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Research Article

EFFECT OF SODIUM VALPROATE AND DOCOSAHEXAENOIC ACID ON INFLAMMATION IN RATS

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ABSTRACT

Objective: To evaluate the anti-inflammatory activity of sodium valproate and docosahexaenoic acid (DHA) supplementation using various experimental models in albino Wistar rats.

Method: A total of 48 adult Wistar albino male rats were divided into 8 groups of 6 rats each. Group I was control (distilled water 1 ml/kg), Group II received intraperitoneal (i.p.) injection of indomethacin (10 mg/kg), Groups III-V were injected (i.p.) with sodium valproate 100, 200, and 400 mg/kg water, and Groups VI-VIII were given sodium valproate 100, 200, and 400 mg/kg plus DHA 300 mg/kg (i.p.), respectively. Anti-inflammatory activity was assessed using carrageenan induced paw edema and the cotton pellet granuloma models.

Results: We found that higher doses of sodium valproate (400 mg/kg) used either alone or with a combination of DHA (300 mg/kg) showed a significant anti-inflammatory activity when compared to control in both the models of inflammation.

Conclusion: Combination of sodium valproate along DHA has shown promising anti-inflammatory activity.

Keywords: Anti-inflammatory drugs, Sodium valproate, Rat model.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) that include both traditional cyclooxygenase (COX) inhibitors and the newer selective (COX-2) ones are at present the mainstay drugs for treating pain and inflammation. While at one end there is no doubt that these drugs are effective in the management of conditions such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis, on the other hand, they are known to cause adverse effects. While traditional agents are known to cause gastrointestinal adverse effects with chronic use, the COX-2 inhibitors possess a cardiotoxic potential [1].

Corticosteroids are known to possess anti-inflammatory and immune modulatory effects. Like NSAIDs, they are useful in the management of painful inflammatory conditions such as rheumatoid arthritis and gout [2]. However, they cause gastritis, proximal muscle weakness, diabetes, and they predispose to infections [3].

The quest for an ideal anti-inflammatory, which is effective and nontoxic at the same time, has got the researchers thinking. Various avenues have been explored.

A commonly used antiepileptic drug, sodium valproate has been found to be effective in many nonepileptic conditions like bipolar disorder and migraine prophylaxis, thanks to its multiple mechanisms of action [4]. One such mechanism of action is inhibition of histone deacetylation (HDAC). It is reported that valproate has neuroprotective and anti-inflammatory properties due to HDAC inhibition [5].

Omega-3 fatty acids, such as eicosapentaenoic acid and the DHA, have shown to decrease the risk of developing many diseases such as cancer, hypertension, and cardiac disease. They have been shown to compete against arachidonic acid for lipooxygenase and COX thereby preventing production of proinflammatory mediators. In addition, this being a natural product is less likely to cause adverse effects compared to the traditional anti-inflammatory drugs [6]. In view of these reports, a study was initiated with a plan to assess the effect of sodium valproate alone and in combination with DHA in rat models of inflammation.

METHODS

An *in vivo* experimental study conducted in the Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India.

Selection and animal handling

A total of 48 adult Wistar albino healthy rats were selected for this study, which were locally bred in the Central Animal House of Manipal University, Manipal. These animals weighed 150-200 g and they were around 6 months old and were housed under controlled conditions with temperature of about 23±2°C, 50±5% humidity, and 10-14 hr of light and dark cycle, respectively. These animals were individually housed in polypropylene cages which contained paddy husk (procured locally) which was sterile, as bedding throughout the study and had easy access to animal chow (sterile food) and water *ad libitum*. This study was initiated after taking approval from the Institutional Animal Ethics Committee (IAEC/KMC/75/2014 dated August 22, 2014). The experiments were conducted in accordance to CPCSEA guidelines.

Identification of individual animals

Rats from each treatment group were marked using different colored marks on their tails, for easy identification and experimentation, according to the group allotted.

Drugs, reagents, and other materials

Three drugs were used during the study. Indomethacin, sodium valproate, and DHA were used. The pure powder form was used, dissolved in distilled water.

