Short Communication

Effect of diclofenac and paracetamol on anticonvulsant activity of phenytoin and sodium valproate in laboratory animals

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ABSTRACT

Background: Neuroinflammation is a major contributor to neurological and neurodegenerative diseases, and inflammatory processes have been implicated in both acute and chronic conditions such as epilepsy. Cyclooxygenase (COX) inhibitors, are used largely to treat febrile condition, pain state and inflammation, through their role in the inhibition of prostaglandin synthesis. Thus, drugs that reduce the production of prostaglandins may have beneficial effect in epilepsy.

Objective: The present study is aimed at evaluating possible effects of diclofenac and paracetamol on the anticonvulsant outcome of phenytoin and sodium valproate in chicks and mice.

Methods: Electrically and chemically-induced seizure models, using maximal electroshock test (MEST) in chicks and pentylenetetrazole (PTZ) test in mice, respectively, were used for the study.

Results: Co-administration (*i.p.*) of diclofenac (20 mg/kg) and paracetamol (100 mg/kg), each with phenytoin (15 mg/kg, *i.p.*), potentiated the anticonvulsant outcome of phenytoin in MEST. Diclofenac-phenytoin and paracetamol-phenytoin administrations exhibited 90% and 80% protection, respectively, as against the 0% protection produced when diclofenac and paracetamol were administered alone. Similarly, there was statistical significant decrease ($p<0.05$) in the recovery time from seizures between the groups co-administered with the two drugs and those that received individual drugs. In PTZ-induced seizure test, there was slight decrease in seizure protection (60%); with significant increase ($p<0.05$) in the mean onset of seizures when diclofenac (20 mg/kg) and sodium valproate (100 mg/kg) were co-administered; while valproate (100 mg/kg) exhibited 80% protection with no significant increase ($p<0.05$) in the mean onset of seizures. Also, similar protection of 80% was obtained when paracetamol (100 mg/kg) and valproate (100 mg/kg) were co-administered showing no potentiation or antagonism in PTZ-induced seizure test.

Conclusion: The results of this study showed potentiating effect when diclofenac and phenytoin; paracetamol and phenytoin, were co-administered. Therefore, the co-administration may be beneficial in generalized tonic-clonic seizures.

Key words: Diclofenac; Paracetamol; Phenytoin; Valproate ; Cyclooxygenase

Effet du diclofénac et du paracétamol sur l'activité anti-convulsivante de la phénytoïne et du valproate de sodium chez les animaux de laboratoire

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RESUME

Contexte: La neuro-inflammation est un facteur majeur des maladies neurologiques et neuro-dégénératives, et les processus inflammatoires ont été impliqués dans des conditions aiguës et chroniques telles que l'épilepsie. Les inhibiteurs de la cyclo-oxygénase (COX) sont largement utilisés pour traiter l'état fébrile, l'état de la douleur et l'inflammation, par leur rôle dans l'inhibition de la synthèse des prostaglandines. Ainsi, les médicaments qui réduisent la production de prostaglandines peuvent avoir un effet bénéfique sur l'épilepsie.

Objectif: La présente étude vise à évaluer les effets possibles du diclofénac et du paracétamol sur les résultats anticonvulsivants de la phénytoïne et du valproate de sodium chez les poussins et les souris.

Méthodes: Des modèles de convulsions électriquement et chimiquement induites, par l'usage d'un test électrochoc maximal (MEST) chez les poussins et le test de pentylènetétrazole (PTZ) chez la souris, respectivement, ont été utilisés pour l'étude.

Résultats: L'administration conjointe (i.p.) de diclofénac (20 mg / kg) et de paracétamol (100 mg / kg), chacun avec de la phénytoïne (15 mg/kg, *i.p*.), a potentialisé le résultat anticonvulsivant de la phénytoïne dans le MEST. Les administrations de diclofénac-phénytoïne et de paracétamol-phénytoïne ont présenté respectivement une protection de 90% et 80% par rapport à la protection de 0% produite lorsque le diclofénac et le paracétamol étaient administrés seuls. De même, il y a eu une diminution significative statistique ($p<0,05$) du temps de récupération des convulsions entre les groupes co-administrés avec les deux médicaments et ceux qui ont reçu des médicaments individuels. Dans le test de convulsion induite par PTZ, il y a eu une légère diminution de la protection contre les crises (60%); avec une augmentation significative ($p<0,05$) du début moyen des crises lorsque le diclofénac (20 mg/kg) et le valproate de sodium (100 mg/kg) ont été co-administrés; tandis que le valproate (100 mg/kg) a présenté une protection de 80% sans augmentation significative ($p<0,05$) dans le début moyen des convulsions. En outre, une protection similaire de 80% a été obtenue lorsque le paracétamol (100 mg/kg) et le valproate (100 mg / kg) ont été co-administrés ne présentant aucune potentialisation ni antagonisme dans le test de crise induite par PTZ.

