Int J Physiol Pathophysiol Pharmacol 2009;1:150-153
www.ijppp.org/IJPPP902008

Short Communication

Effect of trifluoperazine on carrageenan-induced acute inflammation in intact and adrenalectomized rats

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Received February 24, 2009; accepted March 10, 2009; available online March 15, 2009

Abstract: The aim of present study was to investigate the effect of trifluoperazine (TFP) on carrageenan-induced rat's paw edema in intact and adrenalectomized (ADX). TFP (0.2 and 8 mg/kg) were given intraperitoneally just before the intraplantar injection of 0.1 ml of 0.5% carrageenan solution. After four hours, paw edema was assessed by calculating the paw volume changes and extravasations of Evans blue dye as inflammatory indicators. In both ADX and control groups, administration of TFP reduced inflammatory parameters (paw volume and tissue content of Evans blue dye) in inflamed paw. Our findings suggest that TFP can effectively reduce carrageenan-induced paw edema in both ADX and control rats. Therefore, anti-inflammatory effect of these drugs does not need the adrenal gland activity.

Key Words: Paw edema, trifluoperazine, adrenalectomy, carrageenan, inflammation

Introduction

Trifluoperazine (TFP) is one of conventional anti-psychotic drugs which have been used for a long time as treatment of Schizophrenia and related psychoses. In recent years, it has been shown that TFP has some anti-inflammatory effects and its effect in inhibition of experimental inflammatory models has been demonstrated. TFP was found to be potentially effective in the prevention of biochemical changes induced by thermal burn or frostbite [1, 2]. TFP reduce the inflammation caused by delayed hypersensitivity [3] and also can prevent the development of skin lesion induced by 2-chloroethylethyl sulfide [4].

On the other hand, in previous studies it has been shown that neuroleptic drug administration could affect the adrenal activity and the plasma Glucocorticoid concentration [5-7]. For instance, TFP can decrease the Adrenocorticotropic Hormone (ACTH) release by central mechanism [7]. Since the adrenal gland and its releasing hormone, cortisol, play an important role in anti inflammatory processes, the effect of TFP on the adrenal activity may contribute to its anti-inflammatory action. However, the interaction between the adrenal activity and the anti-inflammatory effect of TFP hasn't been elucidated.

The present study was designed to investigate the effect of TFP on carrageenan-induced paw edema. To evaluate the role of Glucocorticoids on anti-inflammatory action of this drug, the test was performed in both intact and adrenalectomized (ADX) rats.

Materials & methods

Animals

All experiments were carried out on adult male Albino rats weighing 250-300g, that were housed four per cage under a 12 h

light/dark cycle in a room with controlled temperature (22±1˚C). Food and water were available adlibitum except in adrenal-ectomized (ADX) rats. Animals were handled daily (between 9:00 and 10:00 a.m) for 5 days before the experiment day in order to adapt them to manipulation and minimize nonspecific stress responses. Rats were divided randomly into several experimental groups, each comprising 6-8 animals. All experiments followed the guidelines on ethical standard for investigation of experimental pain in animals [8].

Drugs

Trifluoperazine (Sigma, CO, UK) were prepared in saline. Carrageenan (Sigma, CO, UK) was dissolved in sterile saline yielding to 0.5% carrageenan solution. Evans blue dye was obtained from Sigma CO, UK.

