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Dual Effects of Interaction Between Meloxicam, Diclofenac Sodium or Tramadol and Nitrogen Species Radicals: *In vitro* Comparative Study

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Abstract: This study aimed to investigate the interaction between synthetic peroxyxynitrite or sodium nitroprusside (nitric oxide donor) and meloxicam, diclofenac sodium or tramadol HCl. Meloxicam, diclofenac sodium or tramadol HCl (100-500 µg) were incubated in phosphate buffer saline in presence or absence of synthetic peroxyxynitrite (180 µM) or sodium nitroprusside as nitric oxide donor (10 mM). The level of peroxyxynitrite and nitric acid in solution were measured using UV-visible spectrophotometer. The results showed that meloxicam scavenged synthetic peroxyxynitrite and involved in peroxyxynitrite mediated phenol nitration when it incubated alone in phosphate buffer. All tested compounds, *in vitro*, behaved like sodium nitroprusside in releasing nitric oxide. Both meloxicam and diclofenac sodium reduced the activity of sodium nitroprusside-releasing nitric oxide. Tramadol HCl was not interacted with sodium nitroprusside at any concentration. We concluded that selective or non selective nonsteroidal anti-inflammatory drugs reduced the activity of nitric oxide donor while tramadol HCl is free from this effect.

Key words: Meloxicam, diclofenac sodium, tramadol, nitric oxide, peroxyxynitrite

INTRODUCTION

Prostaglandins and nitric oxide are important mediators of different physiological and pathophysiological processes (Serhan *et al.*, 2008; Pacher *et al.*, 2007). Nonsteroidal anti-inflammatory drugs (NSAIDs) act on cellular transduction pathway other than those involving prostaglandins (Cho *et al.*, 2005; Miranda *et al.*, 2004). The effects of NSAIDs on nitric oxide or peroxyxynitrite, were studied in several experimental models. Repeated injections of diclofenac, in rats, resulted in increase in serum total antioxidant substances with no significant changes in nitric oxide level (Curcelli *et al.*, 2008). Also it did not attenuate cellular nitric oxide production by liposaccharide (Cho *et al.*, 2005). Interleukin-1 induced nitric oxide production by chondrocytes derived from human cartilage is reduced by selective cyclooxygenase-2 inhibitor, celecoxib (Nakamura *et al.*, 2007). Kowalczyk *et al.* (2006) reported that diclofenac enhanced the production of plasma nitric oxide while acetylsalicylic acid and nimesulide had no effect i.e., the effect was not relevant to selective or non selective cyclooxygenase inhibition. In human beings NSAIDs treatment interact with nitric oxide donors. They

reduce nitroglycerin induced nitric oxide-mediated vasodilatation of brachial arteries (Li *et al.*, 2008). On the other hand l-arginine, the nitric oxide precursor, reduced the anti-nociceptive effect of tramadol, a centrally acting analgesic agent (Yalcin *et al.*, 2005). Accordingly, NSAIDs or centrally acting analgesic interact with nitric oxide resulting in harmful or beneficial effects. There is no report in literature of nitric oxide or peroxyxynitrite interaction property with nonsteroidal anti-inflammatory drugs or centrally acting analgesics *in vitro*. This comparative study aimed to demonstrate the interaction between nitric oxide donor (sodium nitroprusside) or exogenous synthesized peroxyxynitrite with diclofenac (non-selective COX inhibitor), meloxicam (selective COX-2 enzyme inhibitor) or tramadol HCl (centrally acting analgesic) *in vitro* experimental model.

MATERIALS AND METHODS

This study was conducted at Departments of Pharmacology and Biochemistry, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq during 2008. This study was approved by the scientific committee in college.

Peroxynitrite assay: Peroxynitrite (ONOO^-) was prepared by mixing 1 volume cooled hydrogen peroxide (1 M) and 1 volume cooled sodium nitrite (1 M) in dark room, then 2 volumes cooled NaOH (1.5 M) was added to the mixture as prescribed by Whiteman and Halliwell (1996). Peroxynitrite (ONOO^-) was quantified spectrophotometrically at 302 nm wavelength ($\epsilon = 1670 \text{ M}^{-1}\text{cm}^{-1}$). The yield was 1.05 M.

