ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF BENZODIAZEPINES AND THEIR INTERACTION WITH ASPIRIN IN WISTAR RATS.

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Summary

In the present study, analgesic and anti inflammatory activity of three benzodiazepines viz, diazepam, chlordiazepoxide and alprazolam is studied in both acute and sub-acute model of inflammation in rats. The study also aimed to elicit the possible interaction of these benzodiazepines with aspirin, in acute and sub-acute models of inflammation.

All the benzodiazepines in their therapeutic equivalent dose exerted significant anti-inflammatory activity. The lower doses of these benzodiazepines viz., diazepam (1.8 mg/kg), chlordiazepoxide (1.8 mg/kg), alprazolam (0.18mg/kg) when coadministered with subanti-inflammatory (SAI) dose of aspirin (54mg/kg) showed significant anti-inflammatory activity in both acute and subacute model of inflammation.

All the benzodiazepines individually in their therapeutic equivalent dose and their lower dose when coadministered with SAI dose of aspirin showed significant analgesic activity. Lower doses of diazepam or chlordiazepoxide did not produce significant gastric ulceration when combined with SAI dose of aspirin.

Such an interaction of benzodiazepines with NSAIDs like aspirin is worth exploiting clinically, if the present findings could be extrapolated to humans.

Key words: Alprazolam, Analgesia, Aspirin, Chlordiazepoxide, Diazepam, Inflammation, Interaction.

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Introduction

Phenobarbitone like drugs were used in combination with analgesics to potentiate their analgesic[1] activity though, it is well known by themselves they produce hyperalgesia, restlessness, excitement and even delirium when given in the presence of pain[2].Diazepam is being used in combination with analgesics for the same purpose, appears to be devoid of hyperalgesic activity when administered in presence of pain, probably due to its reported analgesic activity[3].

It is quiet surprising to observe that benzodiazepines and barbiturates have discriminating effects on pain though, their pharmacological actions on the CNS are mediated through the GABA induced chloride currents[4].In contrast to phenobarbitone, diazepam has also been reported to possess anti-inflammatory activity[5], anti ulcerogenic activity [6,7] and antipyretic activity[8]. Similar pharmacological actions have been reported for chlordiazepoxide [9,10],while, alprazolam is reported to be devoid of analgesic activity[11]. These reports clearly indicate that various benzodiazepines not only differ from barbiturates but also differ individually in their pharmacological profiles despite possessing the common chemical nucleus.

Kinins and prostaglandins, the mediators of pain are also involved in the pathogenesis of inflammation. Therefore analgesics suppressing these mediators could be expected to suppress the inflammation too.

Studies reporting analgesic activity of diazepam, used very high doses to patients, moreover another clinical study[12] reported variable effects of diazepam on pain threshold. These controversial reports and the poor documentation of analgesic, anti-inflammatory activity of other benzodiazepines prompted the present study.

In the present study, three commonly used benzodiazepines viz., diazepam, chlordiazepoxide, alprazolam have been used in the therapeutic equivalent doses to study their analgesic and anti-inflammatory activity in Wistar rats. These three drugs were also administered in lower therapeutic equivalent doses along with subantiinflammatory dose of aspirin in order to elicit their possible interaction with the latter, since combinations (aspirin like drugs and diazepam) are often used in the clinical practice.

Materials and methods

Animals: The complete course of experiments were carried out using healthy male rats of Wistar strain, weighing between 100-150 grams. The animals were acclimatized to normal laboratory conditions with 12-hr natural light-dark cycle and were maintained on standard laboratory diet with free access to water.

Drugs used and their doses: The adult clinical doses of the drugs were converted into rat equivalent doses with the help of converting table [13]. The drugs (with their adult therapeutic daily dose in parenthesis) used were diazepam 3.6 mg/kg (40 mg), chlordiazepoxide 3.6 mg/kg (40 mg), alprazolam 0.36 mg/kg (4 mg), aspirin 200 mg/kg (2 g). Lower doses of these drugs viz., diazepam 1.8 mg/kg (20 mg), chlordiazepoxide 1.8 mg/kg (20 mg), alprazolam 0.18 mg/kg (2 mg) were coadministered with sub-antiinflammatory dose (SAI) of aspirin 54 mg/kg which was determined in an earlier study [14]

In acute studies all the treatments were administered to different groups of animals (n=6 in each) in a single dose, thirty minutes prior to sub plantar injection of carrageenan while in sub acute studies the treatment was started after implanting the sterile foreign bodies and continued every 24 hours for 10 days. Control animals received equivalent volume of gum acacia suspension. All the drugs were administered orally as a suspension with 1% gum acacia.

