13th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-13), 1-30 November 2009.
http://www.mdpi.org/ecsoc-13/ & http://www.usc.es/congresos/ecsoc/13/

[c015]

ANTI-CONVULSIVE ACTIVITY OF NL197, A DERIVATIVE FROM 4(3*H*)QUINAZOLINON ON CHEMICAL INDUCED SEIZURE MICE

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ABSTRACT

Objectives: NL197 is a derivative from 4(3H)- quinazolinon and it has been reported to possess central and peripheral effect as well as learning and memory enhancing effect in vivo test. In this present study, we focus on the anticonvulsant effect of NL197 on pentylentetrazol or strychnine induced- seizure mice.
Methods: The anticonvulsive activity of NL197 is investigated in experimental seizure models in mice inducing by chemo-convulsant such as pentylenetetrazole (PTZ) 100 mg/kg, SC or strychnine (STN) 2 mg/kg, SC. The anticonvulsive potent of NL197 is also made comparison with that of diazepam 5 mg/kg, PO.
Results: In PTZ induced- seizure model, anticonvulsant activity of NL197 NL was determined at the dose of 50 mg/kg, 73.2 mg/kg, and 100 mg/kg. In strychnine induced- seizure model, NL 197 had anticonvulsant activity at dose of 50mg/kg, 73.2mg/kg. No effect was determined with NL197 at dose of 37mg/kg.
The protective potent of NL197 againsts PTZ- or STN induced seizures is equal to that of diazepam 5mg/kg, PO at the dose of 73.2 mg/kg and 50 mg/kg, SC, respectively.
Conclusions: These present results provided evidence that NL197 has potential anticonvulsant activity in pentylentetrazol or strychnine induced-seizure model in mice as well as diazepam, a standard antiepileptic drug.

BACKGROUND

Epilepsy is a chronic neurological condition, characterized by recurrent seizures that are caused by abnormal cerebral nerve cell activity. The treatment of epilepsy is always a challenge for researchers and clinical practitioners. Over the last decades, several new drugs have been introduced for the treatment of epilepsy. Despite this progress, about 30% of patients with epilepsy are resistant to current pharmacotherapies and many of the available antiepileptic drugs. Based on this reason, there has been a continuous attempt to find new antiepileptic drugs which increases the demand to conduct more studies in this field. Although one could argue that research on human epilepsy should be ideally carried out on humans with epilepsy, this approach is not always possible or practical. Because of the ethical aspect as well as many other difficulties, carrying out research projects on patients would be prohibitive. So that animal models of epilepsy are the most likely essential to epilepsy research. Animal models of epilepsy are most often used to investigate fundamental neuronal mechanisms of seizure as well as mechanism of potential agents. We can use these models for basically estimating or testing the efficacy of new antiepileptic drugs or other novel therapeutic

interventions. Among various type of models, acute seizure model using chemo-convulsants is one of the most common type of model which were used effectively by early researchers and are still widely used today for studying seizure–related phenomenon. Animal models that used chemo-convulsants with specific, known neurotransmitter interactions were used to identify potential therapeutic agents. The mechanisms by which these convulsants produced seizures were thought to be important for the interpretation of a compound's mechanism of action to inhibit seizures. Some common chemo-convulsants include camphor, strychnine, pentylenetetrazole, picrotoxin, bicuculline, N-methyl D-aspartic acid (NMDA), allylglycine, mercaptoproprionic acid, isoniazid , and aminophylline [2],[7] .

In Viet Nam, nowadays, there have been very little laboratory procedures studying about neuropharmacological aspects, as well as screening and testing the efficacy of new drugs in neural science especially antiepileptic drugs. Based on these practical demand, in this research we try to tesing chemically- induced acute seizure using pentylentetrazole (PTZ), strychnine sulfat (STN), then apply these model in basically testing anticonvulsive activity of NL197- a derivative of 4(3H)-quinazolinon. This agent has been recently reported to possess central and peripheral effect as well as learning and memory enhancing activity in vivo test[1].