Induction and measurement of the inflammation

Animals were anesthetized with thiopental (40 mg/kg) intraperitoneally (i.p) and inflammation was induced by intraplantar injection of 0.1 ml of 0.5% carrageenan solution in the left hind paw. The right hind paw was received the same volume of saline [9]. TFP (0.2 and 8 mg/kg) or saline were administered i.p just before the injection of carrageenan. Four hours after carrageenan injection, we measured changes in the hind paw volume and the tissue content of Evans blue dye as indicators of inflammation. The hind paw volume was measured by hydroplethysmometer and the algebraic difference between the volumes of the carrageenan-treated and saline-treated hind paw was calculated [10]. For measuring extravasations of Evans blue in the rat paw, Evans blue dye (20 go/kg) was injected into the femoral vein 30 minutes before the termination of the experiment (That is, 3:30h after carrageenan injection). Four hours after carrageenan injection, the hind paw volume was measured first, then animals were killed quickly by decapitation and the paw was dissected, homogenized and shakes (IKA-WERK Germany at 167rpm) in a solution contain 16 ml acetone plus 4 ml sodium sulfate 1%, for 24 h at 37˚C to allow Evans blue extraction. Each solution was then centrifuged (2000 rpm for 10 min) and the absorbance of the filtrate assessed at 620 nm using spectrophotometer (Milton Roy, Belgium) [11].

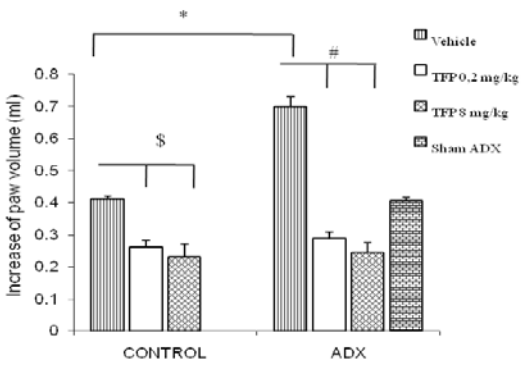

Figure 1. Effects of treatment with TFP on paw edema. *: significant difference between vehicle in control and ADX group (T-test, P= 0.023), $ significant difference between TFP (both doses) with vehicle in control group (ANOVA followed by tukey, all P<0.05), # :significant difference between TFP with vehicle in ADX group (ANOVA followed by tukey, all P<0.01). TFP; Trifluoperazine, ADX; Adrenalectomized.

Adrenalectomy

Animals were anesthetized with thiopental (40 mg/kg) i.p. both adrenal glands were removed through two dorsal incisions. The sham operation consisted of bilateral dorsal incision, plus locating and exposing the adrenals. All adrenalectomized rats were maintained on 0.9% sodium chloride drinking solution; whereas the sham operated rats were kept on tap water. The animals were tested 7 days after adrenalectomy or sham procedure.

Experimental groups

Normal groups consist of: 1. Rats received intraplantar injection of 0.1 ml of saline in the left hind paw. 2. Adrenalectomized (ADX) rats received intraplantar injection of 0.1 ml of saline in the left hind paw. Control groups consist of: 3. Rats received intraplantar injection of 0.1 ml 0.5% carrageenan solution in the left hind paw and saline i.p. 4. ADX rats received intraplantar injection of 0.1 ml 0.5% carrageenan solution in the left hind paw and saline i.p. Experimental groups consist of: 5-6. Rats received intraplantar

injection of 0.1 ml 0.5% carrageenan solution in the left hind paw and TFP (0.2 or 8 mg/kg) i.p. 7-8. ADX rats received intraplantar injection of 0.1 ml 0.5% carrageenan solution in the left hind paw and TFP (0.2 or 8 mg/kg) i.p. 9. Sham operated rats.

Statistical analysis

All results are reported as mean ± SEM. The unpaired Student's t-test was used for statistical evaluation of the results when two groups were compared. When more than two groups were compared, analysis of variance followed by tukey's test was used. Two way ANOVA was used to compare the effects of TFP on measured parameters, in control and ADX rats. A value of $p < 0.05$ was considered as significant.

