Interaction of allopurinol and non-steroidal anti-inflammatory drugs on the carrageenan-induced rat paw edema

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ABSTRACT

Objectives: To find out the effect of combining allopurinol with non-steroidal anti-inflammatory drugs on carrageenan-induced rat paw edema.

Methods: The study was carried out at the College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia, over the period 1999 to 2000. Male wistar rats were randomly divided into 12-16 rats in each group. Edema was induced by subplantar injection of 0.1 ml of carrageenan (10 mg/ml) and the resulting edema volume was measured by plethysmograph, 3 hours after the injections. Saline of 0.9% (0.1 ml/100 g) was administered to the first group serving as control. The second and third groups received variable concentration of allopurinol (12.5, 25, 50 mg/kg) and tenoxicam (0.0625, 0.125, 0.25 mg/kg) 30 minutes before carrageenan injection. The fourth group received a combination of tenoxicam and allopurinol. Similar procedures were carried out with respect to diclofenac at 1.25, 2.5, 5.0 mg/kg and indomethacin at 0.25, 0.5, 1.0 mg/kg. The activities of the drugs were expressed as percentage inhibition of edema.

Results: Pre-treatment of the rats with the 4 drugs individually resulted in dose-dependent reduction of volume of paw edema. The combination of allopurinol and diclofenac acted synergistically to reduce edema. A similar synergistic action was obtained when allopurinol was combined with indomethacin. By contrast, tenoxicam-allopurinol combination resulted in antagonistic action and produced an effect on edema, which was less than their individual inhibitory action.

Conclusion: Combining allopurinol with either diclofenac or indomethacin produced synergistic inhibitory action on rat's paw edema. However, tenoxicam, when combined with allopurinol, produced antagonism.

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Stimulated polymorphonuclear cells elaborate oxygen to form superoxide anion radicals (Free O_2 radicals). These mediate significant inflammation and cause tissue injury and irreversible modification of macromolecules. Among some of the proposed effects of these toxic entities are: destruction of microbes, injury of host cells, tumor promotion, tumor cell injury, stimulation of secretion of products of other components of the blood, generation of chemotactic lipids from arachidonic acid, and generally promotion of inflammation.[1,2]

Allopurinol is a xanthine oxidase inhibitor with free radical scavenging activity.[3] Some of its action may be mediated by increasing the levels of superoxide dismutase.[4] Xanthine oxidase is responsible for free oxygen radicals production in some situations and its inhibition by allopurinol leads to reduced inflammation.[5]

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Its mild suppression of rat paw edema was previously demonstrated.[6] Non-steroidal anti-inflammatory drugs (NSAIDs) exert their action through the inhibition of the cyclooxygenase enzyme system and subsequent reduction in prostaglandin formation.[7] In addition, they have other proposed actions such as inhibition of lipoxygenase, phosphodiesterase, complement activation, decreased granulocyte and monocyte migration and phagocytosis, and inhibition of free radicals.[7] Indomethacin and diclofenac have been shown to reduce rat paw edema when used singularly.[8] The combination of diclofenac or indomethacin with dexamethasone but not ascorbic acid, resulted in potentiation of anti-edematous action.[9] Tenoxicam also reduced paw edema to the same extent when used alone or combined with zinc.[10] The combined effect of allopurinol with diclofenac, indomethacin or tenoxicam on rat paw edema has not been previously documented. In this article, we aim to find out the action and interaction of allopurinol and NSAIDs on the carrageenan-induced rat paw edema.

Methods. The study was carried out at the College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia, over the period 1999 to 2000. Carrageenan and indomethacin were purchased from Sigma Chemical Company (St. Louis, MO, USA); diclofenac and tenoxicam were generously donated by Ciba-Geigy Pharmaceutical Company (Basel, Switzerland) and Hoffman La-Roche Pharmaceutical Company (Basel, Switzerland). Allopurinol was supplied by Glaxo-Smith-Kline Co, UK. Male wistar rats were supplied by the Animal Care Centre, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia. Five animals were housed in a cage at a room temperature of 22.1°C and had free access to water and food. Rats (120-140 gm in weight) were randomly divided into 12-16 rats in each group. The edema was produced by subplantar injection of 0.1 ml of carrageenan (10 mg/ml). The volume of edema was determined by means of a plethysmograph (Ugo, Basile, Italy) 3 hours after injection of carrageenan. Saline of 0.9% (0.1 ml/ 100 gm) was administered to the first group of rats serving as control. The second group received variable concentrations of allopurinol (12.5, 25, 50 mg/kg) 30 minutes before carrageenan. A third group received tenoxicam (0.0625, 0.125, 0.25 mg/kg) 30 minutes before injection with carrageenan (as most of the drugs given usually show their maximum effect early after administration). The fourth group received a combination of tenoxicam (0.0625 mg/kg) and allopurinol (12.5, 25, 50 mg/kg). A similar procedure was carried out with respect to diclofenac at 1.25, 2.5, 5.0 mg/kg and indomethacin at 0.25, 0.5, 1.0 mg/kg. Concentrations of drugs given to rats were arrived at using surface area conversion tables as outlined by Paget and Barnes.[11,12] Upon completion of experiments, the rats were killed by an injection of an overdose of pentobarbitone, intraperitoneally, in accordance with the ethics committee requirement in our institution. The activities of drugs were expressed as percent inhibition of edema. As we found that anti-edematous effect no greater than 70% could be reached, we determined the ED50 value, the dose that inhibits the edema formation by 30% compared to the respective control. Results were expressed as means ± SEM of 12-16 rats in each group. Comparison was carried out by means of one way analysis of variance (ANOVA). As the number of observations in each experiment is <30, we used the t-test for unpaired data (2-tailed) to find the level of significance.

