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## *In vivo* and *in vitro* interaction studies of ibuprofen with enalapril

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### ABSTRACT

**Objective:** To study the *in vitro* interactions of ibuprofen with enalapril.

**Methods:** In this study, we investigated synergistic effect of angiotensin-converting enzyme inhibitor (enalapril) with commonly used nonsteroidal anti-inflammatory drug, ibuprofen in carrageenan induced inflammation rats. Anti-inflammatory response was observed for ibuprofen when given simultaneously with enalapril by comparing decrease in paw size.

**Results:** The results were expressed in percentage reduction in paw size for every hour and were calculated for edema rate and percentage reduction in inflammation. *In vitro* interaction studies were carried out using high performance liquid chromatography and both the drugs were analyzed by assaying both drugs alone and in combination. The combination of enalapril and ibuprofen showed increased action of ibuprofen as shown by the increase of percentage reduction and decrease in edema rate. It was also proved from analytical ultracentrifugation of the chromatogram of combining drugs.

**Conclusions:** This learning has certain restriction as a number of experienced animals were small and the period of inflammatory response was checked for ibuprofen alone and simultaneous with enalapril for 5 h. Further studies may be required to establish the strong relationship.

## 1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) interfere with certain antihypertensive drugs especially with angiotensin-converting enzyme (ACE) inhibitors. It is reported if an angiotensin converting enzyme inhibitor is given with ibuprofen, it will cause significantly increases in systolic and diastolic blood pressures[1].

ACE inhibitors reduce blood pressure by two mechanisms of reducing the formation of angiotensin II and aldosterone, and also increasing the production of kinins. This leads to a decreased vasoconstriction of the kinin and to a potentiation vasodepressor

system with antihypertensive and vasodilatory effects. A rise in kinin levels stimulates the synthesis of prostaglandins[2]. It was also reported an increase in prostaglandin production after captopril administration[3]. Therefore, the prostaglandin system could be involved in the mechanism of action of ACE inhibitors. A human reduction of the antihypertensive activity of captopril was observed when NSAIDs, especially ibuprofen were administered[4].

Over the last two decades, the concern about interactions between anti-inflammatory and antihypertensive agents has been grown and several cases with hypertensive emergency have been reported after using NSAIDs[5]. Taking this into account, we have worked on a number of *in vitro* interactions of enalapril with hypoglycemic agents, enalapril maleate and H<sub>2</sub>-receptor antagonists, enalapril with flurbiprofen, ACE inhibitors with diclofenac and mefenemic acid, and captopril with NSAIDs[6-9]. In this research paper, the *in vivo* and *in vitro* interactions of ibuprofen with enalapril has been studied to investigate the anti-inflammatory response of ibuprofen with enalapril and alone.

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We reported a method to quantitate enalapril with NSAIDs simultaneously using high performance liquid chromatography (HPLC)-UV technique which was used to study the *in vitro* interactions[10].

## 2. Materials and methods

### 2.1. In-vivo study: animals, inflammation induction and drug treatment

Female rats of weighing about 160–250 g were used for interaction studies and ten animals in each set were housed under standard temperature conditions (*i.e.* at 21 °C).

Inflammation was induced in rats using intradermal injection at the foot. Solution prepared for inflammation induction by carrageenan in 0.9 NaCl to the concentration of 1 gm/100 mL. Animals ( $n = 10$ ) were distributed at random into specific groups, which were received their individual treatment orally using 0.5 mL dimethyl sulfoxide (DMSO) prior 1 h to swelling generation and the dose regime of different drugs were also given in Table 1.

**Table 1**

Doses of different groups.

Groups	Treatments	Dose (mg/kg)
CIR saline (control)	Normal saline	---
CIR ENP	Enalapril	2.5
CIR IBU	Ibuprofen	5.0
CIR IBU + ENP	Ibuprofen + enalapril	5.0 + 2.5

ENP: Enalapril; IBU: Ibuprofen; CIR: Carrageenan induced inflammation rat.

The inflammation was assessed by change in paw volume and deliberated by using a plethysmometer (Model 7140, Ugo Basile, Varese, Italy) on time interval of 0 to 5 h of the planned testing. The volumes of paw were deliberated in test and control groups on zero and then on every alternate hour until 5 h when the research finished.