Conclusion: Les résultats de cette étude ont montré un effet de potentialisation lorsque le diclofénac et la phénytoïne; le paracétamol et la phénytoïne, ont été co-administrés. Par conséquent, la co-administration peut être bénéfique dans les crises toniques et cloniques généralisées.
Mots clés: diclofénac; paracétamol; phénytoïne; valproate; cyclo-oxygénase

INTRODUCTION

Epilepsy is the most common neurological disorder affecting individuals at all age groups,[1] and it is considered a public health problem in low and middle-income countries.[2] It is a chronic disorder often requires an additional therapy for underlying diseases, and thus, drug interaction may occur when the effects of one drug are changed by the prior or concomitant exposure to another drug.[3] Monotherapy remains the main stay for the treatment of epilepsy, but antiepileptic drugs may also be combined with other drugs to treat inter-current or associated conditions, thereby increasing the probability of co-prescription.[4] Anti-seizure drugs have low neuroprotective activity or their side effects which are the outcomes of long therapy overcome their therapeutic benefits. Therefore considerations now focus on neuroprotective effects of various components.[5] Cyclooxygenase (COX) inhibitors, are used largely to treat febrile condition, pain state and for prevention of many diseases, through their role in the inhibition of prostaglandin synthesis.[6] Targeting brain inflammation may accordingly represent a novel therapeutic strategy for epilepsy, consistent with efforts to shift focus away from the symptomatic control of seizures to disease-modifying treatments that better target the underlying pathological mechanisms.[7]

Previous studies have shown that COX inhibitors such celecoxib, etoricoxib, rofecoxib, nimesulide, naproxen and aspirin, reduced seizure activity in animal models;[8,9,10] others suggested the intimate involvement of inflammatory mechanisms in the generation of epileptic discharges and in the cellular damage associated with seizures.[7,6] Also, effects of ibuprofen, aspirin, metamizole, indomethacin, piroxicam and paracetamol, on the anticonvulsant activity of phenytoin and sodium valproate, were studied in maximal electroshock-induced seizure test.[11] The present study was aimed at evaluating the possible effects paracetamol and diclofenac, on the anticonvulsant activity of phenytoin and sodium valproate, using both maximal electroshock test (MEST) and Pentylenetetrazole (PTZ) test.

MATERIALS AND METHOD

Animals: Swiss Albino mice (18 - 22 g) of both sexes were used for the experiments. They were obtained from the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria; while day-old cockerels were obtained from Ota Farm, Ogun State, Nigeria. The animals were kept in plastic cages with constant supply of standard animal feeds and water *ad libitum* and maintained at room temperature. The use of animals was in compliance with the National Institute of Health Guidelines for the Care and use of Laboratory Animals (Publication nos. 85–23, revised 1985).

Drugs and equipment: Phenytoin; Pentylenetetrazole (Sigma-Aldrich,St.louisUSA), Diclofenac (Clofenac-Hovid, Malaysia), Paracetamol (SKG-Pharma, Nigeria), Sodium valproate (Epillin-Sanofi, UK) and Electroconvulsive machine (UgoBasile, Model No. 7801), were used for the study. Distilled water was used to dissolve the drugs and all drugs were prepared freshly for each experiment.

