Screening of Analgesic, Antimicrobial, Cytotoxic and Antioxidant Activities of Metal Complexes of Indomethacin

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ABSTRACT: The present study was performed to evaluate the biological activities of metal complexes of indomethacin with cobalt, copper, manganese and zinc. In radiant tail flick method, complexes of indomethacin with cobalt and copper at a dose of 20 mg/kg b.w. showed significant central analgesic activity having 66.09% and 75.45% elongation of time after 30 minutes and complexes of indomethacin with copper at a dose of 20 mg/kg b.w. showed significant central analgesic activity having 62.47% elongation of time after 60 minutes compared to the standard morphine. In this study, indomethacin and its complexes with cobalt, copper and manganese showed mild antimicrobial activity and the indomethacin-manganese complex also displayed highest cytotoxicity with a lowest LC_{50} 1.222 ± 0.21 µg/ml and indomethacin-cobalt, indomethacin-copper and indomethacin-zinc had significant LC_{50} of 1.549 ± 0.39 µg/ml, 1.662 ± 0.17 µg/ml and 1.903 ± 0.64 µg/ml, respectively where standard vincristine sulphate had LC_{50} of 0.824 ± 0.04 µg/ml. The complex of indomethacin with cobalt, copper, manganese and zinc revealed % of inhibition 38.46 ± 1.03, 64.31 ± 0.21, 46.71 ± 0.46 and 30.79 ± 0.30, respectively and also had significant IC_{50} of 17.51 ± 0.62 µg/ml, 12.31 ± 0.58 µg/ml, 15.71 ± 0.16 µg/ml and 19.84 ± 0.08 µg/ml correspondingly. This study indicates that the complexes of indomethacin had analgesic, antimicrobial, cytotoxic and antioxidant activities which could be subjected for further therapeutic evaluation.

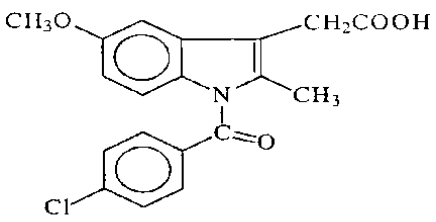
Key words: Indomethacin**,** metal complex, radiant tail flick method, central analgesic activity

INTRODUCTION

NSAIDs are nonsteroidal anti-inflammatory agents which are grouped as a class of drugs that shows antipyretic, analgesic activities and anti-inflammatory activities. The necessity of potent and less gastro intestinal damaging agents has led to the synthesis and clinical trial of metal salts of indomethacin.[1,2]

Nonsteroidal anti-inflammatory drugs are most commonly used drugs for the treatment inflammatory diseases such as osteoporosis and arthritis, after having their serious gastrointestinal and cardio-vascular toxicity.[3,4] Indomethacin is readily absorbed with helps to increase in membrane permeability and a rapid reduction in mucosal potential difference.[5]

Nonsteroidal anti-inflammatory drugs can cause damage to the surface-active phospholipids on the mucosal surface.[6-10] NSAIDs tends to reduce bicarbonate and mucosal secretion which decrease the efficacy of the pH gradient in epithelial protection.[11-13]



Indomethacin

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Also there is suppression in prostaglandin levels which cases damage to the gastric mucous layer.[14] Several trails have been made to reduce the unexpected side effects associated with NSAIDs. Cobalt, copper, manganese and zinc are the d-block elements which may also have the profile of increasing the efficiency of indomethacin in complex form.

So, in this study, we have mentioned about the synthesis and analgesic, antimicrobial, cytotoxic and antioxidant activities of metal complexes of indomethacin with cobalt, copper, manganese and zinc to observe pharmacological activities with good chemical stability.

MATERIALS AND METHODS

Drugs and materials. Indomethacin was obtained from Ningbo-Smart Pharmaceutical, China. Chemical salts were collected at highest purity from Aristopharma Ltd. and ACI Pharmaceuticals Ltd. Morphine was purchased from Gonoshasthaya Pharmaceuticals Ltd. Vincristine sulfate and ciprofloxacin were collected from Becon Pharmaceuticals Ltd and Square Pharmaceuticals Ltd., respectively.