Acute inflammation: Overnight fasted (with water ad lib) animals were subdivided in to a control and 7 treatment groups to receive the dose (mg/kg) of, (i)aspirin 200,(ii) diazepam 3.6 mg/kg, (iii) chlordiazepoxide 3.6,(iv)alprazolam 0.36.Remaining three groups received diazepam 1.8 or chlordiazepoxide 1.8 or alprazolam 0.18 with aspirin 54.

Acute inflammation was produced by subplantar injection of 0.05 ml of 1% carrageenan (from sigma co. St Louis) in left hind paw. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw volume was measured with the help of plethysmograph by mercury displacement method at zero hour (immediately after injecting carrageenan). The same procedure was repeated at 1,3 and 6 hour. The difference between 0 hour and subsequent reading was taken as actual oedema volume.

Subacute inflammation: Subacute inflammation was produced by method D'Arcy et.al [15] with some modification. In overnight starved(with water ad lib) rats after clipping the hair in axillae and groin, under light halothane anaesthesia, two sterile cotton pellets weighing 10 mg were implanted subcutaneously, through a small incision. Wounds were then sutured and animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the procedure. The animals were subdivided in to a control (vehicle) and a standard group (n=6 in each) to receive the dose (mg/kg) of aspirin 200 alone. Remaining groups received diazepam 1.8 or chlordiazepoxide 1.8 or alprazolam 0.18 with aspirin 54.The treatments were started after implantation and was repeated every twenty four hours, regularly for ten days.

On eleventh day the rats were sacrificed with an overdose of anaesthesia to remove cotton pellets, grass piths and stomachs. The pellets, free from extraneous tissue were dried overnight at 60^{o}C to note their dry weight. Net granuloma formation was calculated by substracting initial weights of cotton pellet (10mg) from the weights noted. Mean granuloma dry weight for various groups was calculated and expressed as mg/100 gm of body weight.

Analgesic activity: Janssen's caudal immersion test as described by Turner [16] was adapted in the present study and was carried out in animals subjected for carrageenan induced inflammation study. The reaction time as indicated by complete withdrawal of tail, was noted in various treated groups at an interval of 1, 2 and 3 hour after drug administration to calculate the mean reaction time.

Ulcer index: Stomachs were cut open along the greater curvature and gently washed with normal saline. Gastric mucosa was examined for the presence of erosions, haemorrhagic spots, ulcer and perforation if any, with the help of magnifying lens. To determine the severity of the ulcer, an arbitrary scoring system as described earlier [17] was followed. Ulcer index was calculated as mean score of ulcer severity in all the treated groups and was compared with that of control.

All the procedures were performed in accordance with the CPCSEA guidelines and the study was approved by IAEC.

Statistical Analysis: Data were expressed as Mean ± SEM and analysed by ANOVA followed by Dunnet's test and 'p' value <0.05 was considered as significant.

Results

Carrageenan induced acute inflammation:

Effects of various treatments (dose in mg/kg) on acute inflammation are as follows: aspirin(200), combinations of diazepam(1.8) or alprazolam(0.18) with SAI dose of aspirin(54) showed significant($p<0.05$, $p<0.01$ and $p<0.001$) reduction in the paw volume when compared with that of control at 3 and 6 hours. While chlordiazepoxide (3.6) and combination treatment of chlordiazepoxide (1.8) with SAI dose of aspirin significantly ($p<0.05$, $p<0.01$ and $p<0.001$) reduced paw edema at 1,3 and 6 hours when compared with that of control value. Where as significant($p<0.01$ and $p<0.001$) reduction in paw volume by diazepam(3.6) and alprazolam(0.36) was observed at 6^{th} hour, when compared with that of control. All the treated groups showed significant ($p<0.05$, $p<0.01$ and $p<0.001$) increase in mean reaction time to thermal stimulus when compared with that of control at 1,2 and 3 hour (Table I).

