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Study of Antiparkinson's Activity of Plain and Niosomal Pentoxifylline

AARTI JAGTAP* AND DEEPALI INAMDAR
Department of Pharmacology, Bombay College of Pharmacy
Kalina, Santacruz (E), Mumbai-400 098

The objective of the study was to investigate the antiparkinson's activity of plain and niosomal pentoxifylline. Pentoxifylline was entrapped in niosomes by lipid layer hydration method using Span 60, cholesterol and dicetyl phosphate (10 mg : 10 mg : 2.5 mg).The antiparkinson's activity was investigated on cataleptic score in haloperidol-induced catalepsy model, and locomotor activity in reserpine antagonism and adenosine antagonism at different doses of plain pentoxifylline (40, 80 mg/kg) and niosomal pentoxifylline (5, 10,15 mg/kg). Plain pentoxifylline (80 mg/kg) and niosomal pentoxifylline (5, 10, 15 mg/kg) gave significant improvement in haloperidol-induced catalepsy. Plain pentoxifylline (80 mg/kg) and niosomal pentoxifylline (10, 15 mg/kg) showed significant increase in locomotor avtivity of reserpinetreated mice and adenosine-treated rats. Results suggest that pentoxifylline may prove to be an effective drug in Parkinson's treatment as an adjuvant.

Parkinson's disease is an age-related disease arising from the degeneration of dopaminergic nigro-striatal neurons of the basal ganglia, which results in bradykinesia, tremor and rigidity[1]. The current therapy for this disorder is restricted to symptomatic relief, since no agent capable of inhibiting the neuronal degeneration has yet been found. The most commonly used agents such as L-dopa, muscurinic acetylcholine receptor antagonists, monoamino oxidase inhibitors, benserazide and all of these show unacceptable side-effects[1]. The potential of novel dopamine receptor agonists has recently been reviewed in the treatment of Parkinson's disease[20].

Reported studies suggest that there is a functional interaction between adenosine and dopamine receptors through adenosine receptors[1,2]. Activation of adenosine A_{2A} receptors reduces the affinity of the ligands for agonist binding sites on D_2 receptors in the striatal membranes[3]. The potential efficacy of adenosine receptor antagonists for the treatment of Parkinson's disease has been shown in humans using a non-selective adenosine receptor antagonist, theophylline which improved mental and motor scores of the disease[4]. Intraperitoneal administration of pentoxifylline (PTX), a homologue of theophylline is reported to increase the rate of tryptophan and tyrosine hydroxylation, norepinephrine turnover and dopamine release by rat brain *in vivo*[5].

But PTX has a short half-life of 0.4-0.8 h[6]. Encapsulation of a drug in vesicular structures such as niosomes may prolong the existence of the drug in the systemic circulation and enhance penetration into target tissue[7].

The present study was aimed at the evaluation antiparkinson's activity of PTX in plain and niosomal form using animal models like catalepsy, reserpine antagonism and adenosine antagonism. Catalepsy model was used as the treatment with neuroleptics (haloperidol) produce synptoms similar to Parkinsonism due to excessive blockade of dopamine receptors, while reserpine depletes brain catecholamine stores and the effect can be antagonized by dopamine agonist. Adenosine decreases the locomotor activity and suppresses postsynaptic dopamine receptor activation and a dopamine

*For correspondence

agonist acting through adenosine receptors may show adenosine antagonism[12,14,19].

MATERIALS AND METHODS

Pentoxifylline, haloperidol were gift samples obtained from Sun Pharmaceuticals Ltd., Mumbai, theophylline was a gift sample obtained from Cipla, Mumbai and reserpine was a gift sample obtained from Cyber, Mumbai. Cholesterol and adenosine were purchased from Loba Chemie, Span 60, dicetyl phosphate and chloroform were purchased from Koch Laboratories, Sigma, US, Hi Media, respectively. All animal experiments are approved by the Ethics Committee of the institution.

Preparation of niosomes of PTX:

Niosomes were prepared by lipid layer hydration method[8,9]. Briefly, Span 60, cholesterol, and dicetyl phosphate (10 mg : 10 mg : 2.5 mg) were dissolved in chloroform and the solvent was evaporated using rotary flash evaporator under reduced pressure at 40-50°. The lipid film was hydrated by PTX solution (10 mg/ml) in phosphate buffered saline (PBS; 10 ml, pH 7.4) at 40-50°, followed by gentle shaking. The mixture was shaken on a horizontal shaker bath for 2 h.

