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The effects of 3-benzoyl-1-methyl-4-phenyl-4-piperidinolhydrochloride (C1), indomethacin, nimesulide and rofecoxib on cyclooxygenase activities in carrageenan-induced paw edema model

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Aim: The aim of this study was to investigate the effects of 3-benzoyl-1-methyl-4-phenyl-4-piperidinol-hydrochloride (C1), which is a structural and also non-classical isomer of bis Mannich base, bis (3-aryl-3-oxo-propyl) methylamine hydrochlorides (B1), on cyclooxygenase (COX) activities in 48 rats with inflammation by using carrageenan-induced paw edema, and to compare its effect with other non-steroidal anti-inflammatory drugs (NSAIDs).

Materials and methods: Experimental animals were supplied by the Center of Experimental Research and Practice in Ataturk University. The animals were housed and fed in the laboratory (normal room temparature, food and water) under standard conditions. Rats were randomly assigned to eight groups (n = 6); control, intact, indomethacin, nimesulide, rofecoxib, C1 (50 mg), C1 (100 mg), and C1(200 mg).

Results: C1 significantly inhibited COX-1 ($P < 0.01$ for all) and COX-2 ($P < 0.01$ for all) activities at the doses of 50, 100, and 200 mg kg^{-1} when compared to control group. The inhibitory effect of C1 on COX-1 and COX-2 activities at all doses was similar to those of nimesulide. While C1 at 200 mg kg^{-1} significantly inhibited COX-1 and COX-2 activities ($P < 0.01$ for both), at C1 100 mg kg^{-1} significantly inhibited only COX-1 activity in comparison to rofecoxib ($P < 0.05$). Inhibitory effects of C1 on COX-1 activity at the doses of 50 and 100 mg kg^{-1} were significantly weaker when compared to indomethacin ($P < 0.01$ and $P < 0.05$, respectively).

Conclusion: It may be claimed that C1 has an anti-inflammatory effect, and its COX-2 selectivity is stronger than indomethacin and nimesulide but weaker than rofecoxib.

Key words: 3-benzoyl-1-methyl-4-phenyl-4-piperidinol-hydrochloride, COX enzymes, inflammation

Karagenin ile oluşturulmuş pençe ödemi modelinde 3-benzoil-1-metil-4-fenil-4-piperidinol-hidroklorür (C1), indometazin, nimesulid ve rofekoksib'in siklooksijenaz enzim aktiviteleri üzerine olan etkileri

Amaç: Çalışmanın amacı 3-benzoil-1-metil-4-fenil-4-piperidinol-hidroklorür (C1) molekülünün karagenin ile oluşturulmuş pençe ödemi inflamasyonu modelinde siklooksijenaz (COX) enzim aktiviteleri üzerine etkilerini araştırmak ve bu etkisini diğer nonsteroid antiinflamatuar ilaçlarla (NSAİİ) karşılaştırmaktı.

Yöntem ve gereç: Deney hayvanları Atatürk Üniversitesi Deneysel Araştırma ve Uygulama Merkezinden sağlandı. Hayvanlar standart laboratuar koşullarında beslendi ve her grupta 6 rat olmak üzere 8 gruba ayrıldı. Bu gruplar; kontrol, intakt, indometazin, nimesulid, rofekoksib, C1 (50 mg) C1 (100 mg) ve C1 (200 mg) idi.

Bulgular: Kontrol grubu ile karşılaştırıldığında, C1 50, 100, and 200 mg kg^{-1} dozlarda COX- 1 (hepsi için $P < 0,01$) ve COX-2 enzim aktivitesini (hepsi için $P < 0.01$) önemli ölçüde azalttı. C1'in tüm dozlarda COX-1 ve COX-2 aktiviteleri üzerine olan inhibitor etkileri nimesulidinkine benzerdi. Rofekoksib ile karşılaştırıldığında, C1 molekülü 200 mg kg^{-1}

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dozda hem COX-1 hem de COX-2 aktivitelerini inhibe ederken (her ikisi için $P < 0,01$) 100 mg kg^{-1} dozda yalnızca COX-1 aktivitesini önemli olarak inhibe ettiği gözlendi ($P < 0,05$). 50 ve 100 mg kg^{-1} dozlarda C1 in COX-1 aktivitesi üzerine olan etkisi indometazin ile karşılaştırıldığında daha zayıf olduğu bulundu (sırası ile $P < 0,01$ ve $P < 0,05$).

Sonuç: C1 molekülünün antiinflamatuar etkisinin olduğu ve bu COX-2 seçiciliğinin indometazin ve nimesulitten daha kuvvetli ancak rofekoksibten daha zayıf olduğu söylenebilir.