### 2.2. In vitro studies

Stock solutions of ibuprofen and enalapril were prepared in 80:20 methanol water individually (100 µg/mL). The 1:1 ratio of both drugs solutions was mixed in flasks individually and kept on a water bath at normal body temperature (37 °C) for 3 h with continuous stirring. The samples were withdrawn after 30 min interval for 3 h and solutions were filtered through a millipore filter (0.45 µ) and analyzed by reversed phase-HPLC. The availability of each drug was

**Table 2**

Edema rate in rat's paw.

Groups	Edema in 1 h	Edema in 2 h	Edema in 3 h	Edema in 4 h	Edema in 5 h
CIR (DMSO)	18.69	25.54	28.30	39.12	40.18
CIR (ENP)	6.46	10.29	10.01	27.61	37.36
CIR (IBU)	5.43	6.69	8.28	2.53	6.66
CIR (IBU + ENP)	8.69	1.22	2.57	7.47	7.45

ENP: Enalapril; IBU: Ibuprofen; CIR: Carrageenan induced inflammation rat.

then calculated with respect to their standard samples (ibuprofen and enalapril).

### 2.3. Statistical investigation

The trial outcomes were articulated as mean ± SD of size ( $n = 10$  rats in each group). Rate of edema in paw and fraction reduction was calculated by using ANOVA software (SPSS 19 version). Tucky's *post-hoc* and least significant difference test were conducted to ascertain mean differences of group with significance level at  $P < 0.005$  for edema rate and percent reduction with alone drugs and in combination.

## 3. Results

Analgesic drugs were prescribed in different combinations and normal patients were received multiple drug regimens. But in aged patients, the chances of interaction of multiple drugs were more than young patients. Both analgesic and antihypertensive drugs were prescribed together in elderly patients because hypertension and simultaneous musculoskeletal tribulations were two common conditions[11,12].

Therefore, this study was designed to recognize the anti-inflammatory response of ibuprofen when did a administration with enalapril. The group of rats with carrageenan induced inflammation was used alone and in combination of drugs. Inflammation was induced by carrageenan that is acute, highly reproducible and well-researched. In this study, an altered anti-inflammatory response of ibuprofen was observed when given concurrently with enalapril and by comparing with foot size (edema). The results were expressed in reduction in paw size for every hour and were calculated. The formula which were used to work out edema and fraction decrease was given below.

$$\text{Edema rate (\%)} = V_T - V_0 / V_0 \times 100$$

where,  $V_0$  = rat's posterior foot volume before 1% carrageenan administration;  $V_T$  = rat's posterior foot volume at t hour.

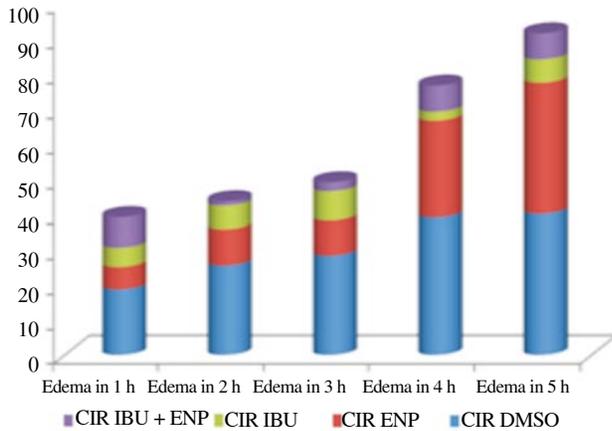
$$\text{Percentage reduction (\%)} = E_C - E_T / E_C \times 100$$

where,  $E_C$  = edema rate of control group,  $E_T$  = test compound of edema rate at t hour.

Rate of edema and reduction data were also analyzed by using One-way ANOVA (SPSS 19). Tukey's and least significant difference test were conducted to find out mean differences of group with significant level at  $P < 0.005$ .

### 3.1. Edema rate

Table 2 and Figure 1 shows the outcome of treatment on edema rate for ibuprofen and enalapril analysis by One-way ANOVA ( $df = 2.27$ ) showed major treatment effect on edema rate in 1st h ( $F_1 = 255484.102$ ), in 2nd hour ( $F_2 = 2704272.360$ ), in 3rd hour ( $F_3 = 779062.490$ ), in 4th hour ( $F_4 = 56477593.618$ ) and in 5th hour ( $F_5 = 50862180.655$ ) with  $P < 0.005$  for both drugs.

