

SPARTEINE INHIBITION OF NON CONVULSIVE SEIZURES AND MEMORY PRESERVATION IN WISTAR RATS

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ABSTRACT

Quinolizidinic alkaloids sparteine and lupanine are the most abundant alkaloids in wild *Lupinus* species (0.5%) as well as in *Lupinus albus* and *Lupinus angustifolius* (0.015%). A hypoglycaemia inducing effect of these alkaloids obtained from wild *Lupinus* has been reported to occur following their oral administration to rats; however, their seizures inhibitory effects have not yet been demonstrated. Therefore, we performed seizure inhibition tests in the following groups: I. 0.9% isotonic solution; II. 40 mg/kg; III. 80 mg/kg; IV. 160 mg/kg of spartein sulfate; and V. 30 mg/kg Carbamazepine orally administered through a metallic cannulae during seven days. On the eighth day Maximal electrostimulation seizure inducing test (MES) and subcutaneously injected pentame thylenetetrazole seizure inducing test (METsc) were performed. Also, these groups received both drugs during the 15th and 30th day periods to study sparteine toxicological effect through the: 1. measurement of blood biochemical metabolites concentrations; 2. morphologically, after performing tissue fixation through intracardiac perfusion with 0.9% NaCl in 0.15M phosphate buffer pH 7.4 containing 4% formaldehyde; and 3. behaviorally, through measurement of spatial learning and memory Morris water maze test from the 31st to the 34th day of sparteine and carbamazepine oral administration. We found an average of 100% protection with SPT in groups IIIa and IIIb was formerly recorded in comparison to 65% (Va) and 55% (Vb). We found no toxicity in groups II, III and V; however, weight loss increased at 80 (III) and mortality at 160 mg/kg. (IV). All water maze parameters for groups II, III, IV and V were normal. In conclusion, sparteine inhibited non convulsive seizures with lesser efficacy (in contrast to discussion?). Under the above conditions SPT was found suitable to become a non convulsive seizures useful drug with greater efficacy than carbamazepine) than carbamazepine. Sparteine displayed no toxicity at 40 and 80 mg/kg in liver, heart and kidneys during the tested periods. Correspondingly, we found that spartein did not modify short-term memory or spatial learning.

KEY WORDS

Lupinus exaltatus, alkaloids, spartein anticonvulsants, morris, toxicity

INTRODUCTION

Sparteine is an heterobicyclononane plant alkaloid (Binnig, 1974; Wynk, 1993) able to display antiarrhythmic activity in several models of cardiac arrhythmia. In isolated guinea pig preparations, sparteine prolongs the action potential duration, and reduces the conduction velocity as well as the maximum rise rate of action potentials (Senges and Ehe, 1973). It also reduces the incidence of ventricular tachycardia and fibrillation evoked by aconitine in rats and ischaemia in dogs (Raschack, 1974). A dose-dependent reduction in heart rate and blood pressure occur over the dose range 1-64 $\mu\text{mol/kg/min}$ of sparteine. The P-R and Q-aT intervals of the electrocardiogram (EKG) and the fibrillation incidence become prolonged when spartein is administered at the EC₅₀ of dose of 110 and 230 μM , respectively. Spartein also decreases the Na⁺ current in a concentration-dependent manner and induces a hyperpolarizing shift of 8 mV for Na⁺ channel inactivation. On the other hand, it has been reported that administration of spartein results in a concentration-dependent blockage of the sustained plateau K⁺ current, and an increase in the rate of decay of the transient outward K⁺ current. This observation suggests that sparteine possess Na⁺ and K⁺ channel blocking properties which may account for their antiarrhythmic actions against electrical and ischaemic arrhythmias (Pugsley *et al.* 1995; Honerjager, P., 1986). Other smooth muscles have shown a dose dependent decrease in K⁺ efflux and inhibition of histamine release with the administration of the K⁺ channel blockers 4-aminopyridine, quinine, sparteine (Beauvais, F., 1992). During clinical trials a phenytoin drug was shown to control partial and generalized seizures. This antiarrhythmic drug, was able to control maximal electroshock induced seizures but not pentylene tetrazol induced seizures by blocking sodium channels, and inhibiting of glutamate and aspartate release (Pellock, J.M.; Finkel, M.J., 1984). Spartein may exhibit anticonvulsant properties since as the anticonvulsant phenytoin have been shown to suppress abnormal action potentials during the cardiac arrhythmia inhibition

process. Therefore, in this study we evaluated the anticonvulsant properties of spartein in an Epileptiform seizures Experimental Models (MECES).