Anahtar sözcükler: 3-Benzoil-1-metil-4-fenil-4-piperidinol-hidroklorür, COX enzimleri, inflamasyon

Introduction

Nonsteroid anti-inflammatory drugs (NSAID) are mainstay for the treatment of pain and inflammation, and are among the most widely used drugs worldwide. Common action mechanism of this class of drugs can be attributed to the inhibition of cyclooxygenase (COX) enzymes. COX is the key enzyme in the biosynthesis of prostaglandins and thromboxane from arachidonic acid. Two izoenzymes of COX, COX-1 and COX-2, have been identified. COX-1 was proposed to regulate physiological functions and COX-2 to mediate pathophysiological reactions such as inflammation and tumorigenic processes (1, 2). A recently found isoenzyme COX-3 is a splice-variant of COX-1, and its RNA is found in central nervous system especially (3). The main limitation in using NSAIDs (especially for COX-1 inhibitors) consists of their side-effects including gastrointestinal ulcerogenic activity and bronchospasm (4). Therefore, development of new more powerful anti-inflammatory drugs with lesser side effects is needed.

3-Benzoyl-1-methyl-4-phenyl-4-piperidinol-hydrochloride (C1) is derived from the bis Mannich base, bis (aryl-3-oxo-propyl) methylamine hydrochloride (B1) (Figure 1). In recent studies, it was found that both C1 and B1 had an anti-inflammatory activity in carrageenan-induced inflammation model (5, 6). Therefore, the aim of this study was to investigate the effects of C1 on COX-1 and COX-2 activities by using carrageenan-induced paw edema model. Additionally, an anti-inflammatory effect of C1 was compared with other NSAIDs [indomethacin, nimesulide and rofecoxib (Figures 2-4)] to have an idea about the selectivity of this compound.

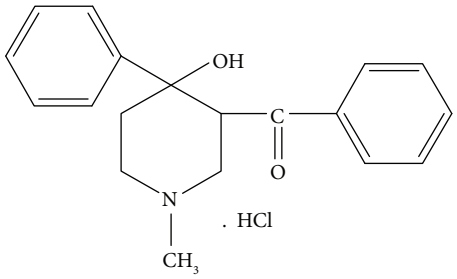

Figure 1. Formula of C1, 3-Benzoyl-1-methyl-4-phenyl-4-piperidinol Hydrochloride

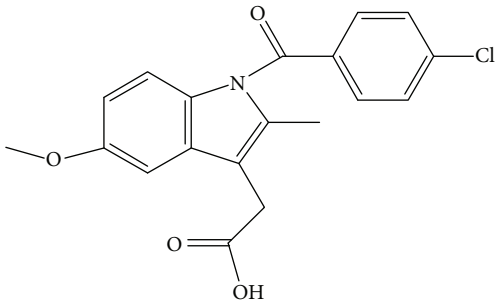

Figure 2. Formula of indomethacin

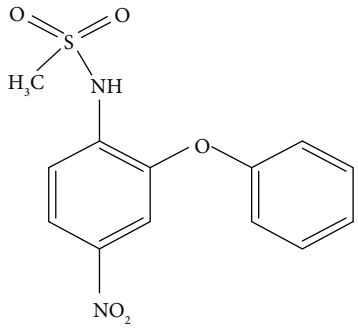

Figure 3. Formula of nimesulide

Materials and methods

Male Wistar albino rats (n = 48, mean weight of 180 ± 10 g) used in this study were fed with standard laboratory chow as groups at 22 °C. All animals were provided by Medical Experimental Practice and Research Center in Atatürk University. Rats were randomly assigned to eight groups (n = 6) as C1 (50

