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In vivo Interaction Studies of ACE Inhibitors with NSAIDs on Carrageenan Induced Inflammation

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Abstract

Both antihypertensive and non steroidal anti-inflammatory drugs are frequently prescribed together since hypertension and co-existing musculoskeletal problems are two of the frequent conditions. *In vivo* interaction studies of ACE inhibitors (enalapril, captopril and lisinopril) were carried out with commonly used NSAIDs (diclofenac sodium and mefenamic acid) in carrageenan induced inflammation in rats (CII) to check the anti-inflammatory response of NSAIDs in such cases. Edema rate and percentage reduction were calculated and data was analyzed by one way analysis of variance using SPSS INC. software. Tukey's post-hoc test was conducted to determine group means differences taking significant level $p<0.05$ and $p<0.005$ highly significant.

Keywords: Pharmacokinetic study; Zaltoprofen; Spherical agglomerates; Spherical crystals; Rabbit model

Introduction

Both antihypertensive and non steroidal anti-inflammatory drugs (Table 1) are frequently prescribed together since hypertension and co-existing musculoskeletal problems are two of the frequent conditions [1,2].

These drugs block the angiotensin converting enzyme that cleaves the terminal two peptides from angiotensin I (decapeptide) to form the potent vasoconstrictor angiotensin II (octapeptide) [3,4] and lower the BP by reducing peripheral vascular resistance without reflex increasing cardiac output rate and contractility. They also inhibit the rate of bradykinin inactivation thus resulting in vasodilatation they also decrease the secretion of aldosterone resulting in decrease of sodium and water retention.

The therapeutic efficacy of NSAIDs is due to their ability for inhibition of prostaglandin endoperoxide synthase or cyclo-oxygenase (COX). COX catalyzes the first two steps in the arachidonic acid cascade that leads to several bioactive lipids including prostaglandins [5,6].

In order to identify the anti-inflammatory response of commonly used NSAIDs when administered concurrently with selected ACE inhibitors (enalapril, captopril and lisinopril), we used the pool of rats with carrageenan induced paw inflammation. Inflammation induced by carrageenan, originally described by Winter [7] is acute, non-immune, well-researched and highly reproducible. In our study the altered anti-inflammatory response of NSAIDs when given simultaneously with ACE inhibitors by comparing decrease in paw size (edema). Results were expressed in % reduction in paw size for every hour and were calculated. Following given formula were used to calculate edema rate and percentage reduction.

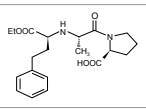
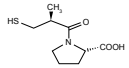
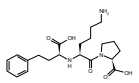
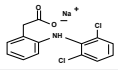
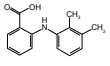
Drugs	Nomenclature	Structure
Enalapril	(S)-1-[N-[1-(ethoxycarbonyl)-3-phenyl propyl]-L-alanyl]-L-proline,(Z)-2-butenedioate salt	
Captopril	1-(3-mercapto-2-dmethyl-1-oxopropyl)-1-proline (S,S)	
Lisinopril	((S)-1-[N2-(1-carboxy-3-phenylpropyl)-1-lysyl]-1-proline dehydrate	
Diclofenac sodium	2-[(2,6-dichlorophenyl) amino] benzene acetic acid monosodium salt	
Mefenamic acid	2-[(2,3-dimethyl phenyl amino] benzoic acid and N-(2,3-xyloyl) anthranilic acid	

Table 1: Antihypertensive and Non steroidal anti-inflammatory drugs.

Edema rate $(E\%) = \frac{V_T - V_O}{V_O} \times 100$

V_O=Rat's hind paw volume before 1% carrageenan administration.