MATERIALS AND METHODS

DRUGS

Pentamethylenetetrazole, spartein sulfate (SPT), carbamazepine (CBZ) propylene-glycol-ethyl alcohol-water. All drugs were purchased from Sigma Co. (St. Louis, USA).

ANIMALS

Male Wistar rats (200 to 350 g) were obtained from the Bioterium of the Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara. Animals were freely fed with standard animal food, and had free access to water. Room temperature, humidity and light/dark cycle were automatically maintained at 22°C, 50% and 12 hr, respectively.

EXPERIMENTAL GROUPS

Male and female rats were assigned to one of five experimental groups of 20 rats each, and were treated for seven consecutive days with the following compound: 0.9% isotonic solution (Ia, Ib), 40 mg/kg (IIa, IIb), 80 mg/kg (IIIa, IIIb), 160 mg/kg (IVa, IVb) of spartein sulfate and 30 mg/kg Carbamazepine (Va and Vb). The experimental compounds were administered through a metallic cannulae. On the eighth day, Maximal electrostimulation seizure inducing test (MES) and subcutaneously injected pentamethylenetetrazole, seizure inducing test (METsc) were performed. The antiepileptic drugs were administered at 7:00 a.m. and 7:00 p.m. and the dosage was adjusted in accordance to body weight and type. After performing the MECES test, the anticonvulsant drugs and experimental compounds were administered daily for eight additional days. On days 15 and 30, rats were subdivided for water maze Morris test, blood sampling and cardiac perfusion with fixative performed.

MAXIMAL ELECTROSTIMULATION SEIZURE INDUCING TEST (MES TEST)

Following Dr Woodbury design, an electrostimulator was built in our laboratory. Briefly, a 200 mA, 115V alternating current electrical pulse lasting for 0.2 s through biauricular saline moistened electrodes was applied. Animals showing a hind limb tonic extensor reflex after the stimulus, which is the main visible feature of this test, were classified as 'positive' [MES (+)]. Parameters recorded for MES (+) animals were: (a) latency (time elapsed between the electrical stimulation and the presentation of the extensor reflex); (b) duration of the extensor reflex; (c) recovery time (time for the animal to be on the orthostatic position again after presenting the extensor reflex), and ambulatory period (time elapsed since ortostatism recovery to initial ambulatory steps). Rats refractory to present the reflex were classified as

'negative' [MES (-)]. The efficacy of an anticonvulsive drug is measured through the inhibition of the hind limb extensor reflex (Woodbury, D.M., 1952).

PENTAMETHYLENETETRAZOLE SEIZURE INDUCING TEST (METSSC)

Pentamethylenetetrazole for METsc was injected subcutaneously in a loose fold of the dorsal skin of rats at a dose of 70 mg/kg (CD99) in a volume of approximately 0.1 mL of 0.89% saline solution. Each animal was placed in isolation cages and observed for the next 60 min for the presence or absence of an episode of clonic spasm persisting for at least 5 seconds. Elapsed time from METsc injection until occurrence of clonic spasms was measured (latency). Animals presenting convulsions were classified as Metsc (+) whereas non-convulsing animals were classified as Metsc (-). Following drug treatment to inhibit clonic spasm appearance of Metsc test induced seizures, an anticonvulsive activity against non-convulsive epilepsies in human have been proven and reported elsewhere (White, H.S. 1997).

MORRIS WATER MAZE (MWM)

The MWM test was performed daily during an 8 days period, when rats reached 50 days of age; stage at which all the animals began a commercial diet feeding regimen. The testing room used (6 x 4 m) was devoid of windows, sound proofed, and lit with diffuse and attenuated white fluorescent light. A black plastic circular tank (170 cm in diameter, 37 cm deep), was filled with warm water (23 ± 3°C) to a depth of 25 cm. The tank was divided into a North, South, East and West imaginary quadrants. At the centre of a randomly selected quadrant, a fixed cylindrical black platform (24 x 10 cm) remained hidden throughout the study. The upper limit of the platform remained 1 cm below water surface and was therefore invisible to the rat. A neutral visual environment was created by placing a beige curtain around the pool. The extra maze spatial cues used to help rats to locate the platform consisted of four distinct geometrical figures (25 cm x 40 cm) placed within each quadrant, 1.50 m above the water surface. The experiments were recorded with a VCR Panasonic video camera connected to a TV. Experimenters were kept out the visual field of the rats both while they were swimming as well as while they remained on the platform. Experimental results were manually extracted from videocassette images without experimenter's knowledge of the regime under which the rats had been maintained. On the first day of the MWM test, a habituation session was performed without recording it. In this session, the rats were deposited into the water in the circular tank facing the wall of the pool, and allowed to swim for a maximum of 90 s in the absence of the escape platform. The session consisted of four consecutive 90 s periods with a 10-12 min interval from the end of one period to the beginning of another. Each of the four intervals was initiated after placing each rat within the pool at a different quadrant, the order of which was randomly