Evaluation of anti-inflammatory activity

A total of 48 adult Wistar albino male rats weighing 150-200 g were equally divided into 8 groups of six rats each. The rats were weighed and marked. Group I was taken as the control (distilled water 1 ml/kg),

Group II was given indomethacin injection (10 mg/kg) intraperitoneal (i.p.), Group III-V, were injected (i.p.) with sodium valproate 100, 200, and 400 mg/kg dissolved in distilled water, and Group VI-VIII were given sodium valproate 100, 200, and 400 mg/kg dissolved with distilled water along with DHA 300 mg/kg (i.p.), respectively. The dose of sodium valproate was chosen as 100 mg/kg, 200 mg/kg, and 400 mg/kg based on a study performed by Raza *et al.* [7]. The dose of DHA was chosen as 300 mg/kg based on a study done by Gao *et al.* [8]

Carrageenan-induced paw edema model

The plethysmometer is a device used to measure small changes in volume. Two interconnected Perspex tubes form the volume transducer and these tubes are filled with conductive solution. The displacement produced as a result of object immersion in the measuring tube is measured in the second tube in between the platinum electrodes.

The albino Wistar rats were starved overnight. The test drug was administered. At 30 minutes post administration of test or reference drug, each animal received 0.1 ml of 1% carrageenan subcutaneously in the hind paw of the right side. The left paw was then injected subcutaneously with the same volume of distilled water. Following this, the volume of the paw was measured using plethysmometer by the volume displacement technique which was sensitive to a volume change of 0.01 ml. The paw volume was measured pretreatment and post-treatment at 60 minutes gap at 60, 120, 180, and 240 minutes. The difference in the paw volume was then measured and values tabulated. Percent inhibition of inflammation was then calculated using the formula:

Percent inhibition=[1-(A-B/C-D)]*100.

Where A and B were the mean paw volume of the rats before and after injection of carrageenan, respectively, in the test group, and C and D are the mean paw volume of rats before and after carrageenan injection, respectively, in the control group [9].

Cotton pellet granuloma model

The albino Wistar rats were weighed and marked. A sterile cotton pellet was weighed, and 30 mg was placed in the subcutaneous plane in the groin of rats. Group I was taken as a control (distilled water 1 ml/kg), Group II was be given an (i.p.) injection of indomethacin (10 mg/kg), Groups III-V were injected (i.p.) with sodium valproate 100, 200, and 400 mg/kg dissolved with distilled water, and Groups VI-VIII were given sodium valproate 100, 200, and 400 mg/kg dissolved with distilled water along with DHA 300 mg/kg, respectively, once daily for seven consecutive days. On day 8, the rats were sacrificed by overdose of ketamine (80 mg/kg; i.p.) followed by cervical dislocation and cotton pellets were removed. Extraneous tissue was separated following, which the pellets were dried overnight at a temperature of 60°C to a constant weight. Any increase in the weight of the pellet was taken as deposition of granuloma tissue [10].

A washout period of 1-month was maintained after studying each inflammation models.

Statistical analysis

The data obtained were analyzed using IBM statistical package for social sciences version 22.0. The results were expressed as mean±standard error of mean. The significance of difference within the groups at different end points was assessed using repeated measures one-way analysis of variance (ANOVA). Similarly, the significance of differences between the various groups were checked for using ANOVA, followed by *post hoc* Tukey's test. Importantly, all test groups were compared against the control and standard groups, to arrive at a conclusion regarding the results. P <0.05 was considered to be statistically significant.

RESULTS

Anti-inflammatory activity

1. Carrageenan-induced paw edema model

The paw volume was significantly lower (0.23±0.05, p=0.010, % inhibition - 58.93%) in the indomethacin (10 mg/kg) treated group, 0.11±0.03, p<0.001, % inhibition - 80.36% in sodium valproate (100 mg/kg) plus DHA (300 mg/kg) group, 0.15±0.03, p=0.001, % inhibition - 73.22% in the sodium valproate (200 mg/kg) plus DHA (300 mg/kg) group, and 0.16±0.04, p=0.001, % inhibition - 71.43% in the sodium valproate (400 mg/kg) plus DHA (300 mg/kg) group at 60 minutes when compared to control (0.56±0.08) as shown in Table 1 and Fig. 1.

The paw volume was significantly lower (0.34±0.06, p<0.001, % inhibition - 68.81%) in the indomethacin (10 mg/kg) treated group, 0.53±0.26, p=0.015, % inhibition - 51.38% in sodium valproate (200 mg/kg) group, 0.46±0.13, p=0.005, % inhibition - 57.80% in sodium valproate (400 mg/kg) group, 0.31±0.06, p<0.001, % inhibition - 71.56% in sodium valproate (100 mg/kg) plus DHA (300 mg/kg) group, 0.32±0.06, p<0.001, % inhibition - 70.65% in the sodium valproate (200 mg/kg) plus DHA (300 mg/kg) group, and 0.25±0.05, p<0.001, % inhibition - 77.07% in the sodium valproate (400 mg/kg) plus DHA (300 mg/kg) group at 120 minutes when compared to control (1.09±0.16) as shown in Table 1 and Fig. 1.