S.No	Groups	Treatment	Dose (mg.kg^{-1})
1	CII saline (Control)	Saline	---
2	CII ENP	Enalapril	2.5
3	CII CAP	Captopril	2.5
4	CII LSP	Lisinopril	2.5
5	CII DIC	Diclofenac sodium	5
6	CII MEF	Mefenamic acid	5
9	CII DIC+ENP	Diclofenac sodium+Enalapril	5+2.5
10	CII MEF+ENP	Mefenamic acid+Enalapril	5+2.5
13	CII DIC+CAP	Diclofenac sodium+Captopril	5+2.5
14	CII MEF+CAP	Mefenamic acid+Captopril	5+2.5
17	CII DIC+LSP	Diclofenac sodium+Lisinopril	5+2.5
18	CII ME+LSP	Mefenamic acid+Lisinopril	5+2.5

Table 2: Experimental design and Drug treatment.

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V_T=Rat's hind paw volume at t hour.

Percentage reduction $(R\%) = \frac{E_C - E_T}{E_C} \times 100$

E_C=Edema rate of control group

E_T=Edema rate of test compound at t hour.

Edema rate and percentage reduction data was also analyzed by using one way analysis of variance using SPSS INC. software. Tukey's post-hoc test was conducted to determine group means differences taking significant level p<0.05 and p<0.005 highly significant.

The principle aim of this work was to study the interaction studies of ACE Inhibitor with NSAIDS. For this purpose adjuvant induced inflammation (AII) rats were used as animal model having similar pathological features as rheumatoid arthritis in human [8,9] as shown in table 1.

Material and methods

Animals

Female rats weighing 180-250 g were used for this study. Six animals per group were housed in an animal room under standard conditions i.e. at 21°C in a controlled temperature and humidity.

Adjuvant induced inflammation

Carrageenan was suspended in normal saline to the concentration of 1 gm in 100 ml. Adjuvant inflammation was induced in animals by a single intra dermal injection of 0.1 ml of the solution at the base of the foot.

Experimental design and drug treatment

Rats were randomly distributed (n=6) into different groups received their respective treatment orally using 0.5 ml dimethyl sulfoxide (DMSO) table as vehicle one hour prior to inflammation induction (Table 2).

The severity of inflammation was assessed by paw volume change. Paw swelling and the general state of the animals were monitored in every hour. Hind paw volumes were measured volumetrically by using plethysmometer (model 7140; Ugo Basile, Varese, Italy) on 0, 1, 2, 3, 4 and 5 hour of the experiment. Paw volumes were deliberated in both the test and control groups on 0 and then on alternate every hour until 5 hour when the experiment ended.

Statistical analysis

The investigational outcomes were expressed as Mean ± S.D of *n*=6 rats in each group. Edema rate and percentage reduction was

GROUPS	ED1	ED2	ED3	ED4	ED5
CII DMSO	18.69 ± 1.11^{++}	25.54 ± 0.5^{++}	28.3 ± 0.43^{++}	39.12 ± 0.35^{++}	40.18 ± 0.27^{++}
CII ENP	6.46 ± 0.06	10.29 ± 0.28**	10.01 ± 0.01**	27.61 ± 0.60**	37.35 ± 0.34**
CII DIC	18.52 ± 0.5**	8.57 ± 0.51**	6.51 ± 0.5**	15.64 ± 0.41**	17.51 ± 0.5**
CII DIC+ENP	1.16 ± 0.01**$^{++}$	1.69 ± 0.12**$^{++}$	4.06 ± 0.03**$^{++}$	1.42 ± 0.06**$^{++}$	1.49 ± 0.06**$^{++}$
CII MEF	12.52 ± 0.5**	8.31 ± 0.42**	6.48 ± 0.5**	14.37 ± 0.4**	15.65 ± 0.41**
CII MEF+ENP	0.30 ± 0.00**$^{++}$	9.8 ± 0.03**$^{++}$	1.25 ± 0.04**$^{++}$	7.07 ± 0.01**$^{++}$	7.06 ± 001**$^{++}$
One Way	F1=26320.135	F2=1596.196	F3=1580.3	F4=3975.545	F5=10292.839
ANOVA (df=8,18)	p<0.005	p<0.005	p<0.005	p<0.005	p<0.005

Values are mean ± S.D.Significant difference by multiple comparision Tukey's test $^{+}$p<0.05, $^{++}$p<0.005 from control and *p<0.05, **p<0.005 from NSAIDs

Table 3: Edema rate in rat's paw after treatment with enalapril NSAIDs.