determined (no quadrant being repeated). Once starting from the chosen quadrant each rat was sequentially deposited in the other three quadrants. After the initial habituation session, the same protocol was followed in the trials that were carried out over the following seven days, in which the rats were required to locate a hidden platform. Once the rat found the platform, it was allowed to remain there for 10 sec before being returned to their holding cage where it was kept warm with an incandescent lamp. If the rat failed to find the platform within the given time period, it was manually guided toward the platform and allowed to remain there for 10 sec. Rats performance in reaching their goal was measured in terms of the elapsed time to find the hidden platform (latency time), the swam distance, the time spent in the quadrant with the platform, and the navigation speed. After finishing the trial, animals were returned to their home cages. Fourteen days after the last trial, a memory retention status evaluation was performed without the platform, allowing rats to swim for a single 60 sec period, registering the time spent in the quadrant where the platform was originally located. All tests were video-recorded and analysed by an independent observer who knew nothing about the group assignment. A connected video camera to a television monitor was used for recording (Morris, R., 1984).

GLUCOSE, CHOLESTEROL AND TRIGLYCERIDES

Plasma glucose, triglycerides and cholesterol concentrations were determined by means of the Glucose oxidase and Peroxidase, cholesterol esterase and cholesterol oxidase and peroxidase and glicerolkinase and gliceraldehyde-3-phosphate-oxidase based methods (Biosystems).

STATISTICAL ANALYSES

Statistical analyses of the individual differences in (a) latency, (b) duration of the extensor reflex, (c) recovery time, and ambulatory period of the anticonvulsive tests and the differences in memory, biochemical tissue markers concentrations were carried out with analysis of variance and student's *t*-test. Values were generally expressed as Means \pm SEM; *P*-values < 0.05 were considered to be significant (Downie N.M., 1973).

RESULTS AND DISCUSSION

Administration of SPT at 40 mg/kg resulted in 100% seizure inhibition (groups IIa and IIb) as compared to 65% (Va) and 55% (Vb) in animals administered CBZ (Table 1).

Table 1. Percentage of seizures inhibition

Subgroup	Percentage	
CBZ/PTZ males	65%	(13/20)
CBZ/PTZ females	55%	(11/20)
ESP/PTZ machos	100%	(0/20)
ESP/PTZ hembras	100%	(0/20)

n = 20/group

The average duration of the extensor reflex stage is shown in Table 2. SPT had no effect on the extensor reflex while CBZ had a small impact on duration indicating a partial protection against seizures. SPT had no toxicity effects at the 40 or 80 mg/kg level (groups II, and III) but caused weight loss and mortality of ~ 40% at the 160 mg/kg level

Table 2. Average duration of each reflex stage

Subgroup	Latency	Reflex	Ortostat.	Recovery
CBZ/PTZ males	03:19 \pm 00:09	00:18 \pm 00:08	00:50 \pm 00:10	00:27 \pm 12.25
CBZ/PTZ females	03:17 \pm 00:12	00:19 \pm 00:12	00:57 \pm 00:10	00:22 \pm 00.14
ESP/PTZ machos	*	*	*	*
ESP/PTZ hembras	*	*	*	*

(IVA AND IVB)

SPT administered at doses of 40 and 80 mg/Kg displayed no toxicity in liver, heart and kidneys during the tested periods. No toxicity effects were observed after CBZ administration. Urea, creatinine, triglycerides, TGO and TGP blood serum levels were normal in animals treated with 40 mg/kg SPT, 80 mg/kg SPT or 30 mg CBZ. However, blood glucose levels were low in animals treated with 80 and 160 mg/kg of SPT (Table 3). A normal morphological pattern was observed in liver, kidney, heart, brain and muscle in groups receiving 40 and 80 mg/kg SPT or 30 mg.