Anticonvulsant studies: Effects of diclofenac and paracetamol on anticonvulsant activity of phenytoin in maximal electroshock test

Hundred (100) chicks were allotted to 10 groups of 10 animals each. Group 1 received distilled water (10 ml/kg) and served as negative. Groups 2 and 3 were administered diclofenac (10 and 20 mg/kg, *i.p.*, each respectively); Groups 4 and 5 received 50 and 100 mg/kg each of paracetamol, respectively. Similarly, chicks in groups 6, 7 and 8 were administered 10, 15 and 20 mg/kg of phenytoin, respectively. All the administrations were via intraperitoneal route. Thirty minutes posttreatment, maximal electroshock was delivered to induce seizures in the chicks, using Ugo Basile electroconvulsive machine (model 7801) with corneal electrodes placed on the upper eyelid of the chicks after dipping them in normal saline. The current, shock duration, frequency and pulse width were predetermined and maintained at 90 mA, 0.2 s, 100 Hz and 1.0 ms-[1]respectively. Absence of an episode of tonic extension of the hind limbs (THLE) of the chicks was considered as protection; recovery time from seizures was recorded for the unprotected animals, as described by[12] and .[13] Based on the outcome of the above, Groups 9 and 10 were administered diclofenac (20 mg/kg, *i.p.*) and paracetamol (100 mg/kg, *i.p.*), respectively. Fifteen minutes later, each group was administered phenytoin at 15 mg/kg (*i.p.*); followed by delivery of shock after another 30 minutes as described above.

Effects of diclofenac and paracetamol on anticonvulsant activity of sodium valproate in pentylenetetrazole (PTZ)-induced seizure test

Mice were divided into 9 groups of 5 animals each. Group 1 received distilled water and served as negative control, while Groups 2 and 3; 4 and 5; 6 and 7, were administered diclofenac (10 and 20 mg/kg, *i.p.*); paracetamol (50 and 100 mg/kg, *i.p.*); sodium valproate

(100 and 200 mg/kg, *i.p.*), each respectively. Thirty minutes later, seizure was induced by administration of PTZ (90 mg/kg, *s.c.*) to each mouse. Onset of seizure and protection from clonic convulsion characterized by loss of righting reflex, were observed and recorded. Similarly, based on the outcome of seizure episodes above, Groups 8 and 9 were administered diclofenac and paracetamol at doses of 20 and 100 mg/kg (*i.p.*), respectively. Fifteen minutes later, sodium valproate was administered to each group at 100 mg/kg (*i.p.*); followed by induction of seizure after thirty minutes, using PTZ as described by.[14]

Statistical analysis

Statistical analysis was carried out using SPSS (Version 20) and data obtained were expressed as Mean ± SEM. They were analyzed using analysis of variance (ANOVA) followed by Dunnette's Posthoc test for multiple comparison. Values obtained with $p \leq 0.05$ were considered significant.

RESULTS

In maximal electroshock test, co-administration (*i.p.*) of diclofenac (20 mg/kg) and paracetamol (100 mg/kg), each with phenytoin (15 mg/kg), potentiated the anticonvulsant outcome of phenytoin in MEST. Diclofenac-phenytoin and paracetamol-phenytoin administrations exhibited 90% and 80% protection, respectively, as against the 0% protection produced by single administration of diclofenac and paracetamol. Similarly, there was statistical significant decrease ($p<0.05$) in the recovery time from seizures between the groups co-administered with the two drugs and those that received individual drugs. However, there was no significant difference in the seizure recovery when diclofenac (10 and 20 mg/kg) and paracetamol (50 and 100 mg/kg) were administered as compared to control (Table 1).

In PTZ-induced seizure test, there was slight decrease in seizure protection (60%), with significant increase ($p<0.05$) in the mean onset of seizures when diclofenac (20 mg/kg) and sodium valproate (100 mg/kg) were co-administered; while valproate (100 mg/kg) exhibited 80% protection with no significant increase ($p<0.05$) in the mean onset of seizures. Also, similar protection of 80% was obtained when paracetamol (100 mg/kg) and valproate (100 mg/kg) were co-administered (Table 2).