GROUPS	ED1	ED2	ED3	ED4	ED5
CII DMSO	18.69 ± 1.11^{++}	25.54 ± 0.5^{++}	28.3 ± 0.43^{++}	39.12 ± 0.35^{++}	40.18 ± 0.27^{++}
CII CAP	7.4 ± 0.2	12.1667 ± 0.3	13.23 ± 0.6	28.3 ± 0.6	38.6667 ± 0.4
CII DIC	18.52 ± 0.5**	8.57 ± 0.51**	6.51 ± 0.5**	15.64 ± 0.41**	17.51 ± 0.5**
CIIDIC+CAP	1.45 ± 0.2**$^{++}$	2.46 ± 0.3**$^{++}$	5.5 ± 0.23**$^{++}$	1.42 ± 0.23**$^{++}$	1.490 ± 0.2**$^{++}$
CII MEF	12.52 ± 0.5**	8.31 ± 0.42**	6.48 ± 0.5**	14.37 ± 0.4**	15.65 ± 0.41**
CII MEF+CAP	12.52 ± 0.2**$^{++}$	9.68 ± 0.5**$^{++}$	6.5 ± 0.6**$^{++}$	15.5 ± 0.6**$^{++}$	17.46 ± 0.6**$^{++}$
One Way	F1=11.01	F2=919.979	F3=16.907	F4=62.687	F5=9893
ANOVA (df=8,18)	p<0.005	p<0.005	p<0.005	p<0.005	p<0.005

Values are mean ± S.D.Significant difference by multiple comparision Tukey's test $^{+}$p<0.05, $^{++}$p<0.005 from control and *p<0.05, **p<0.005 from NSAIDs

Table 4: Edema rate in rat's paw after treatment with captopril NSAIDs.

GROUPS	ED1	ED2	ED3	ED4	ED5
CII DMSO	18.69 ± 1.11^{++}	25.54 ± 0.5^{++}	28.3 ± 0.43^{++}	39.12 ± 0.35^{++}	40.18 ± 0.27^{++}
CII LSP	34.93 ± 0.2	13.3 ± 0.32	15.5 ± 0.2	28.3 ± 0.2	39.2 ± 0.02
CII DIC	18.52 ± 0.5**	8.57 ± 0.51**	6.51 ± 0.5**	15.64 ± 0.41**	17.51 ± 0.5**
CII DIC+LSP	8.33 ± 0.32**$^{++}$	13.23 ± 0.2**$^{++}$	28.3 ± 0.2**$^{++}$	38.66 ± 0.3**$^{++}$	40.4 ± 0.5**$^{++}$
CII MEF	12.52 ± 0.5**	8.31 ± 0.42**	6.48 ± 0.5**	14.37 ± 0.4**	15.65 ± 0.41**
CIIMEF+LSP	8.36 ± 0.3**$^{++}$	12.53 ± 0.3**$^{++}$	15.26 ± 0.3**$^{++}$	29.46 ± 0.5**$^{++}$	39.4 ± 0.65**$^{++}$
One Way	F1=1.07	F2=717.18	F3=5203.54	F4=9119.69	F5=5018.11
ANOVA (df=8,18)	p<0.005	p<0.005	p<0.005	p<0.005	p<0.005

Values are mean ± S.D.Significant difference by multiple comparision Tukey's test $^{+}$p<0.05, $^{++}$p<0.005 from control and *p<0.05, **p<0.005 from NSAIDs

Table 5: Edema rate in rat's paw after treatment with lisinopril NSAIDs.

also calculated by using one way analysis of variance using SPSS INC. software. Tukey's post-hoc test was conducted to determine group means differences with the level of significance chosen at $p<0.05$ or $p<0.005$.