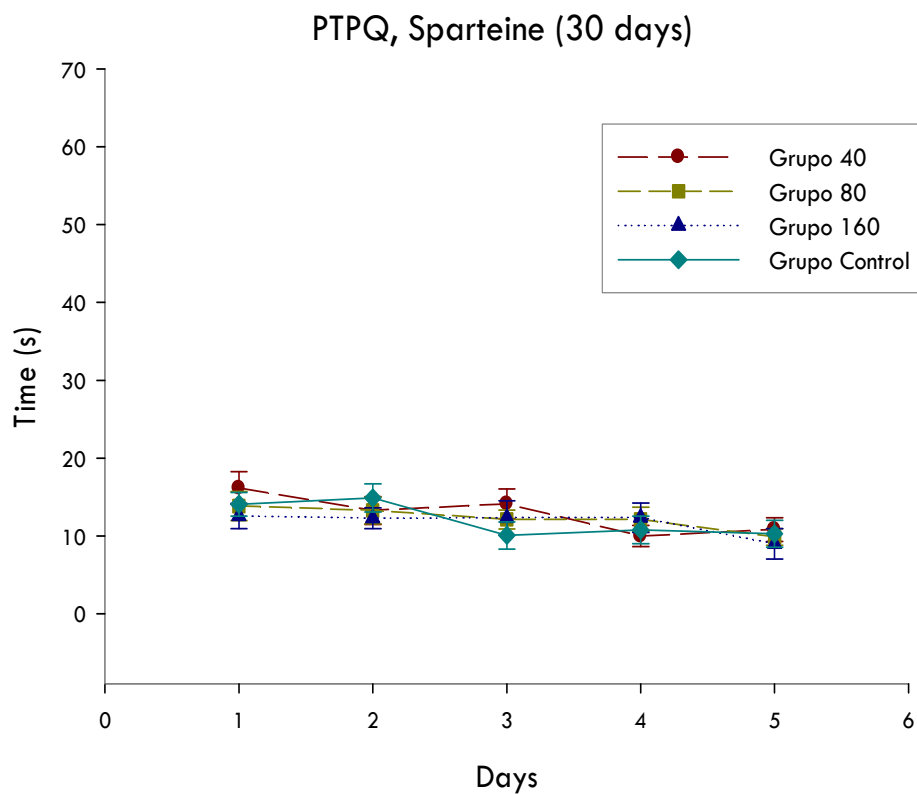
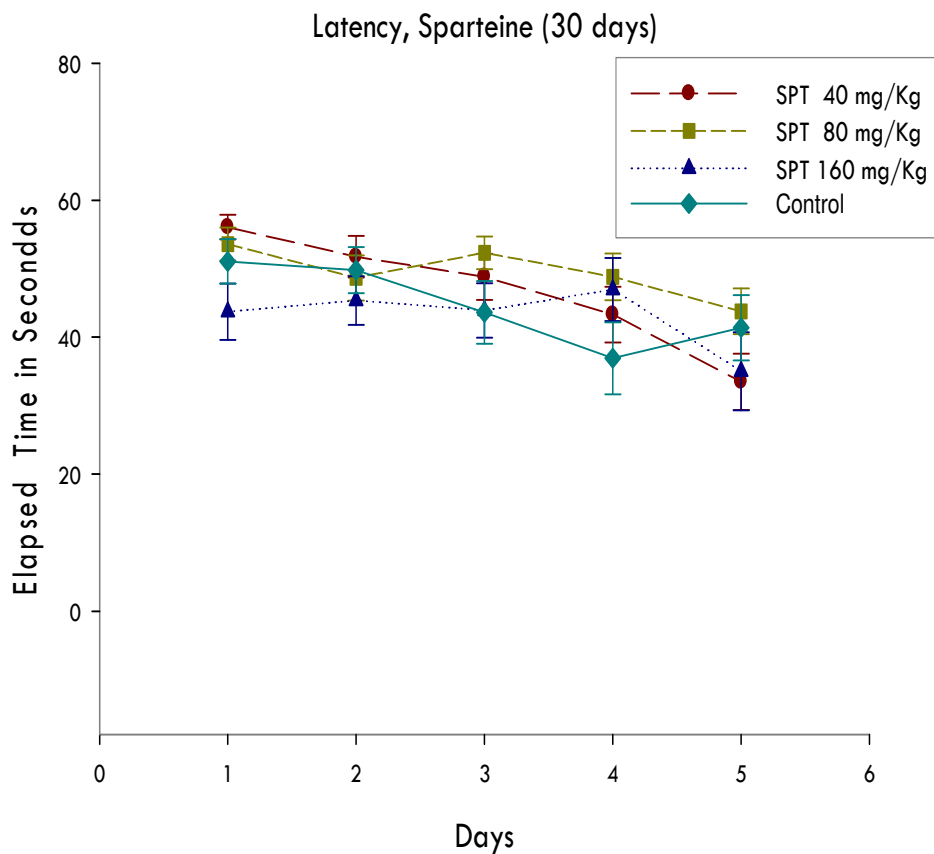
Table 3. Spartein (SPT) treated Wistar rats