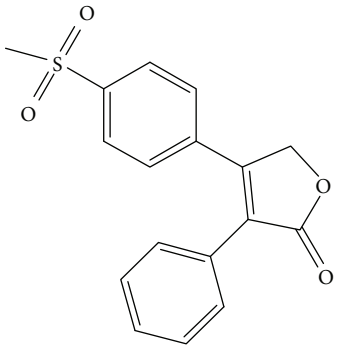

Figure 4. Formula of rofecoxib

mg), C1 (100 mg), C1 (200 mg), control, intact, indomethacin, nimesulide and rofecoxib. C1 at 50, 100, 200 mg kg^{-1} doses, indemethacin 25 mg kg^{-1}, nimesulide 100 $mgkg^{-1}$ and rofecoxib 25 mg kg^{-1} doses were given to rats orally by feeding. The same volume of distilled water used as vehicle was given to the control group. One hour after the last treatment, carrageenan 0.1 ml (1%, w/v) solution in distilled water was subcutaneously injected into the plantar surface of the right hind paw of all rats except the intact group. Four hours after the carrageenan administration, rats were sacrificed by giving thiopenthal sodium (50 mg kg^{-1}, ip). Right hind paw tissues were obtained and washed with Tris buffer (pH 7.4). The tissues were homogenized in 5 mL cold buffer (0.1 M tris-HCl, pH 7.8 containing 1 mM EDTA) per gram tissue and centrifuged at 10,000 x g for 15 minutes at 4 °C. COX activity assay kit (Cayman, USA) was used for the measurement of tissue COX activity. This kit measures the peroxidase activity of COX. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N, N, N', N'-tetramethyl-p-phenylenediamine at 590 nm. COX-2 activity was measured using COX-1 specific inhibitor (SC-560). Results were expressed as units per gram tissue. All of the spectrophotometric measurements were performed using a Beckman DU 500 spectrophotometer (USA).

All data are expressed as the mean ± standard deviation (SD). The significance of differences between the groups was assessed using the Kruskall-Wallis test and Mann-Whitney U test using statistical software SPSS. A P value < 0.05 was considered significant.

Results

As shown in the table below, C1 significantly inhibited COX-1 ($P < 0.01$ for all) and COX-2 ($P < 0.01$ for all) activities at the doses of 50, 100, and 200 $mgkg^{-1}$ compared to control group. In addition, the inhibitory effect of C1 on COX-1 and COX-2 activities at all doses was similar to those of nimesulide. In this study, while C1 at 200 $mgkg^{-1}$ significantly inhibited COX-1 and COX-2 activities ($P < 0.01$ for both); at C1 100 $mgkg^{-1}$ significantly inhibited only COX-1 activity in comparison to rofecoxib ($P < 0.05$). Inhibitory effects of C1 on COX-1 activity at the doses of 50 and 100 $mgkg^{-1}$ were significantly weaker compared to indomethacin ($P < 0.01$ and $P < 0.05$, respectively). However, this effect of C1 on COX-2 activity at all doses was not different from that of indomethacin.

It was found that mean COX-1 activity was significantly higher than mean COX-2 activity in all groups ($P < 0.05$ for all) except the indomethacin and control groups. When compared with the control group, nimesulide and rofecoxib significantly inhibited COX-2 activity only ($P < 0.01$ for both), whereas indomethacin inhibited not only COX-1 but also COX-2 activities ($P < 0.01$ for both).

Discussion

Mannich bases have various biological activities such as antimicrobial, cytotoxic (7, 8), anticancer, analgesic (9) and anticonvulsant activities (10). It has also been reported that Mannich bases have anti-inflammatory activities (11). C1 is derived from the bis Mannich base B1, bis (β-benzoylmethyl) methylamine hydrochloride by an internal aldol reaction. In a previous study, it was found that C1 significantly inhibited carrageenan-induced paw edema (5). Mannich base of benzoxazolinone is dual inhibitor of COX and lipooxygenase. In Chen et al.'s study (12), pharmacological tests showed that Mannich base of benzoxazolinone had a significant inhibiting effect on carrageenan-induced rat paw edema, and this effect of the componud was similar to that of ibuprofen.

From COX enzymes, COX-1 products, prostaglandins (PGI_2 and PGE_2) maintain integrity of the gastrointestinal system by reducing gastric acid

Table. The effects of C1, indomethacin, nimesulid, and rofecoxib on COX enzyme activities in carrageenan-induced paw inflammation model.

