



Adrenergic Agonist Aided Activation of Adrenergic Anti Inflammatory Pathway for Inflammation Modulation

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ABSTRACT

The present study was performed to evaluate the anti-inflammatory activity of amphetamine. Inflammation in experimental animals (Wistar rats) was induced by injecting carrageenan and by insertion of cotton pellet. The above inflicting agents brought derangements in paw volume and biochemical parameters (SGPT, SGOT, ALP) which were statistically improved by our interest drug, amphetamine. This improvement took place may be due to activation of adrenergic anti-inflammatory pathway. Throwing light on this pathway/topic might lead to further new discoveries in the treatment of inflammation and various inflammatory disorders.

Keywords: Amphetamine, rats, adrenergic anti-inflammatory pathway.

INTRODUCTION

Inflammation comes from the Latin word '*inflammaré*' which 'means to set on fire'. Inflammation means, local response of living mammalian tissue to injury due to any injurious agents such as mechanical trauma, heat, radiation etc.

Five cardinal signs of inflammation are redness, swelling, heat, pain, loss of function.^{1,2}

Inflammation can lead to host of diseases like rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, retinitis, multiple sclerosis, psoriasis, atherosclerosis, hay fever, periodontitis, carcinoma etc.

The above is a classical example of inflammatory exaggeration and its sustanation, without apparent benefit, with harmful adverse, side effects. This thing can last throughout the life of the individual person.³

The use of anti-inflammatory drugs such as non steroidal anti-inflammatory drugs (aspirin, indomethacin, ibuprofen, ketorolac, diclofenac etc.) can reduce inflammation to a certain extent but not to zero.

These drugs work by inhibiting cyclo oxygenase pathway. But they do not reduce the levels of various mediators of inflammation such as cytokines, leukotrienes etc. and so do not fully control inflammation.^{4,5}

Stimulation of adrenergic anti-inflammatory pathway find a novel way to cure inflammation and associated inflammatory diseases to a greater extent, with broad anti-inflammatory spectrum.

Use of adrenergic agonist to activate adrenergic receptor stimulate adrenergic anti-inflammatory pathway.

This will reduce levels of various mediators of inflammation and inhibit inflammation.⁶ Hence, throwing light on this pathway/topic might lead to further new discoveries in the treatment of inflammation and various

inflammatory disorders.

Thats why the current research study was carried out to evaluate the anti-inflammatory activity of amphetamine.

MATERIALS AND METHODS

Experimental Animals

Wistar rats of either sex were housed at 25°C ± 5°C, relative humidity 50 ± 5 % in a well-ventilated, environment controlled animal house under 12:12 hour light dark cycle. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional Animals Ethics Committee approved the experimental protocol (SDCP/IAEC-04/2014-2015).

Groupings and Drugs

For carrageenan induced paw edema model, Wistar rats were divided into 5 groups of 6 animals each. Group 1 consisted of normal animals. Group 2 consisted of untreated diseased animals. Group 3 consisted of diseased animals treated with low dose of amphetamine (3.08 mg/kg p.o). Group 4 consisted of diseased animals treated with high dose of amphetamine (6.16 mg/kg p.o). Group 5 consisted of diseased animals treated with standard drug diclofenac sodium (10 mg/kg p.o). The drug solutions were prepared using distilled water.

For cotton pellet induced granuloma model, Wistar rats were divided into 4 groups of 6 animals each. Group 1 consisted of normal animals. Group 2 consisted of diseased animals treated with low dose of amphetamine (3.08 mg/kg p.o). Group 3 consisted of diseased animals treated with high dose of amphetamine (6.16 mg/kg p.o). Group 4 consisted of diseased animals treated with standard drug diclofenac sodium (10 mg/kg p.o). The drug solutions were prepared using distilled water. Oral feeding was carried out with the help of feeding needle.