From this yield, 180 μM ONOO^- was incubated with 100 μL of each drug; diclofenac sodium 100-500 μg or meloxicam 100-500 μg or tramadol HCl 100-500 in phosphate buffer saline for 15 min and the absorbance at 302 nm of the samples was recorded. All experiments were performed in triplicate.

Peroxynitrite mediated nitration of phenol was measured (an index of ONOO^- release) for diclofenac sodium, meloxicam and tramadol as described by Beckman *et al.* (1992) cited Van Uffelen *et al.* (1998). Briefly, diclofenac sodium, meloxicam or tramadol HCl in different concentration (100-500 μg) was added to 5 mM phenol in 50 mM sodium phosphate buffer pH 7.4 in a final volume of 3 mL. After incubation for 2 h at 37°C, 50 μL of 0.1 M sodium hydroxide was added and the absorbance at 412 nm of the samples was immediately recorded. The yield of nitrophenol was calculated from $\epsilon = 4400 \text{ M}^{-1} \text{cm}^{-1}$. All experiments were performed in triplicate.

Nitric oxide donating activity was determined as described by Newaz *et al.* (2003). Briefly Diclofenac sodium 100-500 μg or meloxicam 100-500 μg or tramadol HCl 100-500 μg to 50 μL HCl (6.5 M) and 50 μL sulfunalic acid (37.5 mM). After incubation for 10 min, 50 μL naphthylethylenediamine dihydrochloride (12.5 mM) was added and incubated for further 30 min, centrifuged for 10 min at 1000 g. The reference nitric oxide donating compound was 5 mM sodium nitroprusside. The absorbance at 540 nm was immediately recorded. All experiments were performed in triplicate.

Nitric oxide assay: Nitric oxide radical scavenging was estimated by the use of Greiss reaction (Sundararajan *et al.*, 2006). Greiss reagent was modified by using naphthylethylenediamine dihydrochloride (0.1% w/v). The reaction mixture was (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer (0.5 mL) and each drug with above concentrations or distilled water as negative control (final volume 0.5 mL) was incubated in dark room at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture mixed with 1 mL of sulfunalic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for complete diazotization. Then 1 mL of naphthylethylenediamine dihydrochloride was added, mixed and allowed to stand

for 30 min in dark place at 25°C. A pink colored chromophore is formed. The absorbance of these solutions was measured at 540 nm against corresponding blank. All experiments were performed in triplicate.

All chemicals used in the study were of analytical grade and drugs as pharmaceutical solutions were obtained from local sources. All the chemicals were prepared freshly prior to the experiments.

RESULTS AND DISCUSSION

Meloxicam showed peroxynitrite-mediated phenol nitration that indicated its ability to release peroxynitrite in concentration dependent manner while diclofenac sodium or tramadol HCl were unable to exert this effect (Fig. 1). Synthetic peroxynitrite (180 μM) interacted with meloxicam producing decrease of peroxynitrite absorbance at 302 nm while diclofenac sodium and tramadol HCl produces an increase of the absorbance to many folds (Fig. 2). The interaction between these compounds and synthetic peroxynitrite was independent to their concentrations.

All tested compounds behaved like sodium nitroprusside by releasing nitric oxide. Their ability of releasing nitric oxide was not related to their concentrations (Fig. 3). Sodium nitroprusside interacted with meloxicam or diclofenac sodium producing a quantitative reduction of sodium nitroprusside-releasing nitric oxide property while tramadol HCl was free from this effect (Fig. 4).