Table 1: Effects of Diclofenac and Paracetamol on Anticonvulsant Activity of Phenytoin in Maximal electroshock test

Treatment (mg/kg)	Protection Against Seizures (%)	Recovery from Seizures (min)
Distilled Water (10ml/kg)	0.0	6.1 ± 0.50
Diclofenac (10)	0.0	6.6 ± 0.33
Diclofenac (20)	0.0	9.3 ± 0.53
PCM (50)	0.0	5.5 ± 0.63
PCM (100)	0.0	6.0 ± 0.66
Phenytoin (10)	0.0	5.0 ± 0.59
Phenytoin (15)	0.0	4.3 ± 0.98
Phenytoin (20)	100.0	-
Dic (20) + PHT (15)	90.0	2.0 ± 0.00*
PCM (100) + PHT (15)	80.0	2.5 ± 1.50*

Values are presented as Mean ± SEM; n = 10 PHT = Phenytoin; PCM = Paracetamol; Dic = Diclofenac; * Statistically significant difference at $p<0.05$ when compared to control (ANOVA) followed by Dunnett's Post-hoc test for multiple comparison.

Table 2: Effects of Diclofenac and Paracetamol on Anticonvulsant Activity of Sodium Valproate in Pentylenetetrazole-induced Seizure Test

Treatment (mg/kg)	Mean Onset of Seizures (min)	Protection Against Seizures (%)
Distilled Water (10ml/kg)	7.6 ± 0.60	0.0
Diclofenac (10)	8.4 ± 1.12	0.0
Diclofenac (20)	10.6 ± 1.28	0.0
PCM (50)	7.8 ± 1.11	0.0
PCM (100)	12.8 ± 2.7	20.0
Valproate (100)	7.4 ± 0.7	80.0
Valproate (200)	0.0 ± 0.0	100.0
Dic (20) + VAL (100)	20.5 ± 5.50*	60.0
PCM (100) + VAL (100)	11.0 ± 0.0	80.0

Values are presented as Mean ± SEM; n = 10; PHT = Phenytoin; PCM = Paracetamol; Dic = Diclofenac; VAL = Sodium valproate; * Statistically significant difference at $p<0.05$ when compared to control (ANOVA) followed by Dunnett's Post-hoc test for multiple comparison.

DISCUSSION

Maximal electroshock (MES) causes facilitation of Ca^{2+} entry into the cells and prolongs the duration of convulsions.[15] Also, it facilitates the entry of other positive ions like Na^{+} and thus, its blockade can prevent the MES-induced tonic extension.[16] Currently available anticonvulsant drugs like sodium valproate and phenytoin act by modulation of these ion channels.[17] Protection against hind limb tonic extension (HLTE) in the MEST predicts anticonvulsant activity of antiepileptic drugs that prevent the spread of seizure discharge from an epileptic focus during seizure activity. Antiepileptic agents that could offer protection against HLTE are employed in the treatment of generalized tonic-clonic and partial seizures.[18] Antiepileptic drug (AED), phenytoin, is primarily used in the management of complex partial seizure and generalized tonic-clonic seizure[19] while sodium valproate possesses broad spectrum of antiepileptic activity.[20] Neuroinflammation is a major contributor to neurological and neurodegenerative diseases, and inflammatory processes have been implicated in both acute and chronic conditions such as epilepsy.[21] Cyclooxygenase (COX), which catalyses the production of pro-inflammatory prostaglandins plays a significant role in seizure-induced neuroinflammation and neuronal hyperexcitability.[9] The involvement of COX-2 in acute and chronic neurodegenerative syndromes has promoted the development of neuroprotective treatment strategies involving COX inhibitors such as non-steroidal anti-inflammatory drugs.[22] From the outcome of MEST in this study, diclofenac and paracetamol potentiated the effect of phenytoin when coadministered. This suggests possible potentiation of the effect of phenytoin in the blockade Na^{+}/Ca^{2+} ions entry into the cells.[23]

Pentylenetetrazole is believed to be an antagonist at GABA pathway in the CNS,[24] and seizures induced by PTZ are potentiated by gamma-aminobutyric acid (GABA) antagonists.[25] Therefore, PTZ-induced seizures can be prevented by drugs that enhance gamma amino butyric acid type A ($GABA_A$) receptor-mediated inhibitory neurotransmission such as benzodiazepines and phenobarbital.[26] However, potentiation effect was not observed in PTZ test when diclofenac and paracetamol were co-administered with sodium valproate; suggesting less involvement of sodium and calcium in the pathogenesis of seizures evoked by PTZ as against that of MEST.

CONCLUSION

Co-administration of NSAIDs with phenytoin may be beneficial in generalized tonic-clonic and partial epilepsy and could as well serve as basis for possible dose reduction of the antiepileptic agent.

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