MATERIAL AND METHOD

Animal

Male ddY mice (vaccines and bioproducts Institute, Nha Trang), weighing 18-30g, 4-6 weeks old were used. Animal were housed in groups of six in the plastic cages, in a temperature controlled room (27±1 ^{0}C), under 12 h light-dark cycle (lights on 7:00-19:00). They were allowed free access to food and tap water. Animals were acclimated to the laboratory conditions for at least 2 days prior to any experimentation. All experiments were conducted between 9:00 and 16:00.

Chemical

Pentylentetrazole (PTZ) and strychnine sulfat (STN), supplied by Sigma Aldrich Chemical Co.,were all dissolved in physiological saline solution (0.9% NaCl) and administered subcutaneously. NL197 was suspended in 0.25% carboxymethyl cellulose Na solution (0.25% CMC), 0.2% polysorbat and administered orally. Diazepam (Pharmadic Ltd. Pharmaceutical Co.) was dissolved in 0.9% physiological saline and used orally as reference drug in positive control group a the dose of 5 mg/kg.

Chemically induced seizure

The chemo-convulsants, PTZ 100mg/kg, STN 2mg/kg were administered subcutaneously in a volume of 10 ml/kg. The animals were placed in individual cage (20 cm x 15cm) and observed for 30 min (PTZ, STN) for latency of seizure onset and time to death. The mortality were also evaluated for each group. Diazepam 5 mg/kg - a standard antiepileptic drug, was administered orally 60 min before injecting chemo-convulsants to evaluate the anti-convulsive activity of diazepam on each model.[3,4]

Evaluation of the anticonvulsive activity of NL197 on chemically induced seizure

PTZ 100 mg/kg and STN 2 mg/kg were injected subcutaneously to induce seizures in mice. The protective activity of NL197 against the PTZ-induced seizures (at the doses of 50 mg/kg; 73 mg/kg; 100 mg/kg) and STN-induced seizures (at the doses of 37 mg/kg; 50 mg/kg, 73.2 mg/kg) was evaluated after administering orally 30 min. Distilled water or diazepam 5 mg/kg were administered orally 30 min or 60 min before injecting PTZ and STN, as control and positive control group, respectively. Mice were observed for 60 min after receiving water or testing chemicals. The latency of convulsive onset, time to death and mortality rate was measured.[5]

Statistical analysis

All data were expressed as mean ± SEM. The data was evaluated by Kruskal – Wallis test and MannWithney test using Minitab 14. Differences between groups were considered significant when $p < 0.05$

RESULTS

Evaluation of chemically induced seizure models using eiher PTZ, or STN in screening anticonvulsive agents

Table 1. Evaluate either PTZ or STN induced seizure models in latency of convulsive onset, time to death and motarlity rate

Chemoconvulsants	PTZ 100 mg/kg (n=6)	STN 2 mg/kg (n=6)
Latency of onset (s)	303.7 ±26	341.7 ±19.5
Time to death (s)	659.8 ±40.3	503.3 ±30.7
Mortality rate (%)	100	100

Table 2. Effect of diazepam 5 mg/kg P.O on the latency of convulsive onset, time to death and mortality rate in either PTZ or STN induced- seizure models

Group	ED –STN (n=6)	DZP-STN (n=6)	ED – PTZ (n=6)	DZP-PTZ (n=6)
Latency of onset(s)	365.5 ±21.1	683.4* ± 25.5	275.3 ±10.6	1478.4* ±89.2
Time to death (s)	489.6± 28.8	-	681.9±33.1	-
Mortality (%)	100	0	100	0

ED: Distilled water *P<0.05 as compared to the same chemical- induced seizure group.