The unentrapped drug was separated from the entrapped drug by centrifugation. The pellet of niosomes was resuspended in PBS and sonicated for 2 min. The size of niosomes was determined using a Malvern mastersizer M-S3 and the characteristics were observed using a light microscope. The drug entrapment was determined from the amount of drug remaining in the supernatant using spectrophotometer (Shimadzu 160A UV) at 274 nm.

Catalepsy model:

The experimental groups consisted 11 groups of 6 rats each. Catalepsy was induced in adult Wistar rats of either sex (150-200 g) by i.p. injection of haloperidol (1.5 mg/kg). Catatonia was observed at 5, 15, 30, 45, 60, 90, 120, 150, 180 min after haloperidol injection. To the treatment groups theophylline (2.5, 5 and 10 mg/kg) or plain PTX (40 and 80 mg/kg) or empty niosomes or niosomal PTX (5, 10 and 15 mg/kg) were injected i.p. 30 min before haloperidol injection. One group served as the negative control which received only vehicle. Catalepsy of an individual rat was measured in a stepwise manner by a scoring method as described by Kulkarni[10]. The data was analyzed using Mann-Whitney U test. A value of $P<0.05$ was considered statistically significant[11].

Reserpine antagonism:

Swiss albino mice (20-25 g) were divided into 11 groups of 6 animals each. Mice from all the groups were injected with reserpine (2 mg/kg) i.p. and the effect of drug on locomotor activity was tested 24 h later. Vehicle (PBS), theophylline (2.5, 5 and 10 mg/kg), plain PTX (40 and 80 mg/kg), empty niosomes, niosomal PTX (5, 10 and 15 mg/kg) were injected i.p to different groups of mice 30 min prior to the observation. One group served as the negative control which received only vehicle. The animals were placed individually on the floor of an actophotometer. Horizontal movement was recorded for 10 min. Locomotor activity of drug treated groups was compared with untreated control (vehicle) using ANOVA[12].

Adenosine antagonism:

Wistar rats of either sex were divided into 11 groups of 6 animals each. Rats were injected with adenosine (50 mg/kg) i.p and the effect of drug on locomotor activity was tested. Vehicle (PBS), theophylline (2.5, 5 and 10 mg/kg), plain PTX (40 and 80 mg/kg), empty niosomes, niosomal PTX (5, 10 and 15 mg/kg) were injected i.p. to different groups of mice 30 min after adenosine injection. One group served as the negative control which received only vehicle. The locomotor activity was recorded 30 min later on actophotometer for 10 min. The drug treated group was compared with untreated control (vehicle) using ANOVA[12].

RESULTS

The entrapment efficiency of niosomes of PTX was found to be 9.26%. Niosomes were unilamellar and multilamellar with mean particle size of 4.96 μm and appeared spherical under light microscope.

Preliminary studies with plain PTX (10, 20 and 40 mg/kg) on haloperidol treated rats showed that plain PTX (10 and 20 mg/kg) did not show any improvement in catalepsy score. (data not shown). Hence, plain PTX (40 and 80 mg/kg) dose was selected as plain PTX (160 mg/kg) produced toxicity and animals died of convulsions.

Due to high viscosity of the formulation the niosomal PTX (20 and 40 mg/kg) produced abdominal stretching in rats. So, lower doses of niosomal PTX (5, 10 and 15 mg/kg) were used for the study along with standard drug, theophylline (2.5, 5 and 10 mg/kg).

Haloperidol at 1.5 mg/kg produced catalepsy in rats and the onset was seen at 15 min, the maximum catatonia being reached at 120 min. Pretreatment with theophylline, PTX (plain and niosomal) dose-dependently reversed haloperidol-induced catalepsy. Empty niosomes did not show any effect while standard drug theophylline (5 and 10 mg/kg), plain PTX (80 mg/kg), and niosomal PTX (5, 10 and 15 mg/kg) showed significant reversal of catatonia. The effect being comparable to theophylline. The onset of catalepsy in treatment groups was delayed and maximum catatonic score was also reduced (Table 1).