Results. Pre-treatment of animals with allopurinol alone resulted in reduction of volume of paw edema in a dose-dependent manner in all 3 experiments (**Table 1, 2, & 3**). Pre-treatment with tenoxicam also resulted in a similar inhibition of edema. However, the combination of the 2 treatments (allopurinol and tenoxicam) resulted in a lower inhibition than any of the 2 individual therapies, signifying antagonism (**Table 1**). In the second experiment, indomethacin caused a dose-dependent inhibition of paw edema (**Table 2**). Combining allopurinol and indomethacin resulted in inhibitory responses superseding the predicted values, suggesting statistically significant synergism between allopurinol and indomethacin. **Table 3** shows diclofenac to exert a dose-dependent inhibitory action on rat paw edema reaching high statistical significance ($p<0.01$). This inhibitory action was augmented by allopurinol in the combination therapy group giving higher than predicted inhibitory values, and thus signifying a synergistic mechanism of action.

Discussion. The results in this study show the inhibitory action of allopurinol on the carrageenan-induced edema in rats. This inhibitory action is further potentiated when allopurinol is combined with indomethacin and diclofenac. Carrageenan-induced paw edema has been used extensively to evaluate NSAIDs activity. This property was documented for both non-selective cyclooxygenase-2 and cyclooxygenase selective compounds.[13-16] Non-steroidal anti-inflammatory drugs exert most of their actions by inhibiting cyclooxygenases and thereby blocking the production of prostaglandins.[17,18] Prostaglandin themselves, although important in enhancing and prolonging inflammatory signals produced by pro-inflammatory agents, do not cause inflammation on their own.[19-21] Other agents such as bradykinins, histamine, neurokinins, complements, nitric oxide and oxygen-derived free radicals play a part in the inflammatory process.[1,2,22] This might mean that the anti-inflammatory action of NSAIDs could be increased further by agents acting as inhibitors of these pro-inflammatory mediators. The potentiation of the anti-inflammatory actions of NSAIDs by allopurinol, which was seen in this study, serves as an example of a

Table 1 - Effect of treatment with various doses of allopurinol when given together with tenoxicam on the carrageenan-induced rat paw edema.

Treatment	Dosage (mg/kg)	n of animals used	Paw volume (ml)	Observed	Predicted
Control (saline)	-	16	0.55 ± 0.09	-	-
Allopurinol	12.5	12	0.46 ± 0.12†	16.3	-
	25	12	0.39 ± 0.11†	27.3	-
	50	12	0.33 ± 0.08†	40.0	-
Tenoxicam	0.0625	12	0.46 ± 0.13†	15.7	-
	0.125	12	0.38 ± 0.07†	29.8	-
	0.25	12	0.35 ± 0.08†	35.0	-
Tenoxicam + allopurinol	0.0625				
	12.5	12	0.49 ± 0.12‡	10.9	32.0
	25	12	0.47 ± 0.11‡	14.5	43.0
	50	12	0.43 ± 0.10*	22.0	55.7

Values are expressed as mean ± SEM.
*significant at $p<0.05$ (compared to allopurinol alone at the corresponding dose), ‡not significant (compared to allopurinol at corresponding dose), †significant at $p<0.05$ (compared to control)

Table 2 - Effect of treatment with various doses of allopurinol when given together with indomethacin on the carrageenan-induced rat paw edema.

