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Reason for the aggravation of diseases caused by inflammation and the ineffectiveness of NSAIDs on these diseases in rainy weather

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Abstract:
In this study, the anti-inflammatory activity of indomethacin, diclofenac, meloxicam and nimesulide were investigated on sunny and rainy days. Parallel to these experiments, the question of whether endogenous adrenaline and cortisol (corticosterone in rats) are factors that affect medicinal activity of these anti-inflammatory drugs on sunny and rainy days was examined. Our experimental results show that the drugs used produced significant anti-inflammatory effects on sunny days (76.5, 62.8, 56.9 and 64.7%, respectively) but were less effective on rainy days. On sunny days, adrenaline levels decreased by 83–86% in the groups that received indomethacin, diclofenac, meloxicam or nimesulide, compared to the control group. In contrast, there was no significant difference in corticosterone levels in any of these groups. In addition, the adrenaline and corticosterone levels of intact (versus adrenalectomized) rats decreased by 83% and 58.8%, respectively, on rainy days compared to sunny days.
Indomethacin, diclofenac, meloxicam and nimesulide were found to exert anti-inflammatory effects by decreasing adrenaline levels but not affecting corticosterone levels. The anti-inflammatory effects of the tested drugs was eliminated on rainy days due to the low level of corticosterone.

Key words:
adrenaline, corticosterone, carrageenan, rainy weather, sunny weather

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of inflammatory diseases (such as rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, psoriatic arthritis, systemic lupus, crystal arthritis, gout, rheumatic fever and other rheumatisms) due to their anti-inflammatory, analgesic and antipyretic effects [5, 14]. NSAIDs produce anti-inflammatory effects by inhibiting the synthesis of inflammatory mediators [14]. Studies into the anti-inflammatory effects of NSAIDs show circadian variability [4]. In addition, one theory suggests that the effects of drugs show not only circadian variability, but also day to day, month to month and even season to

season variability [7]. The fact that the same doses of NSAIDs show different anti-inflammatory efficacy on different days [1, 11, 13, 15, 19] supports this day to day, month to month and season to season variability theory. The disappearance of the anti-inflammatory effects of diclofenac sodium and nicardipine in adrenalectomized rats [18] suggests that adrenal gland hormones are important factors affecting the efficacy of NSAIDs. These data also demonstrate that these homones have a role in the mechanism of the anti-inflammatory effect of NSAIDs. Rheumatismal diseases have been reported to be affected by weather conditions [20]. Additionally, it has been reported that patients with arthritis describe the pain becoming intense when the weather is cold, humid or rainy [2, 6]. These data, obtained from the literature, show that cold, humid and rainy weather are among the factors that increase the severity of inflammation. In light of this data, we investigated the anti-inflammatory activity of NSAIDs on both sunny and rainy days. Our results show that the anti-inflammatory effects of NSAIDs peak on sunny days, while these effects are lost on rainy days. Our previous study [18] and preliminary experiments led us to hypothesize an important role for rainy weather and adrenal gland hormones in the anti-inflammatory efficacy and mechanism of NSAIDs effects. In another study, we demonstrated the role of endogenous adrenaline and cortisol in the anti-inflammatory efficacy and effect mechanism of NSAIDs [16]. The absence of anti-inflammatory efficacy of NSAIDs both on rainy days and in the adrenalectomized rats led us to hypothesize that the level of both endogenous adrenaline and cortisol (corticosterone in rats) decrease in rainy weather.

The aims of this study are to investigate the anti-inflammatory effects of NSAIDs on sunny and rainy days and to determine whether the factors affecting the potency of the drugs on sunny and rainy days are endogenous adrenaline and corticosterone.

Materials and Methods

Animals

Male albino Wistar rats (n = 92, 213 ± 2 g) were obtained from the Medical Experimental Research Center, Ataturk University. Before the experiments, the rats were housed and fed as divided groups, under standard conditions at 22°C in a laboratory. All experiments were performed in the same laboratory under standard conditions. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by Ataturk University's local animal care committee.

Chemicals

Indomethacin, diclofenac sodium, meloxicam, nimesulide and carrageenan were provided by Sigma (Germany).