The paw volume was significantly lower (0.39±0.06, p<0.001, % inhibition - 73.65%) in the indomethacin (10 mg/kg) treated group, 0.93±0.1, p=0.001, % inhibition - 37.17% in sodium valproate (100 mg/kg) group, 0.63±0.16, p<0.001, % inhibition - 57.44% in sodium valproate (200 mg/kg) group, 0.31±0.06, p<0.001, % inhibition - 79.06% in sodium valproate (400 mg/kg) group, 0.38±0.06, p<0.001, % inhibition - 74.33% in sodium valproate (100 mg/kg) plus DHA (300 mg/kg) group, 0.40±0.07, p<0.001, % inhibition - 72.98% in the sodium valproate (200 mg/kg) plus DHA (300 mg/kg) group, and 0.34±0.06, p<0.001, % inhibition - 77.03% in the sodium valproate (400 mg/kg) plus DHA (300 mg/kg) group at 180 minutes when compared to control (1.48±0.06) as shown in Table 1 and Fig. 1.

The paw volume was significantly lower (0.41±0.04, p<0.001, % inhibition - 72.85%) in the indomethacin (10 mg/kg) treated group, 0.89±0.28, p=0.037, % inhibition - 41.06% in sodium valproate (100 mg/kg) group, 0.29±0.03, p<0.001, % inhibition - 80.80% in sodium valproate (400 mg/kg) group, 0.57±0.06, p<0.001, %

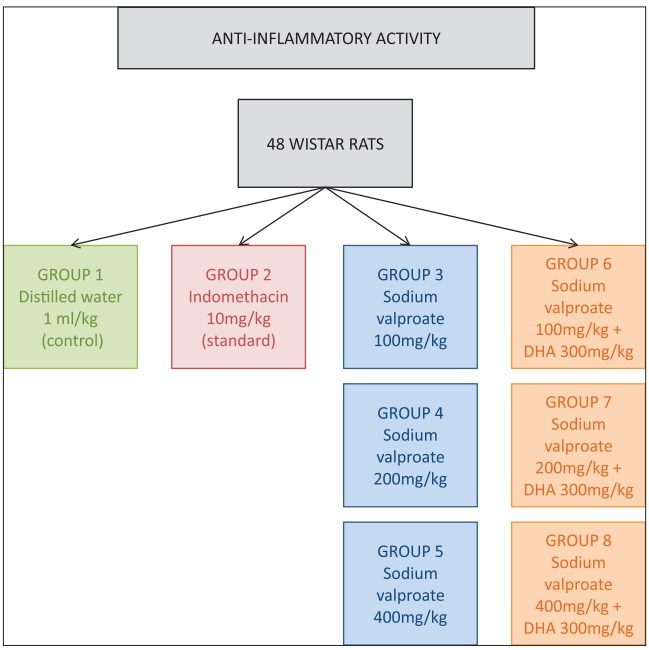

Fig. 1: Anti-inflammatory activity of sodium valproate and docosahexaenoic acid in carrageenan-induced paw edema model, mean difference in paw volume (mL) ± Standard error of mean at different time points. b denotes p<0.05 compared to control p value obtained by one-way analysis of variance followed by *post hoc* Tukey's test

Table 1: Carrageenan-induced paw edema model

Group	Mean difference in paw volume (ml.)±SEM at different time points (percentage inhibition of edema)			
	60 minutes	120 minutes	180 minutes	240 minutes
Control	0.56±0.08 (0)	1.09±0.16 (0)	1.48±0.06 (0)	1.51±0.06 (0)
Indomethacin 10 mg/kg (std)	0.23±0.05[b] (58.93)	0.34±0.06[b] (68.81)	0.39±0.06[b] (73.65)	0.41±0.04[b] (72.85)
Val 100 mg/kg	0.53±0.11 (5.36)	0.78±0.10 (28.45)	0.93±0.11[b] (37.17)	0.89±0.28[b] (41.06)
Val 200 mg/kg	0.31±0.03 (44.65)	0.53±0.26[b] (51.38)	0.63±0.16[b] (57.44)	0.98±0.18 (35.10)
Val 400 mg/kg	0.32±0.05 (42.86)	0.46±0.13[b] (57.80)	0.31±0.06[b] (79.06)	0.29±0.03[b] (80.80)
Val 100 mg/kg+DHA 300 mg/kg	0.11±0.03[b] (80.36)	0.31±0.06[b] (71.56)	0.38±0.06[b] (74.33)	0.57±0.06[b] (62.26)
Val 200 mg/kg+DHA 300 mg/kg	0.15±0.03[b] (73.22)	0.32±0.06[b] (70.65)	0.40±0.07[b] (72.98)	0.45±0.11[b] (70.20)
Val 400 mg/kg+DHA 300 mg/kg	0.16±0.04[b] (71.43)	0.25±0.05[b] (77.07)	0.34±0.06[b] (77.03)	0.35±0.05[b] (76.83)

