Effect of Diazepam on Induced Acute Inflammation in Rat

*Ferdous A,[1] Rashid MU,[2] Habib A,[3] Islam MN,[4] Moosa A[5]

Anti-inflammatory effects of diazepam and other nonsteroidal anti-inflammatory drugs, diclofenac sodium and valdecoxib were evaluated using formalin induced paw oedema model and formalin induced arthritis model test in rat. A significant anti-inflammatory effect was observed with diazepam at a dose of 10 mg/kg in both model tests. The maximum inhibitory effects were observed at 3 hour, in paw oedema model with single dose (10 mg/kg. P,O.) and on day 4, in arthritis model with daily oral dose of diazepam(10 mg/kg, P.O.,7 days). This result suggested that diazepam have exhibited significant anti-inflammatory effect on induced acute inflammation in animal which were comparable with the effects of diclofenac and valdecoxib with minimum side effects

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Introduction

Inflammation is a common biological event experienced by almost all living creature. It may be defined as a complex reaction of vascularised connective tissue to noxious agents**.** Inflammatory diseases affect people of sexes, all ages and ethnic groups. Climate, attitude of life and geography do not appear to influence its prevalence.[1,2] The inflammatory process involves a series of events that can be elicited by numerous stimuli like microbes, trauma, toxins, chemicals, autoimmune reactions etc. Inflammatory response occurs in three distinct phases, each apparently mediated by different mechanism: acute transient phase characterized by local vasodilatation and increased vascular permeability; a delayed sub acute phase, most prominently characterized by infiltration of leukocyte and other phagocytic cells and a chronic proliferative phase in which tissue degeneration and fibrosis occurs.

Many different complex mechanism involved in the inflammatory process to induce response in these phase**.** Interaction of this response produces various chemical substances popularly called chemical mediators responsible for the clinical manifestation of inflammation.[3] Macroscopically inflammatory response is usually accompanied by the historical and familial clinical sign of rubor (redness) calor (increased temperature), dolor (pain), tumor (swelling) and functiolaesa (loss of function).[4] Inflammation is fundamentally a protective response to get rid of the noxious agents but sometimes it may be potentially harmful and needs anti-inflammatory agents to control and or minimize or alleviate its clinical manifestations.[3] On the background knowledge of immunopathogenesis and pharmacological process involved in inflammatory response, many drugs have been tested and developed and introduced to treat and control the symptoms of inflammations.

1. **Dr. Afsana Ferdous, Assistant Professor, Department of Pharmacology and Therapeutics, Rangpur Medical College, Bangladesh. afsanaferd@gmail.com*
2. *Dr. Mamun Ur Rashid, Professor and Head Department of Pharmacology and Therapeutics , Islami Bank Medical College, Rajshahi.*
3. *Dr. Anwar Habib, Professor and Head, Department of Pharmacology and Therapeutics Rajshahi Medical College.*
4. *Dr. Md. Nazrul Islam, Assistant Professor, Department of Biochemistry, Dinajpur Medical College.*
5. *Dr. ASM Moosa, Assistant Professor, Department of Pharmacology and Therapeutics, Sathkhira Medical college.*

** For correspondence*

Diazepam is a long acting benzodiazepine having CNS depressant effect commonly used as anxiolytics, hypnotics and anticonvulsant etc. Diazepam also having anti-inflammatory effect in animal.[5] Diazepam reduce induced acute inflammation in rat. The corticosterone and peripheral benzodiazepine receptors plays important role in reducing acute inflammation.[6] From surveying the literatures and text on diazepam have given a conception that diazepam has reducing effect on acute inflammation in animal. No study has been done to explore its anti-inflammatory action in animal in our context which grows attention for further investigation before committing the final conclusion.

This study aimed to observe the effects of diazepam, diclofenac and valdecoxib on induced acute inflammation in rats by using formalin induced paw oedema and formalin induced arthritis model with single high oral dose and the results obtained were compared to control.