Sub acute inflammation (foreign body induced granulomas):

All the treated groups showed significant ($p<0.05$, $p<0.01$ and $p<0.001$) decrease in granuloma dry weight when compared with that of control (Table II).

Ulcer Index:

Aspirin (200 mg/kg) and combination treatment of alprazolam (0.18 mg/kg) with SAI dose of aspirin(54 mg/kg) showed significant ($p<0.01$,$p<0.001$) increase in ulcer index, where as in other groups no significant change was observed.

Table I: Effect of various treatments on carrageenan induced rat paw oedema and thermal pain (caudal immersion test).

Groups (n=6)	Drugs and Dose mg/kg	Paw volume in ml (Mean ± S.E.M)			Mean value in seconds (Mean ± S.E.M)		
		1 Hr	3 Hr	6 Hr	1 Hr	2 Hr	3 Hr
1	Control	0.17 ±0.01	0.30 ±0.03	0.44 ±0.02	1.67 ±0.09	1.69 ±0.13	1.68 ±0.05
2	Aspirin 200	0.11 ±0.01	0.12 *** ±0.03	0.17*** ±0.02	2.60*** ±0.99	2.74 *** ±0.08	2.82*** ±0.08
3	Diazepam 3.6	0.13 ±0.03	0.23 ±0.03	0.25*** ±0.03	2.37*** ±0.05	2.35 * ±0.21	2.26* ±0.24
4	Chlordiazepoxide 3.6	0.12* ±0.02	0.19* ±0.04	0.21** ±0.08	2.45*** ±0.09	2.67*** ±0.10	2.80*** ±0.12
5	Alprazolam 0.36	0.12 ±0.03	0.21 ±0.02	0.28** ±0.08	2.40 ** ±0.14	2.58*** ±0.09	2.24 ** ±0.14
6	Diazepam 1.8 with Aspirin 54	0.12 ±0.02	0.20* ±0.03	0.24*** ±0.02	2.45 *** ±0.09	2.96** ±0.16	2.08** ±0.08
7	Chlordiazepoxide 1.8 with Aspirin 54	0.12* ± 0.02	0.14* ±0.05	0.18*** ±0.03	2.49*** ±0.01	2.65*** ±0.10	2.75*** ±0.11
8	Alprazolam 0.18 with Aspirin 54	0.13 ±0.02	0.18* ±0.43	0.27** ±0.05	2.42*** ±0.06	2.53*** ±0.05	2.33 *** ±0.12

ANOVA followed by Dunnet's test, $p<0.05$*, $p<0.01$** and $p<0.001$***.

Table II: Effect of various treatments on foreign body induced granulomas, ulcer index.

Groups (n=6)	Drugs and Dose mg/kg	Granuloma dry weight (mg/100 g. B.W) Mean± S.E.M	Ulcer Index Mean± S.E.M
1	Control	75.60 ± 6.74	11.67±4.01
2	Aspirin 200	42.25±1.21***	31.67± 4.77**
3	Diazepam 1.8 with Aspirin 54	43.85±3.90**	18.33±1.96
4	Chlordiazepoxide 1.8 with Aspirin 54	52.74±6.88*	20.67±2.10
5	Alprazolam 0.18 with Aspirin 54	46.27±1.67**	31.60±1.56***

ANOVA followed by Dunnet's test, $p<0.05$*, $p<0.01$** and $p<0.001$***.

Discussion

Of the benzodiazepines used in the present study, anti-inflammatory activity agrees with earlier report about diazepam[18] and chlordiazepoxide[10].But the dose (10 and 20 mg/kg) of diazepam was higher in the earlier study [19]. There is paucity of information about similar activity of alprazolam observed at 6^{th} hour in the present study. Similarly synergistic anti-inflammatory activity between (low dose) benzodiazepines with aspirin in both acute and subacute studies is also poorly documented.

All benzodiazepines used in the present study showed significant analgesic effect when used individually as well as in combination with SAI dose of aspirin. Though the efficacy of diazepam in the short-term management of chronic orofacial muscle pain [19] is reported, analgesic activity of chlordiazepoxide and alprazolam could not be traced in accessible literature.