Plain PTX (40 mg/kg), empty niosomes, niosomal PTX (5 mg/kg) did not show any significant increase in the locomotor activity of reserpine pretreated mice and adenosine treated rats but plain PTX (80 mg/kg), niosomal PTX (10 and 15 mg/kg) showed significant increase in locomotor activity as the standard theophylline (5 and 10 mg/kg) (Fig. 1 and 2).

TABLE 1: EFFECTS OF VARIOUS TREATMENTS ON CATALEPSY SCORE OF HALOPERIDOL PRETREATED RATS

Treatment	Dose (mg/kg)	Catalepsy Score at different Time Intervals (min) after Haloperidol Treatment							
		15	30	45	60	90	120	150	180
Control (PBS)	2 ml/rat	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
PBS + Haloperidol	1.5	1.00 ± 0.62	1.75 ± 1.09	1.917 ± 1.28	2.5 ± 1.14	2.916 ± 0.59	3.5 ± 0.00	3.5 ± 0.00	3.5 ± 0.00
Theophylline*	2.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.083 ± 0.2	0.583 ± 0.58	1.25 ± 0.82	2.33 ± 1.03	3.33 ± 0.41
Theophylline*	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.41	0.667 ± 0.88	1.25 ± 1.5
Theophylline*	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.417 ± 0.8	1.00 ± 1.14
Plain PTX	40	0.00 ± 0.00	0.167 ± 0.41	0.33 ± 0.52	1.33 ± 1.51	1.917 ± 1.77	2.583 ± 1.43	3.25 ± 0.61	3.33 ± 0.4
Plain PTX*	80	0.00 ± 0.00	0.00 ± 0.00	0.1 ± 0.22	0.75 ± 0.88	1.5 ± 1.38	1.917 ± 1.74	1.917 ± 1.77	2.417 ± 1.69
Empty niosomes	2ml/rat	0.083 ± 0.2	1.00 ± 1.28	1.917 ± 1.46	2.25 ± 1.57	2.917 ± 1.2	3.25 ± 0.61	3.25 ± 0.61	3.5 ± 0.00
Niosomal PTX*	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.42	1.417 ± 0.92	2.25 ± 1.29	2.33 ± 1.17	2.667 ± 0.98
Niosomal PTX*	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.3 ± 0.27	1.8 ± 1.18	1.8 ± 1.18	2.4 ± 1.2
Niosomal PTX*	15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.5 ± 0.32	1.167 ± 0.93	2.0 ± 0.95

Each value is expressed as mean ± s.d. (n=6). *indicates the value statistically significant at $P<0.05$ as compared to control by Mann Whitney U-test.

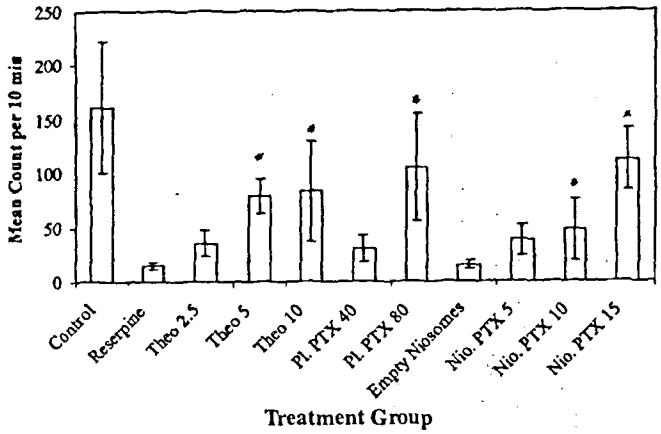

Fig. 1: Effect of various treatments on locomotor activity (Mean counts/10 min) of reserpine treated mice

PBS (0.5 ml/mice) in control group, theophylline, empty niosomes (0.5 ml/mice), pentoxifylline (plain and niosomal) was injected 30 min prior to observation. Each value represents mean ± s.d. (n=6) of locomotor activity in rats. *P<0.05 is considered to be statistically significant as compared to control by ANOVA