All values are expressed as Mean±SEM. [b]p<0.05 compared to control. p value obtained by one-way ANOVA followed by *post hoc* Tukey's test. SEM: Standard error of mean

inhibition - 62.26% in sodium valproate (100 mg/kg) plus DHA (300 mg/kg) group, 0.45±0.11, p<0.001, % inhibition - 70.20% in the sodium valproate (200 mg/kg) plus DHA (300 mg/kg) group, and 0.35±0.05, p<0.001, % inhibition - 76.83% in the sodium valproate (400 mg/kg) plus DHA (300 mg/kg) group at 240 minutes when compared to control (1.51±0.06) as shown in Table 1 and Fig. 1.

2. Cotton pellet granuloma

As shown in Table 2 and Fig. 2, the dry weight of cotton pellet granuloma in control group was 0.13±0.01 g, in indomethacin (10 mg/kg) group was 0.03±0.00 g (p<0.001), in sodium valproate (100 mg/kg) group was 0.13±0.01 g (p=1.000), in sodium valproate (200 mg/kg) group was 0.13±0.01 g (p=1.000), in sodium valproate (400 mg/kg) group was 0.07±0.02 g (p<0.001), in sodium valproate (100 mg/kg) plus DHA (300 mg/kg) group was 0.08±0.03 g (p=0.001), sodium valproate (200 mg/kg) plus DHA (300 mg/kg) group was 0.06±0.03 g (p<0.001), and sodium valproate (400 mg/kg) plus DHA (300 mg/kg) group was 0.07±0.02 g (p<0.001). The drug sodium valproate when given alone in low doses (100 and 200 mg/kg) did not show any statistically significant anti-inflammatory property when compared to control. However, 400 mg/kg of sodium valproate when given alone showed statistically significant anti-inflammatory activity when compared to control. All the three groups where sodium valproate was combined with DHA showed statistically significant anti-inflammatory activity as compared to control. However, the reduction in granuloma formation in these groups was less than that of standard drug indomethacin.

Table 2: Cotton pellet granuloma model

Groups	Mean dry weight of granuloma (mg)
Control	0.13±0.01
Indomethacin 10 mg/kg (std)	0.03±0.00[b]
Val 100 mg/kg	0.13±0.01[c]
Val 200 mg/kg	0.13±0.01[c]
Val 400 mg/kg	0.07±0.02[b]
Val 100 mg/kg+DHA 300 mg/kg	0.08±0.03[bc]
Val 200 mg/kg+DHA 300 mg/kg	0.06±0.03[b]
Val 400 mg/kg+DHA 300 mg/kg	0.07±0.02[bc]

All values are expressed as Mean±SEM. [b]p<0.05 compared to control and [c]p<0.05 compared to standard. p value obtained by one-way ANOVA followed by *post hoc* Tukey's test. SEM: Standard error of mean, DHA: Docosahexaenoic acid

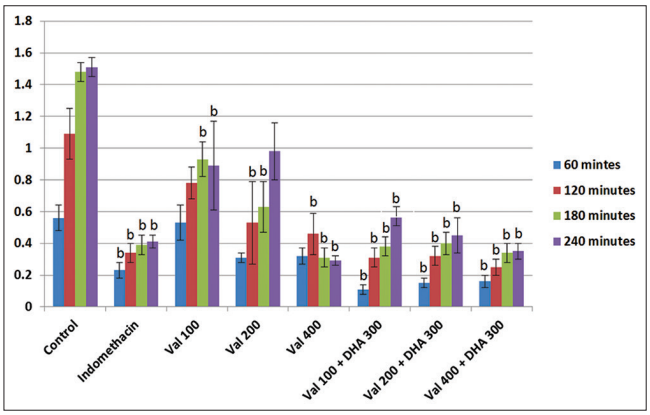

Fig. 2: Anti-inflammatory activity of sodium valproate and docosahexaenoic acid on cotton pellet granuloma model, mean dry weight of granuloma (mg). b denotes p<0.05 compared to control and c denotes p<0.05 compared to standard p value obtained by one-way analysis of variance followed by *post hoc* Tukey's test