Carrageenan test on the sunny day

In this series, the anti-inflammatory effects of indomethacin, diclofenac sodium, meloxicam and nimesulide were investigated on carrageenan-induced paw edemas in rats on a sunny day [3]. We performed the experiment in autumn. The weather conditions can be summarized as follows: temperature of 24°C, pressure at 1016 mb and 12% humidity. The carrageenan-induced inflammation model is commonly used to determine the effects of drugs on the acute phase of inflammation [13]. The rats were divided into five groups before the experiment began. The first four rat groups received either indomethacin (25 mg/kg), diclofenac sodium (25 mg/kg), meloxicam (7.5 mg/kg) or nimesulide (100 mg/kg) by oral gavage. The control group (the fifth group) received an equal volume of distilled water as a vehicle. One hour after the administration of drugs, 0.1 ml (1% w/v) carrageenan solution in distilled water was injected subcutaneously into the plantar surface of the hind paw of all the rats. Before the administration of carrageenan, each animal's paw volume was measured by plethysmometer up to the knee joint. Carrageenan-induced paw edema was measured four times, at one hour intervals. The anti-inflammatory effects of the drugs were evaluated by comparison to the results of the control group.

Parallel to this experiment, indomethacin, diclofenac sodium and meloxicam were administered to three different rat groups on the same day, in the same way, and at the doses mentioned above. The fourth group (control) received an equal volume of distilled water as a vehicle. At the third hour of carrageenan administration, blood samples were obtained from the

hearts of the rats under thiopental anesthesia (20 mg/kg) and concentrations of endogenous adrenaline and corticosterone were measured by a biochemistry laboratory. In this study a total of 46 animals were used.

Carrageenan test on the rainy day

In this series of experiments, the anti-inflammatory effects of the abovementioned NSAIDs were evaluated at the same doses and by the same procedure as on the sunny day; however, these experiments were carried out on a rainy day. We performed the experiment in autumn. The weather conditions can be summarized as follows: temperature of 9°C, pressure at 1018 mb and 81% humidity. Parallel to this experiment, indomethacin, diclofenac sodium and meloxicam was administered to three different rat groups on the same day, in the same way, and at the doses mentioned above. The fourth group (control) received an equal volume of distilled water as a vehicle. At the third hour of carrageenan administration, blood samples were obtained from the hearts of the rats under thiopental anesthesia (20 mg/kg) and concentrations of endogenous adrenaline and cortisol (corticosterone in rats) were measured by a biochemistry laboratory. In this study a total of 46 animals were used.

Biochemical analyses

Adrenaline levels in rats

We collected blood samples from the hearts of rats in 2 ml EDTA vacuum glass tubes to determine the levels of adrenaline and noradrenaline. Within 15 minutes of venesection, the EDTA samples for the adrenaline measurement were placed on ice and centrifuged at 3500 × g for five minutes.

After centrifugation, the plasma adrenaline concentration was measured by an isocratic system using a high performance liquid chromatography (HPLC) pump (model: Hewlett Packard Agilent 1100; flow rate of 1 ml/min and injection volume of 40 µl; analytical run time of 20 min) and an electrochemical detector.

We used a reagent kit for HPLC analysis of catecholamines in plasma serum (Chromsystems, Munich, Germany).

Corticosterone levels in rats

We collected blood samples from the hearts of rats in 2 ml EDTA vacuum glass tubes to determine the corticosterone levels in rats. Samples were centrifuged at 3500 × g for 10 minutes. The samples for the measurement were frozen and kept at –80°C until measurement. Plasma was separated and extracted with 5 ml ethyl acetate (betamethasone was used as an internal standard) and then the extract was washed with sodium hydroxide (0.1 M) and water. After the evaporation of the ethyl acetate, the residue was dissolved in mobile phase (acetonitrile-water-acetic acid-TEA, 22:78:0.1:0.03, v/v) and injected into an isocratic HPLC consisting of a 10 cm C18 column and UV detector at 254 nm. The plasma corticosterone concentration was measured by an isocratic system using an HPLC pump (model: Hewlett Packard Agilent 1100; flow rate of 1 ml/min and injection volume of 150 µl). Pure corticosterone (Sigma; St. Louis, MO) was purchased and dissolved in ethyl acetate. The samples were applied directly and compared with standard pure corticosterone [8].

Statistical analyses

All results were shown as the means ± standard error (SE). One-way analysis of the variance was used to evaluate the results. A value of $p < 0.05$ was considered significant.