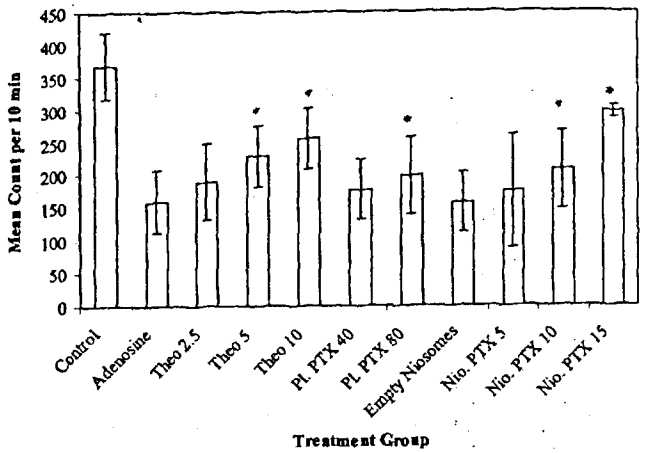

Fig. 2: Effect of various treatments on locomotor activity (Mean counts/10 min) of adenosine treated rats

PBS (2 ml/rat) in control group, theophylline, empty niosomes (2 ml/rat), pentoxifylline (plain and niosomal) was injected 30 min prior to observation. Each value represents mean ± s.d. (n=6) of locomotor activity in rats. *P<0.05 is considered to be statistically significant as compared to control by ANOVA

DISCUSSION

Catalepsy has been defined as an inability to correct an imposed abnormal posture while maintaining the righting reflex[11]. It has been ascribed to decreased brain levels of dopamine[11]. Haloperidol-induced catalepsy is an animal model for Parkinson's disease as neuroleptics like haloperidol produce Parkinson's like symptom in rodents by blockade of striatal dopamine receptors[2].

PTX has shown to decrease the cataleptic score dose dependently delaying the onset of catatonia. The action of PTX may be due to the increased brain level of dopamine. It has been reported that intraperitoneal administration of PTX increases the cerebral levels of amine transmitters by increasing the cerebral adrenalin turnover and dopamine release. The effect of PTX is reported to be more potent than theophylline in bringing about changes in transmitter uptake and release[5]. Our study shows that PTX in higher doses produces anticatatonic effect comparable to that produced by theophylline.

Reserpine induces depletion of central catecholamine stores. It depletes noradrenaline, serotonin and dopamine causing sedation in mice[12]. The prolonged reserpinization leads to adaptational changes i.e., increased dopamine receptor sensitivity. When receptors are rendered supersensitive, behavioural stimulation appears to be induced by dopamine receptor stimulation[14]. This can be acheived by a dopamine agonist[12,13]. PTX has shown to increase the locomotor activity in prolonged reserpinized mice. Thus PTX may have it's effect by acting on the sensitized dopamine receptors and antagonizing the effect of reserpine.

The co-localization of adenosine A_2 receptors and dopamine D_2 receptors in the striatum indicated that this might be the site where the regulation of dopamine receptors could take place[15]. It is reported that A_2 receptors can suppress postsynaptic dopamine receptor activation[3]. Adenosine agonist are known to reduce locomotor activity[16]. Adenosine antagonist reverses catalepsy and synergizes with dopamine agonist under reserpine-induced dopamine depletion and haloperidol-induced dopamine receptor blockade[1,17]. In the present study PTX has reversed the effect of adenosine, it has increased the locomotor activity in rats after adenosine treatment.

PTX probably lowers the cataleptic scores by it's action on dopamine receptors as a dopamine agonist and increases the dopamine levels in the brain. Thus, the ability of PTX to increase dopamine levels in the brain in reserpinized mice (in presence of supersensitive dopamine receptors) may be modulated through adenosine receptors.

Niosomes of PTX produces the same effect as plain PTX in a much lower dose. PTX is reported to have a low brian-barrier permeability[19] and niosomal formulation of methotrexate is shown to increase the brain uptake of methotrexate[7]. The improved effect of niosomal PTX may be due to better distribution of PTX into the brain. Niosomal PTX may be giving a sustained release of the drug, localization into the brain and decreased metabolism of PTX.

In conclusion, results suggest that PTX possesses an antiparkinson's activity. Additional studies to pinpoint the details of the mechanism of PTX are required to be done. Studies should to carried out using MPTP model and specific adenosine receptor agonist should be used to study the effect of PTX on adenosine agonist-induced catalepsy.

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