Solvents and reagents. Dimethyl sulfoxide and sodium bicarbonate were purchased from Merck, Darmstadt, Germany. Acetone, tween-80, methanol and 1, 1-dipheny-l-2-picryl hydrazyl were collected from Sigma Chemicals, USA. Normal saline was obtained from Opsonin Pharma. All chemicals and reagents were of analytical grade.

Synthesis of metal complexes. Indomethacin 1 g (0.0028 mol) and acetone 11.2 ml were mixed in a beaker. In the mean while sodium bicarbonate 0.235 g (0.0028 mol) was mixed with 11.2 ml of water. The solutions were blended in a beaker and after that another solution of cobalt chloride hexahydrate 0.333 g (0.0014 mol) copper sulphate pentahydrate 0.223 g (0.0014 mol), manganese sulphate monohydrate 0.236 g (0.0014 mol) and zinc sulphate heptahydrate 0.226 g (0.0014 mol) were prepared in 11.2 ml water and these solutions were mixed separately with indomethacin and sodium bicarbonate mixed solution in different test tubes slowly and stirred continuously. So there pink, green, white and white precipitations were formed consequently. Precipitates were filtered, washed with water and acetone subsequently. At last, it was dried under vacuum condition to get the metal complexes of indomethacin. The yield values were 69.4%, 68.9%, 65.2% and 76.5% of indomethacin complexes with cobalt, copper, manganese and zinc respectively.

Experimental animals. Adult Swiss-albino mice 25-30 g ages of 4-5 week were used as experimental animal. They were housed properly and kept under well controlled temperature with humidity of 60-70% in the animal house and kept before the test for 5-7 days because of their environmental sensitivity.

Central analgesic activity. Tail flick assay of animal models was used to determine central analgesic response.[15] A hot wire was applied to rat tail, which acted as pain stimulus. When the stimulus exceeded the threshold, rat showed instant withdrawal of its tail.[16] In this experiment, test samples and saline were administrated orally. Tail flicking time was taken by analgesiometer. For making the wire hot, current was passed through the wire. The animals flick the tail aside the tail. The time of withdrawal of the tail was recorded. Percentage of time elongation was calculated using the following formula: % elongation of reaction time = Average reaction time of test group - Average reaction time of control group / Average reaction time of control group**.** The central analgesic activity of the test samples were compared in respect to morphine. Statistical analysis was done using SPSS software by one-way ANOVA considering 95% confidence level at $p<0.05$ being considered as significant.

Antimicrobial activity. Indomethacin and its complexes were tested for antimicrobial activities by the standardized disc diffusion method.[17] *In vitro* antimicrobial screening was done against numerous strains of bacteria and fungi. The obtained results were compared with standard antibiotic, ciprofloxacin.

Cytotoxic activity. Cytotoxicity was evaluated by using brine shrimp lethality test according to the reported method.[18] In this test dimethyl sulfoxide and vincristine sulfate were used as negative control and positive control correspondingly. Ten matured shrimps were applied to each of all test tubes of indomethacin and its complexes. After 24 hours the morbidity of brine shrimps was observed. An approximate linear correlation was observed by plotting logarithm of concentration versus percentage of mortality.

Antioxidant activity. The antioxidant activity of indomethacin and its complexes was assessed by 1, 1-dipheny-l-2-picryldrazyl and estimated by reported methods.[19] Here butylated hydroxyl toluene was used as standards and DPPH solution was used as control. The absorbance was measured by UV spectrophotometer at a wave length of 570 nm. Inhibition of free radical was estimated by following equation:

Inhibition of free radical % = A_c-A_s/ A_c ×100 where, A_c = Absorbance of the control and A_s = Absorbance of the indomethacin and its complexes. The 50% inhibitory concentration IC_{50} was calculated by plotting the inhibition concentration versus standard indomethacin complex concentration.