Results

Carrageenan test on the sunny day

On sunny days, indomethacin, diclofenac, meloxicam and nimesulide inhibited the carrageenan-induced inflammation by 76.5% ($p < 0.001$), 68.2% ($p < 0.001$), 56.9% ($p < 0.001$) and 64.7% ($p < 0.001$), respectively (Tab. 1). The maximal effect of these drugs was observed at the third hour of carrageenan-induced inflammation. Therefore, we show only the results of the third hour in Table 1.

Tab. 1. Effects of indomethacin, diclofenac, meloxicam and nimesulide on carrageenan-induced inflammatory paw edema in rats

Drugs	Dose (mg/kg)	Number of animals	Paw volume of rats (ml)		Increase in inflammatory paw volume (ml)	Anti-inflammatory effect %	p
			Before inflammation	At the 3rd hour of carrageenan injection			
On the rainy day							
Indomethacin	25	6	0.82	1.41	0.59 ± 0.63	–13	> 0.05
Diclofenac Na	25	6	0.84	1.38	0.54 ± 0.41	–1.8	> 0.05
Meloxicam	7.5	6	0.82	1.37	0.55 ± 0.1	–3.7	> 0.05
Nimesulide	100	6	0.83	1.33	0.50 ± 0.087	+5.7	> 0.05
Control	–	6	0.81	1.34	0.53 ± 0.081	–	–
On the sunny day							
Indomethacin	25	6	0.79	0.91	0.12 ± 0.035	+76.5	< 0.001
Diclofenac Na	25	6	0.76	0.95	0.19 ± 0.045	+62.8	< 0.001
Meloxicam	7.5	6	0.75	0.97	0.22 ± 0.042	+56.9	< 0.001
Nimesulide	100	6	0.78	0.96	0.18 ± 0.03	+64.7	< 0.001
Control	25	6	0.77	1.28	0.51 ± 0.029	–	< 0.001

"+" indicates an anti-inflammatory effect and "–" indicates a pro-inflammatory effect

Carrageenan test on the rainy day

While nimesulide produced a slight, but insignificant, anti-inflammatory effect (5%), all other drugs we tested (indomethacin, diclofenac and meloxicam) showed a slight pro-inflammatory effect (Tab. 1). The maximal effect of these drugs was observed at the third hour of carrageenan-induced inflammation. Therefore, we report only the results of the third hour in Table 1.

Results of biochemical analyses

As shown in Table 2, on sunny days, the adrenaline levels were 3088.2 ng/ml ($p < 0.002$), 3978.5 ng/ml ($p < 0.003$), and 3730.7 ng/ml ($p < 0.003$) in the rat groups that received indomethacin, diclofenac or meloxicam, respectively; while the corresponding corticosterone levels were 11.2 μg/dl ($p > 0.05$), 9.2 μg/dl ($p > 0.05$) and 10.4 μg/dl ($p > 0.05$). On the rainy day, the adrenaline levels were 2460.3 ng/ml ($p < 0.05$), 2656 ng/ml ($p < 0.05$), and 2550 ng/ml ($p < 0.05$) in the rat groups that received indomethacin, diclofenac or meloxicam, respectively; the corresponding corticosterone levels were 3.9 μg/dl ($p > 0.05$), 3.1 μg/dl ($p > 0.05$) and 3.6 μg/dl ($p > 0.05$).

On the sunny day, the adrenaline and corticosterone levels were 23590.5 ng/ml and 9.0 μg/dl, respectively, in the healthy intact rat group that received only distilled water as a vehicle. On the rainy day, the adrenaline level was 4023.5 ng/ml while the corticosterone level was 3.5 μg/dl in the control group.

Discussion

In this study, the anti-inflammatory activity of indomethacin, diclofenac, meloxicam and nimesulide were investigated on both sunny and rainy days. In addition, the question of whether endogenous adrenaline and cortisol are the factors that alter the activity of these drugs on sunny and rainy days was examined. Our experimental results show that the NSAIDs we used had statistically significant and clear anti-inflammatory effects on sunny days. However, on the rainy day the anti-inflammatory effects of these drugs disappeared. On the rainy day, only nimesulide showed a slight, but insignificant, anti-inflammatory effect of 5%, while the other NSAIDs used had a slight pro-inflammatory effect. Reports have shown

Tab. 2. Effects of indomethacin, diclofenac sodium and meloxicam on adrenaline and corticosterone levels in rats