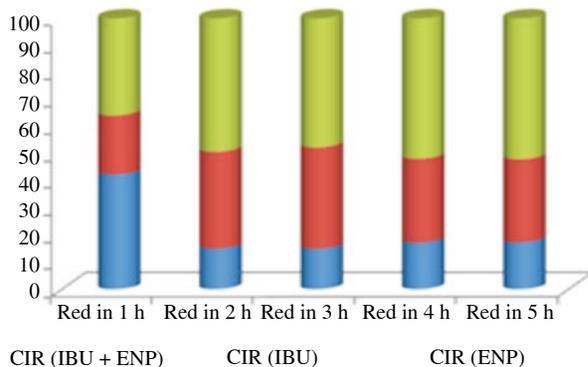


**Figure 1.** Edema rate in rat's paw after treatment.

ENP: Enalapril; IBU: Ibuprofen; CIR: Carragenan induced inflammation rat.

### 3.2. Percent reduction

The results (Table 3 and Figure 2) of percent reduction by One-way ANOVA ( $df 2.27$ ) showed significant effect of treatment in 1st hour ( $F_1 = 126914.0$ ), in 2nd hour ( $F_2 = 95.71$ ), in 3rd hour ( $F_3 = 97.16$ ), in 4th hour ( $F_4 = 236.1$ ) and in 5th hour ( $F_5 = 283.2$ ) with  $P < 0.005$  for ibuprofen enalapril.



**Figure 2.** Reduction in rat's paw after treatment (%).

Red: Reduction.

ENP: Enalapril; IBU: Ibuprofen; CIR: Carragenan induced inflammation rat.

**Table 3**

Reduction rate in rat's paw. %.

Groups	Reduction in 1 h	Reduction in 2 h	Reduction in 3 h	Reduction in 4 h	Reduction in 5 h
CIR (ENP)	70.31	30.10	31.05	32.78	33.03
CIR (IBU)	36.46	72.00	78.29	59.51	59.03
CIR (IBU + ENP)	59.55	99.03	99.43	99.24	99.62

ENP: Enalapril; IBU: Ibuprofen; CIR: Carragenan induced inflammation rat.

### 3.3. Interaction of enalapril with ibuprofen

Reduction in paw edema in the CIR (IBU) group was significantly high ( $P < 0.005$ ) from 1st hour to the 4th hour while insignificantly low in the 5th hour. The percent reduction of this group was  $76.45\% \pm 1.50\%$ ,  $77.16\% \pm 1.04\%$ ,  $74.21\% \pm 1.06\%$ ,  $96.10\% \pm 1.01\%$  and  $82.98\% \pm 1.10\%$  in 1st to 5th hour respectively. Simultaneous administration of ibuprofen with enalapril in the group of CIR (IBU + ENP) showed percent reduction  $59.55\% \pm 0.23\%$ ,  $99.02\% \pm 0.66\%$ ,  $99.42\% \pm 0.30\%$ ,  $99.23\% \pm 0.21\%$  and  $99.61\% \pm 0.33\%$  in 1st to 5th hour respectively which was significantly high when compared to both groups.

Effect of enalapril with ibuprofen administration was studied by observing the groups of CIR (IBU) and CIR (IBU + ENP). Percent reduction of ibuprofen was  $3.00\% \pm 1.00\%$ ,  $72.01\% \pm 1.01\%$ ,  $79.53\% \pm 0.50\%$ ,  $67.20\% \pm 1.06\%$  and  $66.07\% \pm 1.00\%$  in 1st to 5th hour respectively which was significantly low ( $P < 0.005$ ) in 1st hour while significantly high ( $P < 0.005$ ) in the remaining hours. Simultaneous administration of ibuprofen with enalapril in the group of CIR (IBU+ENP) showed percent reduction ( $59.55\% \pm 0.23\%$ ,  $99.02\% \pm 0.66\%$ ,  $99.42\% \pm 0.30\%$ ,  $99.23\% \pm 0.21\%$  and  $99.61\% \pm 0.33\%$ ) in 1st to 5th hour respectively which was significantly high ( $P < 0.005$ ) when compared to both groups.