Statistical analysis. Statistical analyses were done by using the Statistical Package for Social Science version 16.0 software, and statistical differences between groups were analyzed by one-way analysis of variance ANOVA followed by Dunnet's t-tests. Data's were represented as means ± SEM and differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Central analgesic activity. The central analgesic activities of metal complexes of indomethacin in tail flick method are given in Table 1. In this experiment, metal complexes were given at a dose of 10 mg/kg b.w. and 20 mg/kg b.w. individually. Complexes of indomethacin with cobalt and copper at a dose of 20 mg/kg b.w. showed significant analgesic activity having 66.09% and 75.45% elongation of reaction time and complexes of indomethacin with manganese and zinc at a dose of 20 mg/kg b.w. showed significant analgesic activity having 38.78% and 38.89% elongation of reaction time, respectively at 30 minutes compared to the standard morphine 132.52%, where indomethacin at a dose of 10 mg/kg b.w. showed the least elongation reaction time 14.67%. At 60 minutes, for the complexes of indomethacin with cobalt, copper, manganese and zinc at a dose of 20 mg /kg b.w., increased the tail flicking time by 42.52%, 62.47%, 31.79% and 33.11% subsequently and after 90 minutes, the central analgesic activity gradually decreases except the complex of indomethacin with copper at a dose of 20 mg/kg b.w. which showed significant analgesic activity of 37.18%.

Table 1. Central analgesic activity of indomethacin and its metal complexes.

Groups	Dose mg/kg b.w.	Reaction time sec		
		% elongation 30 min	% elongation 60 min	% elongation 90 min
Control	-	9.41 ± 0.38	8.82 ± 0.83	8.15 ± 0.38
Standard	2	21.88 ± 0.41, 132.52***	19.18 ± 0.48, 117.46***	16.8 ± 0.85, 106.13***
Indomethacin	10	10.79 ± 1.10, 14.67	9.82 ± 1.30, 11.33	8.93 ± 0.91, 9.57
	20	11.46 ± 0.51, 21.79	10.38 ± 0.76, 17.68	9.27 ± 0.11, 13.74
Indomethacin-cobalt	10	10.91 ± 1.22, 15.94	9.92 ± 0.92, 12.47	9.07 ± 0.49, 11.29
	20	15.33 ± 0.93, 66.09***	12.57 ± 1.39, 42.52**	10.51 ± 0.67, 28.96*
Indomethacin-copper	10	12.29 ± 0.59, 30.61*	10.86 ± 1.60, 23.13	9.91 ± 0.52, 21.59
	20	16.51 ± 0.19, 75.45***	14.33 ± 0.35, 62.47***	11.18 ± 0.30, 37.18**
Indomethacin-manganese	10	11.06 ± 0.47, 17.53	10.33 ± 0.84, 17.12	9.67 ± 0.62, 18.65
	20	13.06 ± 1.01, 38.78**	11.57 ± 0.51, 31.79**	10.72 ± 0.59, 29.69*
Indomethacin-zinc	10	11.54 ± 0.83, 22.32	9.51 ± 0.44, 7.82	8.62 ± 0.21, 5.77
	20	13.07 ± 1.15, 38.89**	11.74 ± 1.37, 33.11**	10.50 ± 0.29, 28.83*

Each value expressed as the mean ± SEM. Significant at ***$p<0.001$, **$p<0.01$, *$p<0.05$ compared to control group.

Table 2. Antimicrobial activity of indomethacin and its metal complexes.

Test organism	Zone of inhibition in mm					
	CIP	I	ICO	IC	IM	IZ
Gram Positive bacteria						
Bacillus cereus	44	9	7	9	8	-
Bacillus megaterium	44	9	7	8	8	-
Bacillus subtilis	44	10	7	8	8	-
Sarcina lutea	45	9	7	8	8	-
Staphylococcus aureus	44	9	7	8	8	-
Gram Negative bacteria						
Escherichia coli	44	9	7	8	8	-
Pseudomonas aeruginosa	44	9	7	8	8	-
Salmonella paratyphi	45	9	7	8	8	-
Salmonella typhi	45	9	7	8	8	-
Vibrio parahaemolyticus	45	9	7	8	8	-
Shigella boydii	44	9	7	8	8	-
Shigella dysenteriae	44	9	7	8	8	-
Vibrio mimicus	44	9	7	8	8	-
Fungi						
Candida albicans	45	9	7	8	8	-
Aspergillus niger	46	9	7	8	8	-
Sacharomyces cerevacae	45	9	7	8	8	-

CIP = ciprofloxacin; I= indomethacin; ICO = indomethacin-cobalt; IC = indomethacin-copper; IM = indomethacin-manganese; IZ = indomethacin-zinc.