Methods

Study design and materials

The study was carried out in the Department of Pharmacology and therapeutics, Rajshahi Medical College, Rajshahi in collaboration with the department of Pathology, Rajshahi Medical College, Bangladesh, with some technical assistance from the Department of Pharmacy, University of Rajshahi,. The experiments were designed to demonstrate the anti-inflammatory effect of diazepam and commonly used nonsterioidal anti-inflammatory drugs diclofenac and valdecoxib on formalin induced paw oedema and formalin induced arthritis models.[1,2]

Drugs and chemicals

Drugs and chemicals used in the experiments were diazepam, diclofenac sodium and valdecoxib (Square Pharmaceuticals, Bangladesh), formalin as 40% formaldehyde (Doctors chemical company, Bangladesh), Dimethyl sulfoxide (Pharmacy Lab, Rajshahi University, Bangladesh), Normal saline (Beximco Infusion, Bangladesh) and distilled water (Square Pharmaceuticals, Bangladesh).

Preparation of drugs and chemicals

Active ingredients of diazepam and valdecoxib dissolved in dimethyl sulfoxide and diclofenac sodium in distilled water in a definite proportion (diazepam 20 mg,valdecoxib 14 mg, diclofenac sodium 20 mg in 05 ml of solvent) and solution were made for oral administration. Desired concentration of formalin, 2% and 4% respectively were prepared by mixing 40% formaldehyde with normal saline in a definite proportion for injection to induce inflammation.

Animals

Albino rats (150-200 gm) were collected from the ICDDR, B (International Center for Diarrheal Disease Research, Bangladesh) animal house and maintained under standard animal husbandry conditions in the animal of Pharmacology department, Rajshahi Medical College, Bangladesh and has free access to food and water. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups each consist of six animals. Experiments were performed in accordance with the Institutional Animal Ethical Committee guidelines.

Assessment of inflammation

As the exudative inflammatory oedema is the macroscopic hall mark of acute inflammation, in the present study inflammation was assessed by taking measurement of mean increase in the circumferential diameters of rats hind paw oedema and hind paw joint swelling respectively. Microscoipically acute inflammation evidenced by heavy neutrophilic infiltration (Figure-1).The percentages of inhibition of increase circumferential diameter of the paw oedema

and joint swelling in both the control and drugs treated groups were compared by using formula:{(1-T/C)}x100, where T stands for test and C stands for control respectively.

Assessment of anti-inflammatory activity by rat paw oedema model

The normal paw circumferential diameter of all rats were measured initially and were divided into four groups each consistent of six animals treated orally with the vehicle as control (distilled water), diazepam (10 mg/kg), diclofenac sodium(10 mg/kg) and valdecoxib (6 mg/kg) respectively. Formalin (0.1 ml of a 2% solution in normal saline) was injected subcutaneously in the planter surface near the center region of planter aponeurosis. The vehicle and drugs were administered 30 min prior to the injection of formalin. The paw circumferential diameter in mm of all rats were measured at central region using a measuring thread at 3 and 6 hour after formalin treatment. A significant reduction in the paw circumferential diameter compared to vehicle treated control animals was considered as inflammatory response. Percentage (%) of inhibition ={(1-T/C)}x100, where T stands for test and C stands for control respectively.[7]

Assessment of anti-inflammatory activity by rat hind paw arthritis model

The normal hind paw joint circumferential diameter of all rats were measured initially and were divided into four groups each consistent of six animals treated orally with the vehicle as control (distilled water), diazepam(10 mg/kg), diclofenac sodium(10 mg/kg) and valdecoxib (6 mg/kg) respectively daily for consecutive seven days. Formalin (0.1ml of a 4% solution in normal saline) was injected subcutaneously in the sub planter region of the hind paw just close to the insertion of tendocalcaneous ligament. The vehicle and drugs were administered 30 min prior to the injection of formalin and thereafter daily. The hind paw joint circumferential diameter in mm of all rats were measured between the insertion of planter aponeurosis and origin of the extensor hallusis dorsi using a measuring thread daily from day1 to day 7after formalin treatment. Data from day 2, day 4 and day 7 were taken for calculation. A significant reduction in the paw circumferential diameter compared to vehicle treated control animals was considered as inflammatory response. Percentage (%) of inhibition={(1-T/C)}x100, where T stands for test and C stands for control respectively.[7]

Statistical analysis

All values expressed as Mean±SD (Standard deviation). Students unpaired "t" test was performed to analyze the difference between the control data with test data. The p values of 0.05 or less were considered as significant.

Results

The effects of diazepam were determined by using formalin induced rat paw oedema model and formalin induced rat paw arthritis model test and results were compared with the effects of diclofenac sodium and valdecoxib. Single high dose of diazepam (10 mg/kg), diclofenac sodium (10 mg/kg) and valdecoxib (6 mg/kg) were evaluated in paw oedema model test and same daily dose evaluated in hind paw arthritis model.