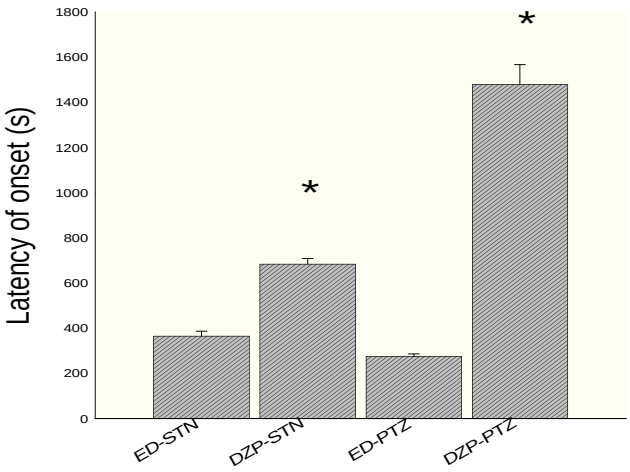

Figure 1. Effect of DZP in latency of convulsive onset in either PTZ, or STN induced seizure models. *P<0.05 as compared to the same chemical- induced seizure group

In both either PTZ or STN-induced seizure models, 100% experimental animals had seizures after administered chemicals about 300 seconds and then seizures exerted mice to death after 500 seconds. Distilled water administered orally 10 ml/kg do not have any effects on latency of convulsive onset, time to death, and mortality rate of mice in groups induced seizures by PTZ 100 mg/kg or STN 2 mg/kg. These models can be used for testing or screening anticonvulsive effect of potential antiepileptic agents as well as interpreting the anticonvulsive activity mechanisms of compounds based on the known chemo-convulsants. While PTZ exerts its action mostly via the t-butylbicyclo-phosphorothionate (TBPS) site of the GABA-A receptors. strychnine has been defined as a blockade of chloride channel associated with glycine receptors. These inhibitory receptors can be found mostly in the spinal cord and brainstem. Therefore strychnine can serve as a model of therapy-resistant seizures arising from the lower brainstem and spinal cord [6,7]. Diazepam 5 mg/kg, PO used to evaluate the efficacy of each model has showed anticonvulsive effect on both seizure models induced by either PTZ 100 mg/kg or STN 2 mg/kg, SC.

Effect of NL197 on mice PTZ and STN-induced seizure model

Table 3. Anticonvulsive effect of NL197 on latency of onset, time to death and mortality rate in STN- induced seizure mice

Group	ED-STN (n=8)	Exp-STN (n=8)	DZP-STN (n=8)	NL197 37mg/kg-STN(n=8)	NL197 50mg/kg-STN (n=8)	NL197 73.2mg/kg-STN(n=8)
Latency of onset (s)	365.5± 21	376.5± 22.1	683.4**,# ±25.5	368.3@ ±22.8	620.5**,# ±61.6	1154.6**,#, @±98.2
Time to death (s)	503.3± 30.7	522.6± 29.3	-	1006.5±32 .9	1680	-
Mortality rate (%)	100	100	0	100	12.5	0

Table 4. Anticonvulsive effect of NL197 on latency of onset, time to death and mortality rate in PTZ- induced seizure mice

Group	ED-PTZ (n=8)	Exp-PTZ (n=8)	DZP-PTZ (n=8)	NL197 50mg/kg-PTZ (n=8)	NL197 73.2mg/kg-PTZ (n=8)	NL197 100mg/kg-PTZ (n=8)
Latency of onset (s)	275.3± 10.6	244±16 .5	1478.4**, #±89.2	546**,#,@ ±161	1011**,# ±183	517.1**,#, @±53.4
Time to death (s)	721.9± 20	744.7± 47.9	-	1744.5±2.5	1739	-
Motarlity rate (%)	100	100	0	25	12.5	0

Exp: Excipient
# p<0.01: as compared to control group receiving distilled water (ED)
**p<0.01: as compared to control group receiving excipient (Exp)
@p<0.01: as compared to positive control group receiving DZP