Table 3. Cytotoxic activity of indomethacin and its complexes.

Test samples	LC_{50} µg/ml
Vincristine Sulphate	0.824 ± 0.04
Indomethacin	1.542 ± 0.13***
Indomethacin-cobalt	1.549 ± 0.39***
Indomethacin-copper	1.662 ± 0.17***
Indomethacin-manganese	1.222 ± 0.21***
Indomethacin-zinc	1.903 ± 0.64***

Data of LC_{50} represents mean ± SEM of triplicate analysis and significant at ***p<0.001 compared to vincristine sulphate.

Table 4. Antioxidant activity of indomethacin and its metal complexes.

Samples	% of Inhibition	Free radical scavenging activity IC_{50} µg/ml
BHT	66.47 ± 0.14	11.45 ± 0.53
Indomethacin	28.51 ± 0.39	21.73 ± 0.64
Indomethacin-cobalt	38.46 ± 1.03**	17.51 ± 0.62**
Indomethacin-copper	64.31 ± 0.21***	12.31 ± 0.58***
Indomethacin-manganese	46.71 ± 0.46***	15.71 ± 0.16***
Indomethacin-zinc	30.79 ± 0.30**	19.84 ± 0.08**

Values are expressed as the mean ± SEM of triplicate analysis and significant at ***p<0.001, **p<0.01, *p<0.05 compared to standard BHT.

Antimicrobial activity. To determine antimicrobial activity of metal complexes of indomethacin were tested against some gram positive and some gram negative bacteria. Here, five gram positive bacteria namely, *B. cereus, B. megaterium, B. subtilis, S. lutea* and *Stap. aureus* as well as eight gram negative bacteria namely, *E. coli, P. aeruginosa, S. paratyphi, V. mimicus, S. dysenteriae* and also three fungi namely, *C. albicans, A. niger* and *S. cerevacae* were used (Table 2). In this study,

indomethacin and its complexes showed mild to moderate antimicrobial activity except zinc complex of indomethacin. The zone of inhibition for indomethacin, indomethacin-cobalt, indomethacin-copper and indomethacin-manganese were 9-10 mm, 7 mm, 8-9 mm and 8 mm respectively which was compared to standard ciprofloxacin with an inhibition zone of 44-45 mm as mentioned in Table 2.

Cytotoxic activity. The results obtained from brine shrimp lethality assay are presented in Table 3. The LC_{50} denoted the concentration by which 50% of shrimps were killed. Here, indomethacin manganese complex showed the highest cytotoxicity with a lowest value of LC_{50} 1.222 ± 0.21 µg/ml. The positive control appeared to have a LC_{50} of 0.824 ± 0.04 µg/ml. As well as, indomethacin, indomethacin-cobalt, indomethacin-copper and indomethacin-zinc had a LC_{50} of 1.542 ± 0.13 µg/ml, 1.549 ± 0.39 µg/ml, 1.662 ± 0.17 µg/ml and 1.903 ± 0.64 µg/ml correspondingly.

Antioxidant activity. The antioxidant activity of indomethacin and its complexes are shown in Table 4. Complex of indomethacin with cobalt, copper, manganese and zinc showed % of inhibition 38.46 ± 1.03, 64.31 ± 0.21, 46.71 ± 0.46 and 30.79 ± 0.30, respectively. In addition, complex of indomethacin with cobalt, copper, manganese and zinc were tested against for the evaluation of free radical scavenging capacity compared to tert-butyl-1-hydroxytoluene as standard. In this case, complex of indomethacin with cobalt, copper, manganese and zinc showed free radical scavenging activity having significant IC_{50} of 17.51 ± 0.62 µg/ml, 12.31 ± 0.58 µg/ml, 15.71 ± 0.16 µg/ml and 19.84 ± 0.08 µg/ml, respectively.

CONCLUSION

Therefore, it can be concluded from the present studies that cobalt and manganese complexes of indomethacin showed strong central analgesic activity and copper and manganese complexes of indomethacin also showed significant antioxidant activity. In case of antimicrobial and cytotoxicity screening mild activities were observed. So, further studies will be focused on the other biological activities of the metal complexes.

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