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Short Communication:

THE EFFECT OF 3-BENZOYL-4-PHENYL-1-METHYL-4-PIPERIDINOL HYDROCHLORIDE ON COX-1 AND COX-2 ENZYME ACTIVITIES

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Abstract

Antiinflammatory activity of 3-benzoyl-4-phenyl-1-methyl-4-piperidinol hydrochloride (C1), which is semi-cyclic Mannich base, has been reported. In this study, the effects of the compound C1 on cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzyme activities have been investigated as a possible mechanism of action for its anti-inflammatory activity. COX-2 activity was inhibited by 55% by C1 at 10 μM by 24 h. On the other hand, inhibition of COX-1 activity by C1 was negligible. Our results showed that C1 has selective inhibition on COX-2 activity, which prevents inflammation without having side effects such as gastric ulcer and hemorrhage. C1 can be a model compound to develop new anti-inflammatory compounds with COX-2 inhibiting activity.

Key words: *COX-1, COX-2, inflammation, Mannich base, piperidine.*

3-Benzoil-4-fenil-1-metil-4-piperidinol hidroklorür`ün COX-1 ve COX-2 enzim aktiviteleri üzerine etkisi

Semi-siklik Mannich bazı olan 3-Benzoil-4-fenil-1-metil-4-piperidinol hidroklorür'ün (C1) antiinflamatuvar etkisi rapor edilmistir. Bu çalışmada, C1 bileşiğinin antiinflamatuvar aktivitesi için olası etki mekanizması olarak siklooksijenaz-1 (COX-1) ve siklooksijenaz-2 (COX-2) enzim aktiviteleri üzerindeki etkileri araştırılmıştır. C1 tarafından COX-2 aktivitesinin inhibisyonu belirgindi: 24 saatte 10 μM'da % 55. Diğer taraftan, C1 tarafından COX-1 aktivitesinin inhibisyonu ihmal edilebilir düzeydeydi. Sonuçlarımız C1'in gastric ülser ve hemorajı gibi yan etkilere sahip olmaksızın inflamasyonu önleyen seçici COX-2 akivitesi inhibisyonuna sahip olduğunu gösterdi. C1 COX-2 inhibe edici etkiye sahip yeni antienflamatuvar bileşiklerin geliştirilmesinde model bir bileşik olabilir.

Anahtar kelimeler: *COX-1, COX-2, inflamasyon, Mannich bazı, piperidin.*

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INTRODUCTION

Chronic inflammation represents a malfunction of normal host defense systems. The most commonly used drugs in the treatment of inflammation are nonsteroid anti-inflammatory drugs (NSAIDs). NSAIDs inhibit prostaglandin (PG) biosynthesis at inflammatory sites. In order to maintain the integrity of the mucosal epithelium in the stomach and intestine cyclooxygenase-1 (COX-1) makes the PGs available. Inhibition of COX-1 may result in ulceration, gastric damage and hemorrhage (1). In the stomach of humans, cytoprotective PGs are synthesized by COX-1, although small quantities of cyclooxygenase-2 (COX-2) are also expressed (2). COX-1 activity is constitutive, present in nearly all cell types at a constant level (3, 4). On the other hand, the situmuli known to induce COX-2 are those associated with inflammation, for example, bacterial lipopolysaccharide (LPS) and cytokines such as interleukin IL-1, IL-2 and tumor necrosis factor (TNF)-α. (3, 5). The discovery and characterization of COX-2 have solved one problem in therapeutics-how to suppress inflammation without the side effects–gastrointestinal ulceration and bleeding, renal damage, and platelet dysfunction-were accepted as inevitable consequences of the inhibition of COX activity required to prevent synthesis of PGs in inflammatory conditions such as rheumatoid or osteo-arthritis. Since COX-2 is clearly associated with inflammation but not with the physiological synthesis of PGs, selective COX-2 inhibitors offer the possibility of inhibition of inflammatory PGs without affecting PGs generated by COX-1 in stomach, kidney or platelet (1). COX-2 inhibitors, which do not cause gastric ulceration, can be used for long-term prophylactic use in certain chronic diseases (6). Improved safety has led to intense efforts in search for potent and selective COX-2 inhibitors, as the next generation of anti-inflammatory agents. The compounds such as oxazoles (7), thiophens (8), pyrazoles (9), and imidazoles (10) are reported to have selective COX-2 inhibitory activity.

Mannich bases are generally formed by the reaction between a compound containing a reactive hydrogen atom, formaldehyde (or other aldehydes) and amine (mostly secondary amine, primary amine or ammonia) (11). We have recently reported the antiinflammatory activity of 3-benzoyl-4-phenyl-1-methyl-4-piperidinol hydrochloride, C1, which is semi-cyclic Mannich base (12). In this study we aimed to investigate the effect of C1 on COX-1 and COX-2 activities as a possible mechanism of action for its antiinflammatory activity. To our knowledge, no study reports the effect of Mannich bases on COX-1 and/or COX-2 activities.

