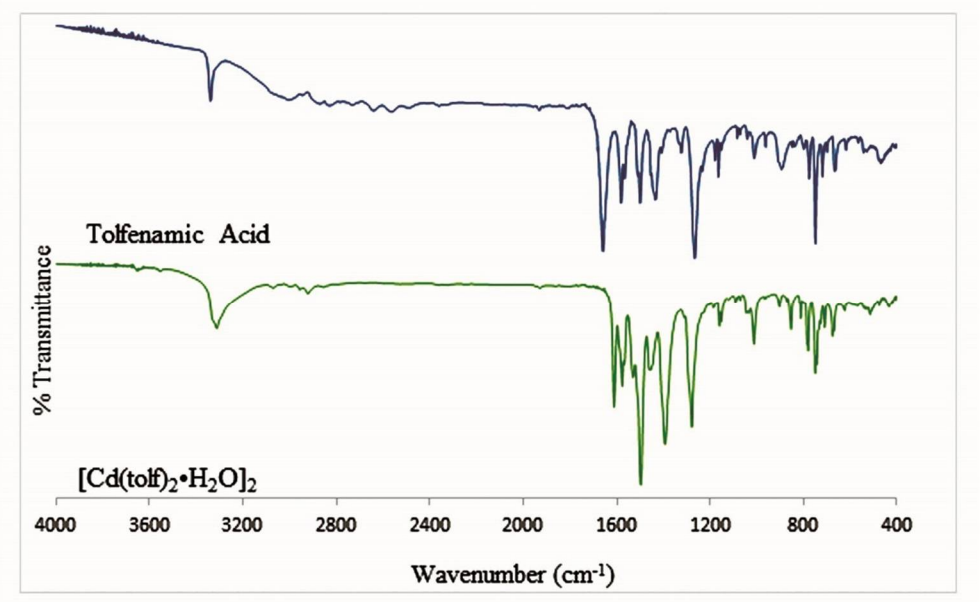

Figure 2. FT-IR spectra tolfenamic acid (dark blue) and $[Cd(tolf)_2 \bullet H_2O]_2$ complex (green).

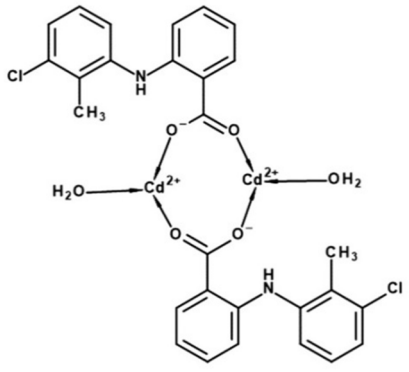

Figure 3. Suggested structure of $[Cd(tolf)_2 \bullet H_2O]_2$.

Peripheral analgesic activity. Here, the test sample ($[Cd(tolf)_2 \bullet H_2O]_2$) showed statistically significant ($p<0.05$) peripheral analgesic activity when compared to that of the control group treated with tolfenamic acid (Table 1). The acetic acid is a pain stimulus and induces pain with biosynthesis of prostaglandins (PGI_2 and PGE). The cadmium(II) complex of tolfenamic acid displayed analgesic activity by inhibiting the biosynthesis of PGE and blocking its receptor, as does its uncomplexed counterpart.[2,6]

Central analgesic activity. The test sample, ($[Cd(tolf)_2 \cdot H_2O]_2$), showed statistically significant ($p<0.05$) central analgesic activity. In the long run, the test sample showed noticeable increase in the central analgesic activity as compared to that of tolfenamic acid (Table 2). Tail flick test is used to test central analgesic activity of the drug-metal complex. Reduction of late pain (at 60 and 90 minutes) by the drug-metal complex was similar to that of tolfenamic acid. However, the test sample's increase in central analgesic activity in diminishing early pain than tolfenamic acid indicated its improved central analgesic activity.[23]

Table 1. Peripheral analgesic activity of tolfenamic acid and its complex with cadmium.

Group	Dose (mg/kg b.w.)	Number of writhing	% Inhibition of writhing
Negative Control (0.9% NaCl in distilled water)	-	19.6 ± 0.51	-
Positive Control (Tolfenamic Acid)	25	9.2 ± 1.11*	53.06
$[Cd(tolf)_2 \cdot H_2O]_2$	25	7.6 ± 1.12*	61.22

Values are expressed as Mean ± SEM from the experiments *p< 0.05 vs. control; n = 5

Table 2. Central analgesic activity of tolfenamic acid and test sample.

Group	Dose (mg/kg b.w.)	Reaction time (sec), % Elongation		
		after 30 mins (sec)	after 60 mins (sec)	after 90 mins (sec)
Negative Control (0.9% NaCl solution in distilled water)	-	2.52 ± 0.06	2.52 ± 0.11	2.73 ± 0.15
Standard (Morphine)	2	15.3 ± 0.49, 506.18*	10.22 ± 0.61, 305.23*	6.48 ± 0.31, 137.19*
Tolfenamic Acid	10	2.88 ± 0.21, 13.95	5.76 ± 0.18, 128.47*	8.35 ± 0.36, 205.49*
$[Cd(tolf)_2 \cdot H_2O]_2$	25.67	5.31 ± 0.17, 110.46*	6.52 ± 0.23, 158.37*	9.38 ± 0.47, 243.19*

Values are expressed as Mean ± SEM from the experiments; *p < 0.05 vs. control; n = 5

The metal-complexes have been emerged as new classes of therapeutic agents.[24] In this experiment, cadmium(II) was complexed with tolfenamic acid to observe the change in analgesic activity of the drug. Promising outcome of the experiment indicates that the drug-metal complex might give better pharmacological activity in the treatment of migraine than its un-complexed counterpart. However, this was a preliminary study and further comprehensive experiments are required to establish its safety and efficacy in the molecular level.

AUTHORS' CONTRIBUTIONS

TS and MSR were involved in conception and design of the study. SS helped in acquisition of data. SS, MSR and TS analyzed and interpreted data. All the authors have read and approves the version to be submitted.

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Conflict of interest

The authors declare no conflict of interest.

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