Results of rat paw oedema model test were shown in Table-I. Diazepam shown significant ($p<0.001$) inhibitory effect on paw inflammatory oedema and was maximal at 3 hour (54.45%) in comparison to control. Diclofenac sodium and valdecoxib shown 56.86% ($p<0.001$) and 57.60% ($p<0.001$) inhibition of paw oedema at 3 hour respectively in comparison to control.

Results of formalin induced hind paw joint arthritis model test were shown in Table-II. Diazepam shown significant ($p<0.001$) inhibitory effect on hind paw joint swelling and was maximal on day 2, (61.33%) in

comparison to control. Diclofenac sodium and valdecoxib shown 66.66% ($p<0.001$) and 64.60% ($p<0.0001$) respectively on day 2.

Table I: Effects of diazepam, diclofenac sodium and valdecoxib on paw oedema test in rat

Group	O hour	3 hour	6 hour	Increment in paw CD		% inhibition	
				3 hour	6 hour	3 hour	6 hour
Control	16.58±0.29	27.00±0.18	26.33±0.30	10.80±0.39[a]	10.16±0.21[a]		
Diazepam(10mg/kg,p.o)	16.08±0.31	21.00±0.12	22.00±0.31	4.92±0.56[a]	5.75±0.38[a]	54.45	43.41
Diclofenac sodium(10mg/kg,p.o)	16.16±0.30	21.00±0.28	22.50±0.25	4.66±0.24[a]	6.33±0.43[a]	56.86	37.70
Valdecoxib(6mg/kg,p.o)	16.66±0.81	21.25±0.21	23.00±0.18	4.58±0.81[a]	6.33±0.10[a]	57.60	37.70

Values are in mean SD; (n=6); a=$p<0.001$, CD=circumferential diameter.

Table II: Effects of diazepam, diclofenac sodium and valdecoxib on formalin induced hind paw joint arthritis

Group	Day 0	Day 2	Day 4	Day7	Increment in joint CD			% inhibition		
					D2	D4	D7	D2	D4	D7
Control	21.00±0.34	24.00±0.18	27.41±0.57	28.50±0.36	3.00±0.34[a]	6.41±0.59[a]	7.33±0.24[a]			
Diazepam (10mg/kg,p.o)	21.08±0.26	22.41±0.19	24.25±0.28	26.00±0.12	1.16±0.39[a]	3.08±0.43[a]	5.16±0.20[a]	61.33	51.95	29.61
Diclofenac sodium (10mg/kg,p.o)	20.92±0.50	24.25±0.37	24.25±0.37	26.16±0.09	1.00±0.18[a]	3.33±0.20[a]	5.25±0.16[a]	66.67	48.04	28.38
Valdecoxib (6mg/kg,p.o)	21.16±0.27	24.50±0.18	24.50±0.18	26.50±0.18	1.08±0.34[a]	3.34±0.46[b]	5.33±0.31[a]	64.00	47.89	27.28

Values are in mean SD; (n=6); a=$p<0.001$, b=<0.01,CD=circumferential diameter, D=Day

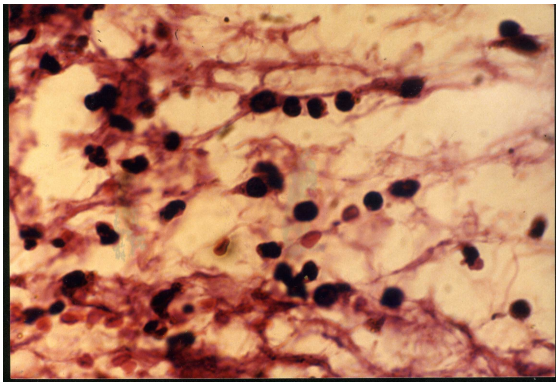

Figure 1. Showing heavy soft tissue neutrophil infiltration after 06 hours of formalin injection in control group

Discussion

Diazepam is a long acting benzodiazepam with CNS depressant activities popularly used as anxiolytics, hypnotics, anticonvulsant and preanaesthetic medication etc. It has also anti-inflammatory effects in animal. The effect of diazepam on induced acute inflammation in rat has described by Lazarrini et al .[6] The present study was designed to evaluate the anti-inflammatory effect of diazepam and commonly used NSAIDs , diclofenac sodium and valdecoxib on formalin induced acute inflammation in rat. Two experimental models used in this study, formalin induced

paw oedema model and formalin induced arthritis model. In formalin induced paw oedema model single high dose (10 mg/kg) of diazepam and other drugs were evaluated and in arthritis model ,daily high oral dose were tested.