Drugs	Dose (mg/kg)	Number of animals	Adrenaline level (ng/ml)	p	Corticosterone level (μg/dl)	p
			On the sunny day			
Indomethacin	25	4	3088.2 ± 1656	< 0.001	11.2 ± 1.99	> 0.05
Diclofenac Na	25	4	3978.5 ± 1934	< 0.002	9.2 ± 2.08	> 0.05
Meloxicam	7.5	4	3730.7 ± 2060	< 0.003	10.4 ± 3.25	> 0.05
Intact (control)	–	4	23590.5 ± 1471.4		9.0 ± 1.04	–
			On the rainy day			
Indomethacin	25	4	2460.3 ± 468	< 0.05	3.9 ± 1.4	> 0.05
Diclofenac Na	25	4	2656 ± 339	< 0.05	3.1 ± 0.7	> 0.05
Meloxicam	7.5	4	2550 ± 524	< 0.05	3.6 ± 0.5	> 0.05
Intact (control)	–	4	4023.5 ± 1541	< 0.004	3.5 ± 1.62	< 0.025

that rheumatic pain, which is caused by inflammation, was affected by moisture [12]. In another study, performed by Sato et al., they demonstrate that simulated meteorological changes augmented behavioral abnormalities in a rheumatic pain model. Their observations support reports from humans with chronic rheumatic pain indicating that pain is aggravated by an approaching low pressure system or exposure to a mildly cold environment [10].

Pain is known to be a major indicator of inflammation and is reduced by NSAIDs [9]. The loss of the anti-inflammatory activity of NSAIDs in adrenalectomized rats may come from a large reduction in adrenal gland hormone production. On rainy days, the appearance of the anti-inflammatory activity NSAIDs may also originate from decreased adrenaline levels.

In order to determine whether adrenal gland hormones have a role in mechanism of the anti-inflammatory effect of NSAIDs, adrenaline and corticosterone levels were measured in the rats in which the drugs were administered on the sunny day, when the anti-inflammatory effects of NSAIDs were the highest. These measurements were compared with the intact rats which were not given any medicine. The results show that adrenaline levels were reduced 83–86% in rats that were given the aforementioned drugs, in comparison with intact rats. However, there were no alterations in corticosterone levels. In our previous study, we showed that nimesulide significantly reduced adrenaline levels and did not alter corticosterone levels [17]. In another study, we demonstrated that prednisolone had a 95.7% anti-inflammatory effect in adrenalectomised rats, while the anti-inflammatory effect in rats given propranolol was 36.2%. In addition, we showed that a glucocorticoid (prednisolone) had a stronger anti-inflammatory activity *via* β_2-adrenergic receptors in the absence of adrenaline (adrenalectomized rat) [16].

In the above study, we demonstrated that prednisolone was ulcerogenic in intact rats, but was anti-ulcerogenic in adrenalectomised rats. In addition, we proved that prednisolone had an anti-ulcerogenic effect *via* α_2-adrenergic receptors in the absence of adrenaline (adrenalectomised rats) [17]. Thus, we theorize that NSAIDs exert anti-inflammatory effects by reducing adrenaline levels without affecting corticosterone levels. We point out the importance of reducing the adrenaline amount and keeping the corticosterone concentration at a normal level for the emergence of significant anti-inflammatory activity in the body. To verify our hypothesis, adrenaline and corticosterone levels were separately measured on the sunny and the rainy day. Compared to the data from the sunny day, we determined that the adrenaline level on the rainy day was reduced by 83% while the corticosterone was reduced by 58.8%. Taken together, these data led us to hypothesize that when both adrenaline and corticosterone are decreased, the β_2-adrenergic receptors which are normally responsible for the anti-inflammatory effects, could no longer be stimulated in physiological grade and natural anti-inflammatory mechanisms are weakened. Therefore, we demonstrated that relapse and aggravation of inflammatory diseases (such as rheumatism) on rainy days

arose from the reduction of adrenaline and cortisol levels. We clearly demonstrated that NSAIDs did not create a reduction in the inflammation severity in rats with a low corticosterone level (on the rainy day).

Consequently, NSAIDs created an anti-inflammatory effect by reducing adrenaline levels but not by changing corticosterone levels. On rainy days, the loss of the anti-inflammatory effects of NSAIDs arose from the low level of corticosterone. In this study, we show that on rainy days, the use of NSAIDs for rheumatic pain was useless; to prevent relapses and reduce the severity of inflammatory diseases on rainy days, cortisol must be used in addition to NSAIDs.

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