Amphetamine (Adderall, Shire Pharmaceuticals), diclofenac sodium (Voveran, Novartis) and rest of the chemicals/reagents used were of analytical/laboratory pure grade. The dosage were determined from human dose by using human dose to animal dose conversion formula.⁷

Carrageenan Induced Paw Edema Model^{8,9}

Wistar rats were injected with 0.1ml carrageenan (1%) in normal saline into sub plantar area of right hand paw. The drugs (low, high doses of amphetamine and diclofenac sodium) were given orally 1 hour prior to carrageenan injection.

The paw volume was measured by using plethysmometer. The mean change in paw volume was calculated at 0, 1, 2, 3 and 4 hours time interval. After 4 hours, the rats were sacrificed under anaesthesia. Blood was collected accordingly and serum was separated by centrifugation. SGPT (serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase) and ALP (alkaline phosphate) estimations were done using the serum (with kits).

Cotton Pellet Induced Granuloma Method¹⁰

4 cotton pellets (10mg) were implanted on either side (2 on each side) of the ventral region of rats. The animals were administered with vehicle, standard drug (diclofenac sodium), low and high doses of the test drug (amphetamine), 30 minutes prior to the cotton pellet implantation.

After 30 minutes of first dosing, 10±5mg of cotton pellet were inserted. Treatment period were for seven consecutive days accordingly. On the eighth day, the cotton pellets were removed surgically. Pellets were separated from extraneous tissue and weighed immediately for wet weight. Then they were dried at 60°C until the weight became constant.

The animals were sacrificed and blood was collected accordingly. The serum was separated by using centrifugation and used for the estimation of SGPT, SGOT and ALP (with kits). Also the difference between dry weight from the wet weight were estimated.

Statistical Analysis

Statistical analysis was done using Graph Pad Prism version 4 software (Graph Pad Inc, USA). ANOVA followed by Turkey-Kramer Multiple Comparison test (compare all) was applied. Data presented as MEAN±SEM. Confidence level was taken as 95%.

RESULTS

Carrageenan Induced Paw Edema Model - Paw Volume Parameter

Carrageenan brought extremely significant increase in paw edema when compared with normal animals. Both low and high doses of amphetamine brought extremely significant reduction in paw edema at 2, 3 and 4 hours

when compared with diseased animals. Similar result was seen with standard drug, diclofenac sodium treated animals. See Table 1.

Carrageenan Induced Paw Edema Model - Biochemical Estimations Parameters

Carrageenan brought extremely significant increase in SGPT, SGOT, ALP levels when compared with normal animals. Both low and high doses of amphetamine brought significant decrease in elevated SGPT, SGOT, ALP levels when compared with diseased animals. Similar result was seen with standard drug, diclofenac sodium treated animals. See Table 2.

Cotton Pellet Induced Granuloma Method - Granuloma Weight Parameter

Both low and high doses of amphetamine showed extremely significant decrease in granuloma weight when compared with normal animals. Similar result was seen with standard drug, diclofenac sodium treated animals. See Table 3.

Cotton Pellet Induced Granuloma Method - Biochemical Estimations Parameters

Like normal animals, low and high doses of amphetamine showed extremely significant improvement in SGPT level. Like normal animals, low dose of amphetamine showed significant improvement in SGOT level. See Table 4.

DISCUSSION

The current study was undertaken to evaluate the anti-inflammatory activity of amphetamine by using wistar rat animal models of carrageenan induced paw edema and cotton pellet induced granuloma.

Amphetamine showed statistically significant anti inflammatory activity.

Amphetamine is a phenethylamine class of drug acting by binding both on alpha and beta adrenergic receptor. It is a long acting (4 to 6 hours), orally active MAO resistant drug.¹¹

In carrageenan induced paw edema model, edema formation in rats is due to carrageenan, which is a biphasic event. 1st phase of edema is due to release of histamine, serotonin etc. 2nd phase is due to release of prostaglandins, proteases, lysosomal enzymes etc.¹²

Cotton pellet induced granuloma method causes damage by targeting and increasing levels of various mediators of inflammation such as lysosomal enzymes etc.^{13,14}

Carrageenan and cotton pellet brought derangements in paw volume and biochemical parameters (SGPT, SGOT, ALP). There levels were statistically improved by amphetamine.