Blood serum metabolites	SPT 40 g/kg	SPT 80 g/kg	SPT 160 g/kg
Creatinine	0.69 \pm 0.06	0.61 \pm 0.03	0.45 \pm 0.05
Urea	55.60 \pm 3.50	42.95 \pm 2.57	44.38 \pm 1.84
Glucose	100.3 \pm 6.03	85.00 \pm 5.69	69.57 \pm 5.38
Triglycerides	31.21 \pm 2.01	86.25 \pm 4.94	81.46 \pm 7.32
Cholestrol	36.74 \pm 1.90	44.86 \pm .80	45.82 \pm

CBZ

Both groups receiving 160 mg/kg of SPT showed liver and kidney degenerative processes. Results of the memory and spatial learning studies can be seen in Fig. 2. SPT nor CBZ had an effect on: latency, distance swam, permanence within platform quadrant or swimming speed (groups II, III, IV and V). These results indicate that oral administration of SPT has no effect on short-term memory or spatial learning.

Under the above conditions SPT was found suitable to become a non convulsive seizures useful drug with greater efficacy than carbamazepine; however, it is necessary to reproduce this data in other species before performing any clinical trials, although it appears that SPT has already been used in humans as an antiarrhythmic drug.



LITERATURE CITED

- Beauvais, F., T. Shimahara, I. Inoue, C. Hieblot, C. Burtin and J. Benveniste. 1992. Regulation of human basophil activation. II. Histamine release is potentiated by K⁺ efflux and inhibited by Na⁺ influx. *J Immunol.* 148(1): 149.
- Binnig, V.F. 1974. Zur Chemie des Sparteines, *Arzneim.-Forsch.* 24: 52.
- Downie, N.M. and Heath. 1973. Métodos estadísticos aplicados. Ed; Harla, S.A. de C.V. Mexico.
- Finkel, M.J. 1984. Phenytoin revisited. *Clin Ther.* 6(5): 577.
- Honerjager, P., E. Loibl, I. Steidl, G. Schonsteiner and K. Ulm. 1986. Negative inotropic effects of tetrodotoxin and seven class 1 antiarrhythmic drugs in relation to sodium channel blockade. *Naunyn Schmiedebergs Arch Pharmacol.* 332(2): 184.
- Morris, R. 1984. Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* 11: 47.
- Pellock, J.M. 1994. The clinical efficacy of lamotrigine as an antiepileptic drug. *Neurology.* 44(11 Suppl 8): S29.
- Pugsley, M.K., M.J.A. Walker, G.L. Garrison, P.G. Howard, R. Lazzara, E. Patterson, W.P. Penz, B.J. Scherlag and M.D. Berlin. 1992. The cardiovascular and antiarrhythmic properties of a series of novel sparteine analogs. *Proc. West. Pharmacol. Soc.* 35: 87.
- Raschack, V.M. 1974. Wirkung von Sperteine und Sparteinderivaten auf Herz und Kreislauf, *Arzneim.-Forsch.* 34: 753.
- Senges, J. and L. Ehe. 1973. The effects of sparteine on membrane potential and contraction of mammalian heart muscle. *Naunyn-Schmied. Arch. Pharmacol.* 280: 253.
- White, H.S. 1997. Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. *Epilepsia* 39 (1): S9.
- Wink, M. 1993. IN: *The Alkaloids*, Vol. 43 (Cordell, G.A., Ed.) pp. 1-118, Academic Press, Orlando, FL.
- Woodbury, D.M. 1952. Effect of adrenocortical steroids and adrenocorticotrophic hormone on electroshock seizure threshold. *J. Pharmacol. Exp. Ther.* 105(1): 27.