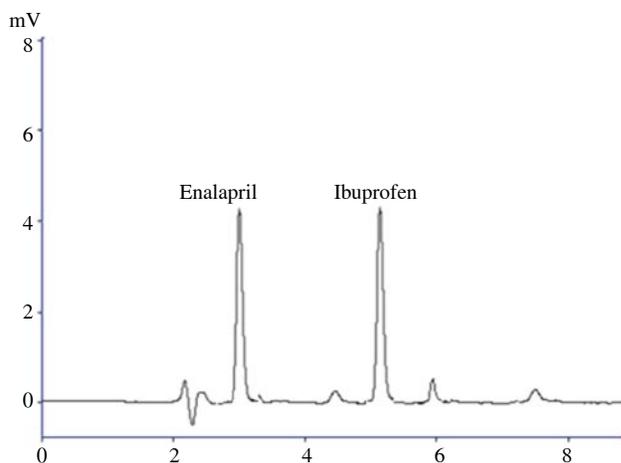
### 3.4. In vitro interactions

To develop a precise, accurate and suitable HPLC method for the simultaneous estimation of ENP and IBU, different mobile phases were tried and the proposed chromatographic conditions [short analysis time ( $< 5$  min)] also enabled its application in routine and quality-control analysis of the finished products. Mobile phase containing methanol: water (80:20) with pH 3.0 while UV detection was performed at 227 nm. The retention time for ibuprofen was found to be 5.2 min, while that of enalapril maleate was 3.3 min (Figure 3). The mobile phase was used after filtration and then sonicated for 10 min; flow rate was set to 1.0 mL/min at constant column temperature ( $25 \pm 2$ )°C.

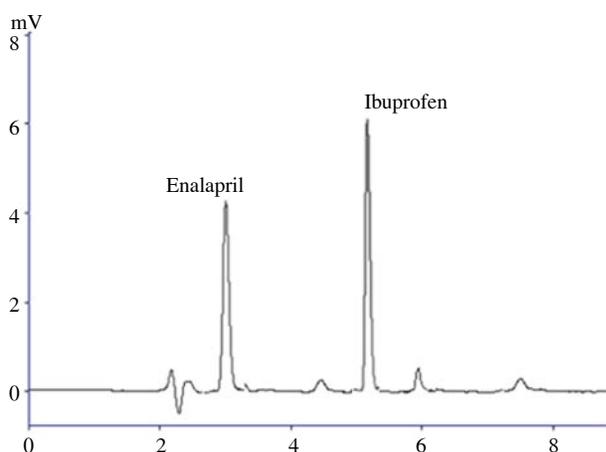
## 4. Discussion

The interaction results showed that the availability of ibuprofen

was increased in the presence of enalapril (Figure 4 and Table 4). This suggested that for the co-administration of enalapril with ibuprofen, administration requires continuous monitoring and a proper interval should be given to avoid such interactions.



**Figure 3.** Chromatogram of enalapril with ibuprofen before interaction.



**Figure 4.** Chromatogram of enalapril with ibuprofen after interaction.

**Table 4**

Availability of enalapril and ibuprofen by HPLC %.

Analytes	Resolution	Capacity factors (K')	Theoretical plates (N)	Availability after 180 min
ENP (3.3)	3.5	2.07	3405	101.2
IBU (5.2)	3.8	2.19	3463	100.0
ENP + IBU	3.6	2.40	4400	106.0

ENP: Enalapril; IBU: Ibuprofen; CIR: Carrageenan induced inflammation rat.

The simultaneous administration of enalapril and ibuprofen shows increased action of ibuprofen as depicting by increase of percent reduction and decrease in inflammation. It was also proved from peak of combining drugs. It is also reported that ibuprofen can potentially interact with drugs that include  $\beta$ -blockers, ACE inhibitors, aspirin and corticosteroids. Therefore, it would always be advisable to consult healthcare professionals about the use of combination drugs. Hence, this study has certain limitations due to limited number of tested animals as

well as duration of anti-inflammatory response studied.

### Conflict of interest statement

We declare that we have no conflict of interest.

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