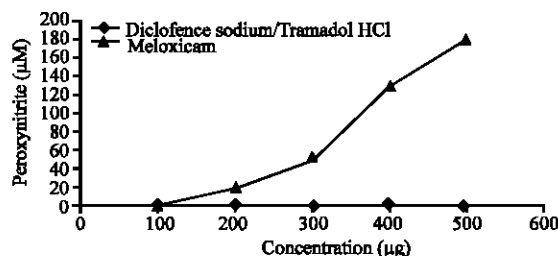


Fig. 1: The effect of drugs in releasing peroxynitrite

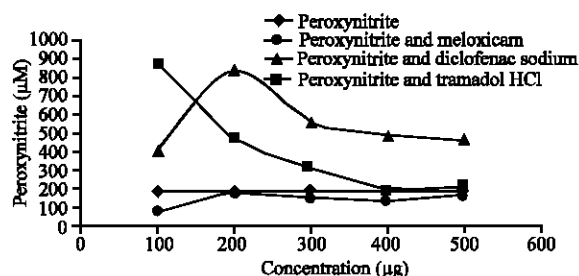


Fig. 2: The interaction between peroxynitrite and tested compounds

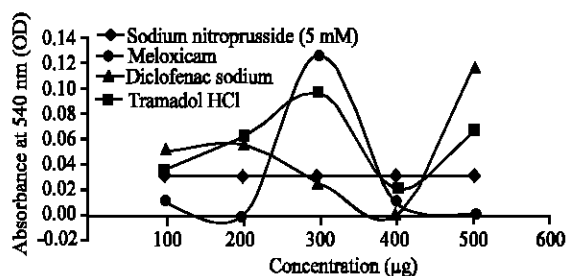


Fig. 3: The ability of tested compound in releasing nitric oxide in reference to sodium nitroprusside

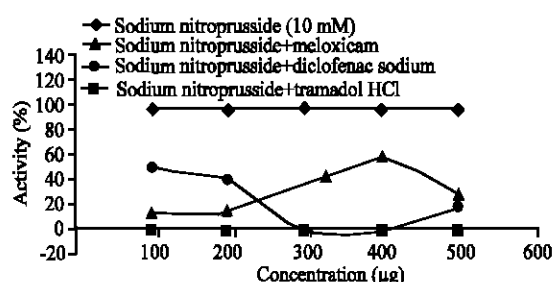


Fig. 4: Interaction between tested compounds and sodium nitroprusside

The interaction between nitrogen species radicals and meloxicam, diclofenac sodium or tramadol HCl is well observed and there is general consensus that it targets their mechanism of action or efficacy. All tested compounds, *in vitro*, donate nitric oxide which may give false interpretation when nitric oxide is used as a marker for the assessment of drug effect. The finding of phenol nitration by meloxicam in the present study adds to earlier *in vivo* finding that meloxicam elevates superoxide anion, a reactive oxygen species shared with nitric oxide in formation peroxynitrite (Burak Cimen *et al.*, 2003).

Also the finding of peroxynitrite scavenging property by meloxicam complements the *in vivo* finding that meloxicam scavenged the reactive oxygen species (Van Antwerpen and Nève, 2004). As a result of high peroxynitrite level induced by meloxicam, the level of nitric oxide is low. Meloxicam, *in vivo*, does activate nitric oxide synthases that involved in production of nitric oxide (Di Girolamo *et al.*, 2003). The direct effect of meloxicam in releasing nitric oxide is shared in antinociceptive effect of meloxicam (Aguirre-Bañuelos and Granados-Soto, 2000). Moreover the interaction of meloxicam and sodium nitroprusside (a nitric oxide donor) explained the undesirable effect of meloxicam in ischemia (Rossoni *et al.*, 2002).

Diclofenac sodium *per se* does not released nitric oxide or peroxynitrite but it accelerates their production in

certain conditions. The present study documented the earlier report that nitric oxide donor potentates the effect of diclofenac sodium (Strapkova *et al.*, 2006) and elevates the peroxynitrite level (Li *et al.*, 2008). Tramadol HCl as with diclofenac, it donates nitric oxide that resulted in high peroxynitrite level. The present finding is in agreement with Kaya *et al.* (2003) which demonstrated that the vasodilator effect of tramadol is related to production of nitric oxide. Interestingly, Tramadol is not interacted with nitric oxide donor, sodium nitroprusside, as with meloxicam or diclofenac sodium. This finding supported the earlier report showed that racemic tramadol had no effect on dose response curve of sodium nitroprusside in rat aorta (Shin *et al.*, 2006).

It concludes that meloxicam, diclofenac sodium or tramadol HCl *per se* produce high nitric oxide level *in vitro*. Non steroidal anti-inflammatory drugs (selective COX-2 or non selective) interact with nitric oxide donor that may adversely the therapeutic effect of nitric oxide while tramadol HCl is free from this effect.

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