Subplanter injection of formalin in rat paw oedema model test showed to a time dependent increase in paw diameter. This increment was observed maximum at 3 hour after formalin injection in the vehicle treated control group. There were a time dependent inhibitory activity observed in formalin induced paw inflammation at all assessment period with diazepam, diclofenac sodium and valdecoxib treatment.
In present study inhibition of paw oedema with diazepam were 54.45% and 43.41% after 3 hour and 6 hour of induced inflammation respectively, which is significant in comparison to control and also comparable with the effects of diclofenac sodium and valdecoxib. Diclofenac sodium shown 56.86% and 37.7% at 3 hour and 6 hour and valdecoxib shown 57.60% and 37.7% inhibition of paw oedema at 3 hour and 6 hour respectively after formalin treatment.

The effects of diazepam on reducing acute inflammation was comparable to that exhibited by Lazarrini et al [6] where they found inhibition of acute inflammatory paw oedema by diazepam were 48% after 3 hours and 43% after 6 hours of induction of inflammation . Results of present study consistent with that of Lazzarini et al.[6] But small difference in result with previous study was also observed. Probable reasons of this facts may be there.

Firstly, earlier study was done on carrageenan induced paw oedema model, where carrageenan was used as a induced agent of inflammation but in present study formalin is used as an induced agent of inflammation, there might be small variation in the biological responses to the inflammatory agents to treatment. Secondly, earlier study was done on Wister spices of rats but present study was done on Albino rats, so there might be species variation in response to treatment. Thirdly, in earlier experiment diazepam was used through intraperitoneal route but in present study diazepam used through oral route, so there might be small phamacokinetic variation in response to treatment.

Another experimental model used in present study was formalin induced arthritis model, where joint swelling (arthritis) was produced by injection 4% formalin and single high dose of all drugs were administered daily orally for consecutive 7 days and progress of joint swelling was measured . It was found that diazepam also showed time dependent inhibitory activity in formalin on hind paw joint swelling. Diazepam (10mg/kg, daily, P.O.) showed significant reduction in joint swelling on day 4 after induce arthritis with percent inhibition of 61.33% in comparison to control and it is also comparable to the effects with diclofenac sodium and valdecoxib , 66.67% and 64.00% on day 4 respectively.

Conventional NSAIDs like diclofenac sodium and valdecoxib exerts their anti-inflammatory action through the inhibition of enzymes involved in the pathogenesis of inflammation.

The precise mechanism by which diazepam produces its anti-inflammatory effect is not yet fully understood. But some possible mechanisms are suggested. Several stressors have been shown to affect the humoral and cellular components of the immune/inflammatory response in laboratory animals and humans.[8] It was suggested that the increased secretion of glucocorticoids during the inflammatory response serves to check and control the development of inflammation.[9,10]

Diazepam exerts its anti-inflammatory action through increasing stress related steroidogenesis .[11,12]. It binds with benzodiazepine receptors in the adrenal gland cells and facilitates the substances such as STAR (a steroidogenic acute regulatory protein) and PBR (peripheral benzodiazepine receptor complex molecule) responsible for the transport of cholesterol from cytoplasm to the inner mitochondrial membrane where the rate limiting catalyzing enzyme (cytochrome P450scc) is located (Lazzarini et al.,2001 and Papadoplous et al.,1993).[6,13] It also increases the hypothalamic-pituitary-adrenocortical (HPA) axis acitvity by cyclic AMP-dependent mechanism.[14] Diazepam treatment increases the serum concentration of corticosterone.[10]

Diazepam also exerts its anti-inflammatory effect by inhibiting phagocytic activity and production of cytokine, IL-1 & TNF-α.[15] On the other hand as diazepam have anxiolytic & hypnotic action may produce additional beneficial role in inflammation.

Conclusion

Therefore, the overall findings of present study shown that diazepam has almost similar anti-inflammatory activity like those of diclofenac and valdecoxib in comparison to control in animal. Of course, extensive clinical trail as well as biochemical and pharmacological experiments are recommended to explore the anti-inflammatory effect and possible mechanism of action of diazepam on human beings for safety dose modification.

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