In combination studies, ulcerogenic potential of alprazolam in contrast to diazepam and chlordiazepoxide differs from earlier clinical report, wherein diazepam[20,21],chlordiazepoxide[7,22] and alprazolam[23] have been shown to decrease gastric acid secretion. The discrepancy could be due to species variation and duration of treatment, as alprazolam was used for 12 weeks in peptic ulcer patients. Participation of central GABA receptors in such activity could be ruled out to some extent, as phenobarbitone unlike benzodiazepines failed to influence the anti-inflammatory activity of aspirin (54 mg/kg-SAI).Pre synaptic GABA receptors ($GABA_B$) on peripheral sympathetic nerve terminals have been described [24].

These receptors are said to suppress the transmitter release by inhibiting voltage sensitive calcium channels[24].Proposed anti-inflammatory mechanisms involved with benzodiazepines include inhibition of Ca^{+} channels. However peripheral benzodiazepine receptors have been reported to be linked with voltage gated calcium channels and their activation by diazepam like benzodiazepines leads to decrease calcium influx and attenuation of dopamine release[25].It has been reported that anti-inflammatory activity of diazepam may be due to its direct action on peripheral-type benzodiazepine receptor (PBR) present in the endothelium. Decreased TNF-alpha activity [26] and reduced prostaglandin synthesis due to their inhibitory effect on norepinephrine [27] could also contribute for their anti-inflammatory activity. The synergistic anti-inflammatory activity observed with benzodiazepines aspirin could be attributed to their additive effects on PG synthesis.

The analgesic activity of the diazepam and chlordiazepoxide as observed in the present study, is in agreement with earlier observation in man[28].Variable effect on pain threshold as reported earlier [12] in contrast to the present findings appears to be due to the lower dose (0.15 mg/kg) used in the earlier study.

The analgesic activity observed in the present study could be explained on the same basis of their interference with synthesis and release of prostaglandins, bradykinins etc [29] and activation of opiod kappa receptors [30].

A study indicated the involvement of NO-cGMP pathway in the analgesic activity of benzodiazepines[31] and adenosine receptor activation [32].

Alprazolam combination with SAI dose of aspirin showed significant ulceration which could be compared to that of aspirin 200 mg/kg treated group. Decreased gastric HCl secretion and reduced gastric ulcers by diazepam in man [20,21], chlordiazepoxide in rabbits [7] and rats [22], lorazepam in rats and alprazolam in man [23] has been reported. Insignificant increase in ulcer index of the animals treated with diazepam-aspirin and chlordiazepoxide-aspirin combination as observed in the present study is in agreement with these earlier reports. Significant increase in the ulcer index in the animals treated with alprazolam-aspirin combination in contrast to control is difficult to explain with the help of the present findings. Discripancy in the anti-ulcerogenic activity of alprazolam in the present study and earlier study could be due to species variation or duration of treatment; as treatment continues for twelve weeks in the peptic ulcer patients in earlier study [23].

Mechanism of anti ulcer activity of benzodiazepines has been suggested to be due to their sedative,anxiolytic and antisecretory action[22].GABA mimetic drugs like N-phthaloyl GABA and sodium valproate have been reported to suppress cold restrained ulcers in rats[33].Since benzodiazepines augment GABA activity, GABA ergic mechanism could be proposed to explain their anti ulcerogenic effect[34]. The sedative and anxiolytic activity of diazepam and chlordiazepoxide contributing for their antiulcer effect in the present study appear to be insignificant, since alprazolam in combination with aspirin significantly elevated ulcer index. It is also possible that the inhibitory effect of benzodiazepines on calcium channels leading to decreased gastric acid secretion could be responsible for their anti ulcer effect, since calcium channel blockers have been reported for their anti ulcer and anti secretory (HCl) activity[35,36].

The findings of the present study clearly indicate that not only diazepam and chlordiazepoxide as reported earlier but also alprazolam have significant analgesic and anti-inflammatory activity.

If the results of the present study are true for man they could also be useful in the treatment of inflammatory disorders. However sedation caused by them would be a major limitation for such use. Aspirin, a well established anti-inflammatory agent has also limitations like gastric irritation and hyper acidity. It therefore appears that combination of smaller doses of aspirin and benzodiazepines like diazepam or chlordiazepoxide would be an effective and safer regimen for the treatment of inflammatory disorder if not in all at least in some selected individuals. Further clinical trials are essential to confirm the same.

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