Results

The effect of TFP on carrageenan-induced paw volume changes in intact and ADX groups

Carrageenan injection significantly increased the hind paw volume and tissue content of Evans blue dye, as indicators of inflammation, in ADX and control rats compared to normal groups (Data not shown). In addition, following carrageenan injection, paw volume significantly enhanced in ADX rats compared to control (T-test, P= 0.023) (**Figure 1**). As shown in figure 1, administration of different doses of TFP significantly decreased paw volume in both control group (ANOVA followed by tukey, all P<0.05) and ADX group (all P<0.01) animals. There was no significant difference between different doses of TFP on paw volume reduction in both control and ADX groups. TFP induced paw volume reduction was greater in ADX animals compared to control group (two-way ANOVA, P<0.05).

Tissue content of Evans blue dye

Tissue content of Evans blue dye was reduced effectively by different doses of TFP in control group (ANOVA followed by tukey, all P<0.05). In adrenalectomized rats, only TFP (8 mg/kg) reduced tissue content of Evans blue dye (P=0.03) (**Figure 2**).

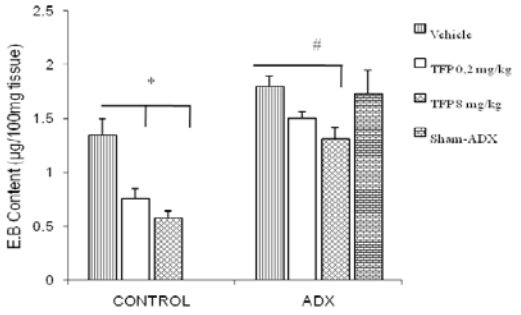

Figure 2. Effect of treatment with TFP on vascular permeability. Vascular permeability was evaluated by the accumulation of Evans blue dye in the left hind paw as described in methods. *: significant difference between TFP (both doses) with vehicle in control group (ANOVA followed by tukey, all P<0.05), #: significant difference between TFP (8mg/kg) with vehicle in ADX group (ANOVA followed by tukey, P= 0.03). TFP; Trifluoperazine, ADX; Adrenalectomized.

Discussion

The present study was designed to determine the effect of trifluoperazine, a conventional anti psychotic drug, on acute inflammation response induced by carrageenan, and possible role of Glucocorticoids in this effect. Our findings suggest that TFP can effectively inhibit carrageenan induced inflammatory edema and this anti-inflammatory effect does not need the adrenal activity, as evidenced by its effectiveness in ADX rats.

The anti-inflammatory effect of TFP was similar to the report of Beitner and Kim, though they used different inflammatory model (skin burn) [1, 2, 4]. Since some reports, following Marone et al. [12] described the role of calmodulin in inflammatory reaction; it is possible that the effect of TFP as calmodulin antagonists [2] may be involved in their anti-inflammatory effect. In addition, as it has been shown that the rate of accumulation of brain 5-HT and Noradrenalin (NA) were enhanced during carrageenan induced inflammation [13] it can postulated that the anti-inflammatory action of TFP is, at least partially, mediated by the inhibition of central dopaminergic and serotonergic systems. However, our results don't allow us to completely clarify the mechanisms of anti-edema effect of these

drugs. Further peripheral and central actions of these drugs are conceivable.

Since TFP could affect the adrenal gland activity and cortisol release, it could be assumed that its anti-inflammatory action of TFP needs the intact adrenal activity. However the result of our study does not support this theory and show that removal of adrenal glands could not influence the anti-inflammatory effect of TFP.

In ADX rats, the anti-inflammatory effect of TFP increased compared with control. HPA and their glucocorticoids have the natural anti-inflammatory effect and several lines of evidence indicate that neuroleptic drugs such as TFP have an inhibitory effect on HPA activity in different levels [5-7], so they have both pro-inflammatory and anti-inflammatory effects in intact rats. By removal of adrenal glands, the effect of drug on this system will be blocked. Therefore, their pro-inflammatory action may be restricted and their anti-inflammatory effect will be dominant.

In conclusion, the present study showed that TFP reduced acute carrageenan-induced inflammation in both intact and ADX rats. Further study to determine the relevance of this finding in human should be undertaken.

Acknowledgements

This experiment was supported by Rafsanjan University of Medical Sciences.

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