Results and Discussion

Edema rate

Tables 3-5 show the effect of treatment on edema rate for enalapril, captopril and lisinopril with NSAIDs. Data was analyzed by one way ANOVA (df 8, 18) showed significant treatment effect on edema rate (F_1=26320.1, p< 0.005) in first hour, (F_2=1596.1, p<0.005) in second hour, (F_3=1580.3, p<0.005) in third hour, (F_4=3975.5, p<0.005) in fourth hour and (F_5=10292.8, p<0.005) in fifth hour for enalapril and NSAIDs. Similarly (F_1=11.01, p< 0.005), (F_2=919.9, p<0.005), (F_3=16.9, p<0.005), (F_4=62.6, p<0.005) and (F_5=9893.0, p<0.005) for captopril and NSAIDs and for lisinopril-NSAIDs (F_1=1.07, p< 0.005), (F_2=717.1, p<0.005), (F_3=5203.5, p<0.005), (F_4=9119.6, p<0.005) and (F_5=5018.1, p<0.005) for first to fifth hour respectively.

Post hoc analysis

Interaction of ACE inhibitors (enalapril, captopril and lisinopril) with diclofenac sodium: Tukey's post hoc analysis showed that diclofenac sodium also reduced carrageenan induced paw edema but it was significantly low (p<0.005) in first hour (3.33%) data observation shows that percent reduction of diclofenac sodium was 72.16 ± 0.77%, 78.5 ± 0.5%, 55.09 ± 1.02% and 57.19 ± 1.05% in second, third, fourth and fifth hour respectively indicating that as experiment proceeded percent reduction increased up to third hour where it was significantly high (p<0.05) then became significantly low (p<0.05) in fourth and fifth hour (Table 6). Effect of enalapril on diclofenac sodium induced anti-inflammatory response was observed in the group CII DIC+ENP where percent reduction was 100.34 ± 0.04%, 94.76 ± 0.48%, 88.54 ± 0.38, 96.57 ± 0.32, 97.52 ± 0.24 in the first, second, third, fourth and fifth hour respectively that showed significant high reduction (p<0.005) in diclofenac sodium anti-inflammatory response when compared to the group treated with diclofenac sodium alone i.e. CII DIC.

Effect of captopril on diclofenac sodium induced anti-inflammatory response was observed in the group CII DIC+CAP where percent reduction was 93.2 ± 0.008%, 94.76 ± 0.48%, 95.4 ± 0.173, 96.57 ± 0.32, 97.52 ± 0.24 in the first, second, third, fourth and fifth hour respectively that showed significant high reduction (p<0.005) in diclofenac sodium anti-inflammatory response when compared to the group treated with diclofenac sodium alone i.e. CII DIC (Table 7). Effect of lisinopril on diclofenac sodium induced anti-inflammatory response was observed in the group CIIDIC+LSP where percent reduction was 76.66 ± 0.02%, 64.76 ± 0.05%, 53.33 ± 0.003%, 41.66 ± 0.05%, 22.33 ± 0.02% in the first, second, third, fourth and fifth hour respectively that showed significant decrease in reduction (p<0.005) in diclofenac sodium anti-inflammatory response when compared to the group treated with diclofenac sodium alone i.e. CII DIC (Table 8).

GROUPS	%R1	%R2	%R3	%R4	%R5
CII ENP	70.31 ± 0.08	30.09 ± 0.05	31.04 ± 0.0	32.77 ± 0.15	33.03 ± 0.0
CII DIC	3.33 ± 0.57^{++}	72.16 ± 0.77^{++}	78.5 ± 0.5^{++}	55.09 ± 1.02^{++}	57.19 ± 1.0^{++}
CII DIC +ENP	100.3 ± 0.04^{++}	94.76 ± 0.48^{++}	88.54 ± 0.3^{++}	96.57 ± 0.32^{++}	97.52 ± 0.2^{++}
CIIMEF	36.46 ± 0.5^{++}	72.0 ± 1.0^{++}	78.29 ± 1.1^{++}	59.51 ± 0.5^{++}	59.03 ± 1.0^{++}
CII MEF+ENP	59.23 ± 0.21^{++}	69.56 ± 0.31^{++}	96.32 ± 0.3^{++}	85.91 ± 0.72^{++}	87.39 ± 0.0^{++}
One Way	F1=136914.0	F2=9.713	F3=9.162	F4=256.157	F5=2883.26
ANOVA (df=8,18)	p<0.005	p<0.005	p<0.005	p<0.005	p<0.005

Table 6: % Reduction in rat's paw after treatment with enalapril NSAIDs.