Groups	COX-1 (U/g tissue)	COX-2 (U/g tissue)
Indomethacin (25 mg kg^{-1})	6.9 ± 3	8.5 ± 2.5
Nimesulide (100 mg kg^{-1})	18.6 ± 6.7	13.8 ± 5.5
Rofecoxib (25 mg kg^{-1})	26.4 ± 9.7	13.6 ± 0.8
C1 (50 mg kg^{-1})	15.5 ± 4.6	10.8 ± 3.3
C1 (100 mg kg^{-1})	13.4 ± 4.4	11.6 ± 2.7
C1 (200 mg kg^{-1})	10.3 ± 2	9.1 ± 2.5
Control	27.5 ± 6.8	30.7 ± 7.4
Intact	19.4 ± 12.3	6.1 ± 7.2
Statistical Comparisons	**P value**	**P value**
C1(50 mg kg^{-1})- C1(100 mg kg^{-1})	N.S.	N.S.
C1(50 mg kg^{-1})- C1(200 mg kg^{-1})	< 0.05	N.S.
C1(100 mg kg^{-1})- C1(200 mg kg^{-1})	N.S.	N.S.
Rofecoxib-C1 (200 mg kg^{-1})	< 0.01	< 0.01
Rofecoxib-C1 (100 mg kg^{-1})	< 0.05	N.S.
Rofecoxib-C1 (50 mg kg^{-1})	N.S.	N.S.
Rofecoxib-Indomethacin	< 0.01	< 0.01
Rofecoxib- Nimesulid	N.S.	N.S.
Indomethacin-C1 (50 mg kg^{-1})	< 0.01	N.S.
Indomethacin -C1 (100 mg kg^{-1})	< 0.05	N.S.
Indomethacin -C1 (200 mg kg^{-1})	N.S.	N.S.
Indomethacin-Nimesulid	< 0.05	N.S.
Control-Intact	N.S.	<0.01
Control-Nimesulid	N.S.	< 0.01
Control-Rofecoxib	N.S.	< 0.01
Control- Indomethacin	< 0.01	< 0.01
Control-C1 (50 mg kg^{-1})	< 0.01	< 0.01
Control-C1 (100 mg kg^{-1})	< 0.01	< 0.01
Control-C1 (200 mg kg^{-1})	< 0.01	< 0.01

Results are presented as Mean ± SD.
n:6 in each group.
N.S.: Not significant.

secretion, increasing the thickness of mucus layer, stimulating bicarbonate secretion, and enhancing mucosal blood flow. Thus, it has been suggested that COX-2 inhibition is responsible for the therapeutic effects of NSAIDs, while COX-1 inhibition causes the gastrointestinal side-effects (13).

Tomlinson et al. (14) showed that COX activity peaked 2-6 hours after carrageenan injection and COX activity was significantly reduced at the 24th hour. Similarly, Suleyman et al. (5) found that an anti-inflammatory effect of C1 reaches to the highest point at the 4th hour of carrageenan-induced inflammation model and C1 had low acute toxicity. Thus, we investigated the effects of C1 on COX-1 and COX-2 activities in paw tissue 4 hours after carrageenan injection.

In the present study, C1 significantly inhibited COX-2 activity at all doses in the inflamed paws of rats. In other words, C1 is a COX-2 inhibitor in a dose-independent manner. C1 at 200 mg kg^{-1} more markedly inhibited COX-1 activity than the other doses (at 50 mg kg^{-1}). Based on this result it may be suggested that C1 particularly at 50 mg kg^{-1} (and 100 mg kg^{-1}) may have less adverse effects on gastrointestinal system when compared to C1 at 200 mg kg^{-1}. Additionally, it may be speculated that low concentrations of C1 is a selective inhibitor of COX-2 (especially at 50 mg kg^{-1} dose).

Suleyman et al (5) reported that C1 inhibited the inflammation almost equally to that of indomethacin, and that the lowest dose of C1 having on anti-inflammatory effect was 50 mg kg^{-1}. In our study, in the indomethacin group, COX-1 activity was lower than those of C1 groups (at 50 and 100 mg kg^{-1} doses), while COX-2 activities were similar at all doses of C1.

Nimesulide has analgesic, antipyretic and anti-inflammatory effects, and is a relatively selective COX-2 inhibitor (15). Investigations showed that nimesulide had a 5 times stronger inhibitory effect on COX-2 than on COX-1 (16). In our study, inhibitory effects of nimesulid on COX activities were similar to those of C1 at all doses.

While C1 (200 mg kg^{-1}) significantly inhibited COX-2 activity than rofecoxib, in other doses, inhibition degree was similar to the rofecoxib. Pharmacologic studies have shown that rofecoxib is the most selective COX-2 inhibitor available for clinical use (17). Previous studies showed that selective COX-2 inhibitors, such as rofecoxib, did not have any deleterious effect on gastrointestinal system (18). But, the use of selective COX-2 inhibitors may potentially play a significant role in adverse cardiovascular outcomes, and led to the voluntary withdrawal of rofecoxib from international markets (19).

The present study revealed that, indomethacin was the most strong inbitor of COX-1 activity among the NSAIDs used. By estimating the remaining % activity in comparison to the control group, the COX-2 selectivity order of the drugs or compounds, from high to low, was found as rofecoxib nimesulid, C1 and indomethacin respectively; that finding was in agreement with previous studies (20).

In conclusion, it may be claimed that C1 has an anti-inflammatory effect, and if it is proven that C1 has lower toxic effect than the other NSAIDs, this agent may occupy a place among the other safe NSAIDs.

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