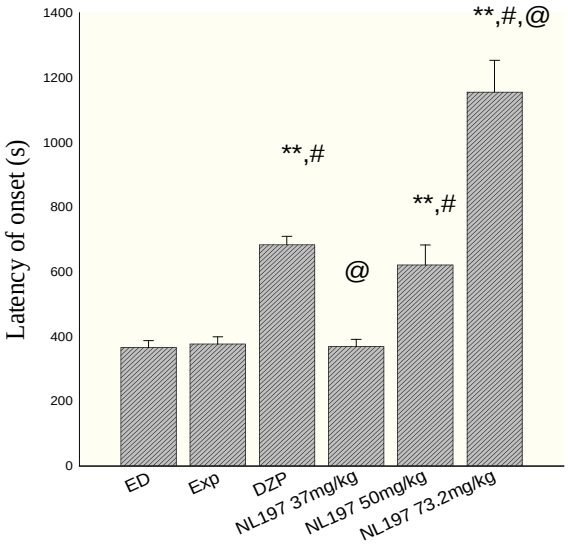

Figure 2. Effect of NL197 on STN induced seizure mice

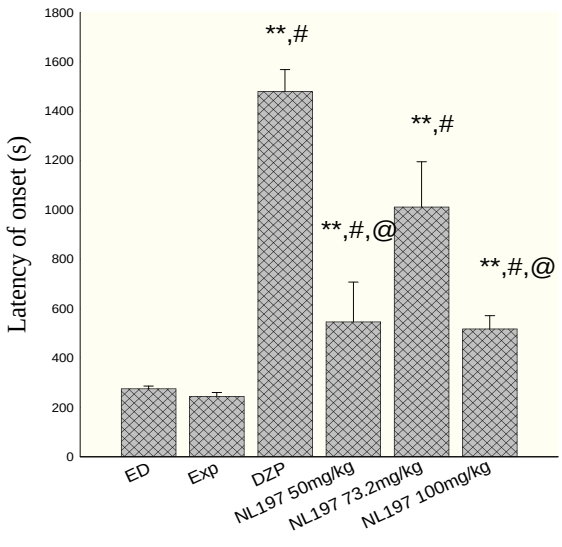

Figure 3. Effect of NL197 on PTZ-induced seizure mice

Latency of convulsive onset, time to death and mortality rate of mice in groups induced seizures by either PTZ 100 mg/kg or STN 2 mg/kg were decreased when mice were pretreated with NL197 at the different doses.

In PTZ induced- seizure model, anticonvulsion activity of NL197 NL was determined at the doses of 50 mg/kg, 73.2 mg/kg, and 100 mg/kg in both latency of onset and time to death. At higher dose of PTZ, 100 mg/kg, mice were protected completely from death. In STN induced-seizure model, NL 197 had anticonvulsive activity at doses of 50 mg/kg and 73.2 mg/kg. Mice

induced seizures by STN 2 mg/kg was protected completely from death by NL197 at dose 73.2 mg/kg. No effect was determined with NL197 at dose of 37 mg/kg.
The protective potential of NL197 against either PTZ- or STN- induced seizures is equal to that of diazepam 5 mg/kg, PO at the dose of 73.2 mg/kg and 50 mg/kg, SC, respectively. NL197 at dose 73.2 mg/kg increased latency time of convulsive onset more significantly than that of diazepam 5mg/kg, PO against STN- induced seizures.

CONCLUSIONS

The present results provided evidence that NL 197 - a derivative of 4(3H)-quinazolinon has significant anticonvulsive activity in adult mice induced seizures by either STN or PTZ. This agent has showed effect in inhibiting GABA-related chemoconvulsant induced seizures like other antiepileptic drugs involve in enhancement of GABAergic systems, such as valproic acid, benzodiazepines, and barbiturates. For compounds which have activity through GABA-A receptors, PTZ-induced seizure model should be chosen. Similarly, for screening compounds having activity through glycine receptors- chloride channel, strychnine induced seizure model should be used.
We suggest continuing testing anticonvulsion activity as well as other pharmacological activities of NL197 on neurvous system in different models using other chemoconvulsants such as picrotoxin, N-methyl D-aspartic acid, bicucucline,... to orientate and find out the other activity mechanisms of NL197 in animals at the different ages.

Acknowledgement: The authors thank to HCM city school of pharmacy for funding this study. And also thank to Prof. Truong The Ky and Dr. Nguyen Ngoc Vinh supplying the chemicals.

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