DISCUSSION

The anti-inflammatory action of sodium valproate along with DHA was assessed using cotton pellet granuloma as well as paw edema model induced by Irritant carrageenan which are standard models for screening agents for anti-inflammatory activity. Paw edema model with carrageenan in rats is the standard model to evaluate acute inflammation. Carrageenan is the irritant that is preferred for testing drugs for anti-inflammatory activity as it is nonantigenic and also due to the absence of systemic effects. It induces edema in two phases; the first phase is characterized by serotonin release and also the release of kinins and histamine, whereas the second phase is due to the release of prostaglandins and slow reacting substances.

In the carrageenan induced paw edema model, a combination of sodium valproate all three doses used with 300 mg of DHA showed anti-inflammatory activity compared to control at 60 minutes. Sodium valproate 200 mg, sodium valproate 400 mg, combination of sodium valproate 100mg with 300 mg of DHA, combination of sodium valproate 200 mg with 300 mg of DHA, and combination of sodium valproate 400 mg with 300 mg of DHA showed anti-inflammatory activity compared to control at 120 minutes. Sodium valproate 100 mg, sodium valproate 200 mg, sodium valproate 400 mg, combination of sodium valproate 100 mg with 300 mg of DHA, combination of sodium valproate 200 mg with 300 mg of DHA, and combination of sodium valproate 400 mg with 300 mg of DHA showed anti-inflammatory activity compared to control at 180 and 240 minutes. The similar anti-inflammatory activity of sodium valproate was reported by earlier workers [7].

In the cotton pellet granuloma model, sodium valproate given alone at 400 mg, combination of sodium valproate 100 mg with 300 mg of DHA, combination of sodium valproate 200 mg with 300 mg of DHA, and combination of sodium valproate 400 mg with 300 mg of DHA showed anti-inflammatory activity as compared to control. However, the anti-inflammatory activity was less than that of standard drug indomethacin.

The carrageenan injection into the paw of the rat is said to raise the levels of interleukin 6 (IL-6), IL-1 β, tumor necrosis factor alpha (TNF α), and cytokine-induced neutrophil chemoattractant 1 levels [11]. In addition, nitric oxide is also involved in the carrageenan-induced inflammation. Sodium valproate, an HDACI inhibits the cytokines like

IL-6 and TNF α. Migration of leukocytes to the inflammatory site is also inhibited [12]. Sodium valproate is also an antioxidant and inhibits the production of reactive oxygen species [13]. These effects of sodium may be responsible for its anti-inflammatory activity.

Studies were done by Ferrucci *et al*. have shown that there exists a reciprocal connection in between the omega-3 fatty acids levels and the inflammatory cytokines like TNF α and IL-6 and C-reactive protein [14]. Another study was done by Rao *et al.* in omega-3 fatty acid deficient rats has shown up-regulation of enzyme which converts arachidonic acid to prostaglandin E2 in these rats [15]. A study was done by McNamara *et al.* have shown that deficiency of omega-3 fatty acids increases the central serotonin turnover [16].

DHA suppresses nuclear factor Kappa Beta mediated nuclear translocation thereby inhibiting cytokine (TNFα, vascular endothelial growth factor, and IL-1β) mediated adhesion molecule expression [17]. DHA is also a peroxisome proliferator activated receptor gamma activator. In addition, they produce resolvins and protectins through pathways involving lipoxygenase and COX enzymes, which have an anti-inflammatory role. These protectins and resolvins prevent the neutrophilic infiltration by preventing transendothelial migration [18]. All these effects probably contribute to the anti-inflammatory effects.

In conclusion, the combination of sodium valproate and DHA has shown better anti-inflammatory activity. A well planned clinical trial using this combination may be worthwhile to evaluate the possibility its therapeutic use in various inflammatory conditions.

CONCLUSION

Higher doses of sodium valproate, either used alone or along with DHA showed statistically significant anti-inflammatory activity in both the models of inflammation. The combination of sodium valproate and DHA has shown promising anti-inflammatory activity and was almost comparable to standard drugs used in this study. Clinical studies on this combination may be worthwhile.

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