GROUPS	%R1	%R2	%R3	%R4	%R5
CII CAP	76.66 ± 0.1	64.76 ± 0.15	53.33 ± 0.25	41.66 ± 0	22.33 ± 0.57
CII DIC	3.33 ± 0.5^{++}	72.16 ± 0.77^{++}	78.5 ± 0.5^{++}	55.09 ± 1.02^{++}	57.19 ± 1.05^{++}
CII DIC+CAP	93.2 ± 0.0^{++}	94.76 ± 0.15^{++}	95.4 ± 0.17^{++}	96.5 ± 0.5^{++}	97.52 ± 0.6^{++}
CIIMEF	36.46 ± 0.5^{++}	72.0 ± 1.0^{++}	78.2 ± 1.12^{++}	59.5 ± 0.5^{++}	59.03 ± 1.0^{++}
CII MEF+CAP	68.44 ± 0.2^{++}	70.46 ± 0.3^{++}	80.6 ± 0.3^{++}	89.2 ± 0.6	89.73 ± 0.5^{++}
One Way	F1=8.255	F2=8.85	F3=13.81	F4=582.639	F5=991.56
ANOVA (df=8,18)	p<0.005	p<0.005	p<0.005	p<0.005	p<0.005

Table 7: % Reduction in rat's paw after treatment with captopril NSAIDs.

GROUPS	%R1	%R2	%R3	%R4	%R5
CII LSP	75.33 ± 0.03	64.76 ± 0.1	54 ± 0.2	43 ± 0.02	20.26 ± 0.02
CII DIC	3.33 ± 0.57^{++}	72.16 ± 0.77^{++}	78.5 ± 0.5^{++}	55.09 ± 1.02^{++}	57.19 ± 1.05^{++}
CII DIC +LSP	76.66 ± 0.02^{++}	64.76 ± 0.05^{++}	53.33 ± 0.00^{++}	41.66 ± 0.05^{++}	22.33 ± 0.02^{++}
CIIMEF	36.46 ± 0.5^{++}	72.0 ± 1.0^{++}	78.29 ± 1.12^{++}	59.51 ± 0.5^{++}	59.03 ± 1.0^{++}
CII MEF+ LSP	80.1 ± 0.02^{++}	64.76 ± 0.06^{++}	54.1 ± 0.02^{++}	42.53 ± 0.07^{++}	18.56 ± 0.3^{++}
One Way	F1=21123.4	F2=305.26	F3=529.18	F4=1062.70	F5=2079.4
ANOVA (df=8,18)	p<0.005	p<0.005	p<0.005	p<0.005	p<0.005

Table 8: % Reduction in rat's paw after treatment with lisinopril NSAIDs.

Interaction of ACE inhibitors (enalapril, captopril and lisinopril) with mefenamic acid: Tukey's post hoc analysis showed that mefenamic acid reduced carrageenan induced inflammation but it was significantly low (p<0.005) in first hour 36.46 ± 0.5%. Data observation told that percent reduction of mefenamic acid was 72.16 ± 0.77%, 78.29 ± 1.12%, 59.51 ± 0.5% and 59.03 ± 1.0% in second, third, fourth and fifth hour respectively indicating as experiment proceeded percent reduction increased up to third hour where it was significantly high (p<0.05) then became significantly low (p<0.05) in fourth and fifth hour. Effect of enalapril on mefenamic acid induced anti-inflammatory response in the group CII MEF+ENP where percent reduction was 59.23 ± 0.21%, 69.56 ± 0.31%, 96.32 ± 0.35%, 85.91 ± 0.72%, 87.39 ± 0.05% in the first, second and third respectively and in fourth and fifth hour showed significant decrease (p<0.005) in mefenamic acid anti-inflammatory response and this reduction response is high as compared to the group treated with mefenamic acid alone i.e. CII MEF (Table 6).