Treatment	Dose (mg/kg)	n of animals used	Paw volume (ml)	Observed	Predicted
Control (saline)	-	16	0.60 ± 0.14	-	-
Allopurinol	12.5	12	0.50 ± 0.13	16.3	-
	25	12	0.43 ± 0.11†	27.2	-
	50	12	0.36 ± 0.09†	40.0	-
Indomethacin	0.25	12	0.51 ± 0.12	14.5	-
	0.5	12	0.40 ± 0.11†	32.7	-
	1.0	12	0.31 ± 0.06†	47.2	-
Indomethacin + Allopurinol	0.25				
	12.5	12	0.39 ± 0.07*	35.0	30.8
	25	12	0.34 ± 0.08*	43.0	41.7
	50	12	0.25 ± 0.04‡	58.3	54.5

Values are expressed as mean ± SEM.
*significant at $p<0.05$ (compared to allopurinol alone at the corresponding dose), †significant at $p<0.05$ (compared to control), ‡significant at $p<0.01$ (compared to allopurinol alone at the corresponding dose)

Table 3 - Effect of treatment with various doses of allopurinol when given together with diclofenac on the carrageenan-induced rat paw edema.

Treatment	Dose (mg/kg)	n	Paw volume (ml)	Observed	Observed predicted
Control (saline)	-	16	0.55 ± 0.11	-	-
Allopurinol	12.5	12	0.46 ± 0.10*	16.3	-
	25	12	0.40 ± 0.09*	27.2	-
	50	12	0.33 ± 0.05*	40.0	-
Diclofenac	1.25	12	0.45 ± 0.11*	18.0	-
	2.5	12	0.38 ± 0.09*	30.0	-
	5.0	12	0.33 ± 0.07*	40.0	-
Diclofenac + allopurinol	1.25				
	12.5	12	0.24 ± 0.02†	56.3	34.3
	25	12	0.21 ± 0.03†	61.8	45.2
	50	12	0.17 ± 0.01†	69.0	58.0

Values are expressed as mean ± SEM. *significant at $p<0.05$ (compared to control), †significant at $p<0.01$ (compared to allopurinol alone at the corresponding dose)

potential clinical use. Allopurinol, a xanthine oxidase inhibitor, has been shown to decrease inflammation in many systems.[23-25] Allopurinol does this mainly by acting as an oxygen radical scavenger of these radicals, which are produced via myeloperoxidase catalysis.[23-25] Why should allopurinol potentiate the inhibitory action of indomethacin and diclofenac, but not tenoxicam, is probably due to the different actions these compounds have on oxygen species. Previous studies showed different behaviors of NSAIDs towards oxygen species.[26,27] Acetaminophen and sodium salicylate reacted with superoxide competitively, whereas naproxen and fluberprofen did not.[26] In particular, tenoxicam failed to show synergistic action on the chemiluminescence response of polymorphonuclear leukocytes when combined with verapamil or diltiazem, in contrast to the synergism observed when diclofenac or indomethacin were combined with verapamil or diltiazem.[28] Tenoxicam has also been shown to act differently to other NSAIDs with regard to random migration of cells and its reaction with hydroxyl radicals.[29,30] While the random migration of polymorphonuclear leukocytes was inhibited by piroxicam, diclofenac sodium and tiaprofenic acid, it was not inhibited by tenoxicam.[29] Other investigators observed tenoxicam to have pro-oxidant effects and had a non-linear competition plots in the deoxyribose assay for determination of rate constants for reaction of NSAIDs with hydroxyl radicals and in the opposite direction to that of indomethacin.[30] It has also been shown that tenoxicam when added to leukocytes undergoes peroxidative metabolization, which may constitute one mechanism by which it exerts its anti-inflammatory action when given alone. In addition, it may interfere with the bioassay used to determine anti-oxidant properties when combined with other drugs as had been suggested by other investigators.[31,32] Tenoxicam is one of the least lipophilic NSAIDs, and it has been suggested that this may restrict its penetration into certain tissues. This low lipophilicity may be reflected in lower concentration of the drug gaining access to the interior of leukocytes and therefore, may be related to the inability of tenoxicam to act on some intracellular events responsible for genesis of edema, and may have at the same time, interfered with the entry of allopurinol into the cells and the exit of anti-inflammatory mediators from the cells when given in the higher concentration. It is also possible that tenoxicam, by inhibiting cyclooxygenase enzyme, has directed the inflammatory process towards the lipoxygenase system and thereby, increasing inflammation. The diversion of arachidonate metabolism into non-cyclooxygenase pathways has been demonstrated experimentally by using NSAID inhibition of cyclooxygenase and resulting in a surge of the inflammatory process in kidneys.[33] These observations may help to explain the paradoxical, pro-inflammatory effect witnessed when combining tenoxicam with allopurinol.

In conclusion, allopurinol acts synergistically with diclofenac and indomethacin to reduce inflammation, while antagonizing the effect of tenoxicam on inflammation in the rat paw edema model. This may invite useful clinical application of this synergistic combination between allopurinol, indomethacin, diclofenac, and the avoidance of the antagonistic combined action of allopurinol and tenoxicam.

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