Beta two adrenergic receptor agonist (such as amphetamine etc.) binds to and activate beta two adrenergic receptor on the surface of the cells of various mediators of inflammation such as CD4, monocyte,



macrophage etc. This inhibit, activation of expression of inflammatory genes and pro inflammatory cytokines, inter leukine 2, interferon gamma etc.⁶

CONCLUSION

Amphetamine showed anti inflammatory activity. It improved inflammation related various biochemical

aberrations. Scope is there for future studies on adrenergic anti inflammatory pathway and related drug discovery-development.

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Table 1: Effect of Amphetamine on Paw Volume in Carrageenan Induced Paw Edema in Rats

Time (hour)	Normal Group	Diseased Group	Standard Drug Treated Group	Low Dose Amphetamine Treated Group	High Dose Amphetamine Treated Group
0	2.4 ± 0.3	2.5 ± 0.1	2.5 ± 0.2	2.6 ± 0.2	2.5 ± 0.3
1	2.5 ± 0.1	5.1 ± 0.2***	3.2 ± 0.3###	4.3 ± 0.1	3.7 ± 0.2
2	2.5 ± 0.2	7.0 ± 0.2***	3.1 ± 0.1###	4.1 ± 0.1###	3.4 ± 0.3###
3	2.5 ± 0.2	7.6 ± 0.3***	2.9 ± 0.3###	3.8 ± 0.3###	3.1 ± 0.2###
4	2.5 ± 0.3	8.2 ± 0.1***	2.8 ± 0.1###	3.6 ± 0.1###	2.9 ± 0.2###

All values are MEAN±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 when compared with normal, #p<0.05, ###p<0.01, ### p<0.001 when compared with diseased.

Table 2: Effect of Amphetamine on Biochemical Parameters in Carrageenan Induced Paw Edema in Rats

Groups	SGPT (U/L)	SGOT (U/L)	ALP (U/L)
Normal	87 ± 1.5	32 ± 2.3	35 ± 1.4
Diseased	170 ± 1.7***	77 ± 1.9***	84 ± 1.6***
Standard drug treated	92 ± 3.2###	37 ± 2.3###	39 ± 2.7###
Low dose amphetamine treated	125 ± 2.9###	57 ± 1.7###	53 ± 3.1###
High dose amphetamine treated	106 ± 3.1###	42 ± 2.3###	47 ± 1.9###

All values are MEAN±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 when compared with normal, #p<0.05, ###p<0.01, ### p<0.001 when compared with diseased.

Table 3: Effect of Amphetamine on Granuloma Weight in Cotton Pellet Induced Granuloma Method in Rats

Groups	Granuloma Weight	
	Wet Weight	Dry Weight
Normal	289 ± 6.4	151 ± 5.9
Standard drug treated	123 ± 5.6***	74 ± 5.3***
Low dose amphetamine treated	232 ± 4.5***	118 ± 5.1***
High dose amphetamine treated	163 ± 5.3***	97 ± 4.9***

All values are MEAN±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 when compared with normal.

Table 4: Effect of Amphetamine on Biochemical Parameters in Cotton Pellet Induced Granuloma Method in Rats

Groups	SGPT (U/L)	SGOT (U/L)	ALP (U/L)
Normal	81 ± 4.2	33 ± 3.9	49 ± 5.2
Standard drug treated	89 ± 4.8	39 ± 4.2	60 ± 3.1
Low dose amphetamine treated	123 ± 3.1***	51 ± 3.2*	68 ± 3.2
High dose amphetamine treated	111 ± 4.2***	42 ± 4.2	61 ± 3.2

All values are MEAN±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 when compared with normal.



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