EXPERIMENTAL

Synthesis of 3-benzoyl-4-phenyl-1-methyl-4-piperidinol hydrochloride (Figure 1), which is a semi-cyclic Mannich base and also nonclassical structural isomer of bis Mannich base, bis(β-bezoylethyl) methylamine hydrochloride, has been reported in our previous study (13).

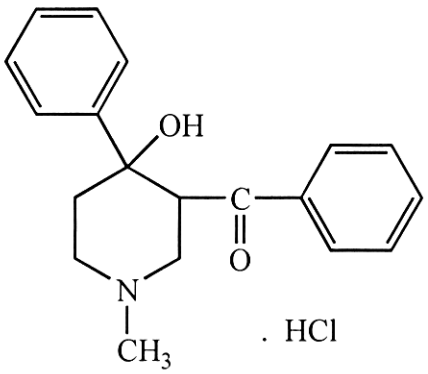

Figure 1 : Chemical structure of 3-benzoyl-4-phenyl-1-methyl-4-piperidinol hydrochloride

Biological material was human blood obtained from two healthy human volunteers with heparin for COX-2 and COX-1 activity assays. Other materials used were: lipopolysaccaride (LPS, from *Escherichia coli*, ref. 0127:B8, DIFCO, Detroit, MI), A23187 calcium ionophore (Sigma, St. Louis, MO, USA), PGE_2 prostaglandin E_2 and TXB_2 enzyme immunoassay (EIA) kits (Amersham, London, U.K.). Modified human whole blood assay, originally developed by Patrignani et al (14), is considered to be the more biologically relevant way to assess the inhibition of the cyclooxygenase isoenzymes, COX-1 and COX-2, by a test compound (15). In this assay, platelets stimulated by calcium ionophore are believed to be the main source of COX-1, whereas monocytes stimulated with LPS are thought to be the source of COX-2. COX-1 activity is determined by the production of thromboxane B_2 (TXB_2), while COX-2 activity is determined by the production of prostaglandin E_2 (PGE_2).

For the inhibition of COX-1 assay, the blood was obtained from two healthy volunteers who had not taken NSAIDs in the previous week. The blood was heparinised (20 U/ml) and distributed in 0.5 ml aliquots in tubes containing vehicle, dimethylsulfoxide, or the test compound, dissolved in dimethylsulfoxide. C1 was evaluated at 100 μM concentration in triplicate determinations. The samples were incubated at 37 ^{0}C with gentle shaking for 23 h 40 min. Calcium ionophore (5 μl) was added for a further 20 min and the reaction was then stopped by submerging the tubes in a cold bath and centrifuging at 13000 rpm for 10 min at 4 ^{0}C. Levels of TXB_2 in the supernatants were determined by EIA.

The blood was heparinised (20 U/ml) and distributed in aliquots in tubes containing 10 μg/ml of LPS together with vehicle or test compound for the inhibition of COX-2 assay. C1, dissolved in vehicle, dimethylsulfoxide, was evaluated at 10 μM concentration in triplicate determinations. The samples were incubated in a bath at 37 ^{0}C for 24h; during this time COX-2 was induced in mononuclear cells. The reaction was stopped by submerging the tubes in a cold bath and centrifuging at 13000 rpm for 10 min at 4 ^{0}C. Levels of PGE_2 in the supernatant were determined by EIA. IC_{50} values for COX-1 and COX-2 inhibition of the reference compound, celecoxib, (Celebrex, Pfizer), in our laboratory were 12.2 and 1.06 μM, respectively.

RESULTS AND DISCUSSION

COX-2 activity was inhibited by 55.0 ± 8.5 % (mean ± SD) by C1 at 10 μM by 24 h. Inhibition of COX-1 activity by the compound studied (C1) was negligible: 0.4 ± 0.07 % inhibition by 24 h incubation at 100 μM.

This is the first study, which shows that a Mannich base, C1, inhibits COX-2 activity. We have previously shown that C1 has anti-inflammatory activity against early (acute paw edema) and also late (cotton pellet granuloma) phases of inlammation comparable with anti-inflammatory drug, indomethacin (12). In that study, C1 significantly inhibited carrageenan induced paw edema at doses of 50, 100 and 200 mg kg^{-1} by 61.98%, 80.84% and 90.32%, respectively, while this inhibition was found to be 89.93% by indomethacin. Antiproliferative effects of C1 (at 100 mg kg^{-1} dose) and indomethacin (at 20 mg kg^{-1} dose) were found to be 46.1% and 43.1%, respectively in cotton pellet granuloma test. C1 (100 mg kg^{-1}) was found to be more effective compared to indomethacin (at 10 mg kg^{-1}) in terms of preventing hyaluronidase induced capillary permeability increase for 30 min (12).

In this preliminary study, inhibition of COX-2 of Mannich base C1 has been shown, which may have contributed to its anti-inflammatory activity in addition to preventing hyaluronidase induced capillary permeability. Selective inhibition on COX-2 activity by C1 may prevent inflammation without having side effects such as gastric ulcer and hemorrhage. C1 can be a model compound to develop new anti-inflammatory compounds with selective COX-2 activity.

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