Effect of captopril on mefenamic acid induced anti-inflammatory response was observed in the group CII MEF+CAP where percent reduction was 68.44 ± 0.2%, 70.46 ± 0.3%, 80.6 ± 0.3%, 89.26 ± 0.6%, 89.73 ± 0.5% in the first, second, third, fourth and fifth hour respectively that showed significant high reduction (p<0.005) in inflammation occurred when compared to the group treated with mefenamic acid alone i.e. CII MEF (Table 7).

Effect of lisinopril on mefenamic acid induced anti-inflammatory response was observed in the group CII MEF+LSP where percent reduction was 80.1 ± 0.02%, 64.76 ± 0.06%, 54.1 ± 0.02%, 42.53 ± 0.07% and 18.56 ± 0.3% in the first, second, third, fourth and fifth hour respectively which showed that in the first hour significant increase (p<0.005) was observed in MEF induced anti-inflammatory response after that this response reduced significantly (p<0.005) from second hour to the final hour when compared with CII MEF (Table 8).

It has been observed while comparing the anti-inflammatory response of commonly used NSAIDs alone and in combination with certain ACE inhibitors (enalapril, captopril and lisinopril) that the

activity of NSAIDs was enhanced by the addition of captopril and enalapril as % reduction got higher and edema rate decreased, whereas in case of lisinopril our findings were different from that of other two ACE inhibitors as the combination of lisinopril and NSAIDs showed decreased activity of NSAIDs as depicted by inhibition of % reduction and increase in edema rate. But since this study has certain limitations as a number of tested animals were small and the duration for which the anti-inflammatory response was checked for NSAIDs alone and in combination with ACE inhibitors was just few hours, further studies required to establish this relationship.

Conclusion

In vivo interaction studies of ACE inhibitors with commonly used NSAIDs in carrageenan induced inflammation (CII) revealed that the anti-inflammatory response of NSAIDs as concurrent administration with enalapril and captopril is high as compared to lisinopril; they produced synergistic effect however more studies are required to establish this relationship.

References

1. Johnson AG (1997) NSAIDs and increased blood pressure. What is the clinical significance? Drug Saf 17: 277-289.
2. Roth SH (1988) NSAIDs and gastropathy: a rheumatologist's review. J Rheumatol 15: 912-919.
3. Mary JM, Richard AH, Pamela CC (1997) Pharmacology: Lippincott's illustrated reviews. Lippincott-Raven Publishers, Philadelphia, 151-162.
4. Bertam GK (2003) Basic and Clinical Pharmacology. McGraw Hill Professional.
5. Smith WL, Marnett LJ (1991) Prostaglandin endoperoxide synthase: Structure and catalysis. Biochim Biophys Acta 1083: 1-17.
6. Smith WL, Marnett LJ, DeWitt DL (1991) Prostaglandin and thromboxane biosynthesis. Pharmacol Ther 49: 153-179.
7. Gugasyan R, Clouston D, Mandel T, Wicks I (1997) Prevention of splenic granuloma formation in adjuvant arthritis by 2-acetyl-tetrahydroxybutylimidazole (THI). Immunol Lett 58: 133-138.
8. Khaliq S, Haider S, Ahmed SP, Perveen T, Haleem DJ (2006) Relationship of brain tryptophan and serotonin in improving cognitive performance in rats. Pak J Pharm Sci 19: 11-15.
9. Cai X, Wong YF, Zhou H, Xie Y, Liu ZQ, et al. (2006) The comparative study of Sprague-Dawley and Lewis rats in adjuvant-induced arthritis. Naunyn-Schmiedeberg's Arch Pharmacol 373: 140-147.