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Comparative Effects of Aspirin and Celecoxibon PTZ-induced Seizure in Rats

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ABSTRACT

Background: Prostaglandin has been shown to increase seizure threshold and seizure onset time and selective blockade of cyclooxygenase 2 $(COX)_2$ activity decreases the effect of prostaglandin. Celecoxib selectively blocks COX_2 but is expensive and not readily available in many countries of Africa. The aim of this study was to compare the antiseizure activities of aspirin and celecoxib on PTZ-induced seizures in rats. **Materials and Methods:** A total of 30 adult Wister rats of either sex and of an average weight of 100 g were used for the study. Five animals were used for convulsion threshold titration and the remaining 25 divided into 5 groups (A-E) of 5 animals group^{-1}. Groups A-E were given 2 mg kg^{-1} of celecoxib, 100 mg kg^{-1} of aspirin, 2 mg kg^{-1} of celecoxib and 10 mg kg^{-1} of valproic acid (15 min apart), 100 mg kg^{-1} of aspirin and 10 mg kg^{-1} of valproic acid (15 min apart) and 2 mL kg^{-1} of clean drinking water, respectively, orally, daily. The 90 mg kg^{-1} of Pentylenetetrazole (PTZ) was administered intraperitoneally, 30-45 min after each drug(s) to induce seizures and the animals observed for 24 h. Time of onset of myoclonic jerks, righting of hind limb extension and/or death were noted and taken as signs of seizures. **Results:** Celecoxib and aspirin treated animals, groups A and B exhibited convulsive signs and died at an average time of 32 and 32.02 min, respectively post PTZ administration ($p<0.01$ compared to negative controls). There was however, no significant difference in seizure onset time between groups A and B ($p>0.79$). Celecoxib plus valproic acid and aspirin plus valproic acid treated animal (groups C and D) exhibited no signs of convulsive seizures and remained alive throughout the experimental period. **Conclusion:** Celecoxib, did not exhibit a superior inhibitory activity of PTZ-induced seizures over aspirin. Cyclooxygenase inhibitor plus valproic acid combination therapy appears to completely abolish seizures.

Key words: Inflammation, prostaglandins, celecoxib, aspirin, seizure, epilepsy, epileptogenesis

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INTRODUCTION

Prostaglandins are lipid-derived autacoids that modulate many physiological systems including the CNS, cardiovascular, gastrointestinal, genitourinary, endocrine, respiratory and immune systems. In addition, prostaglandins have been implicated in a broad array of diseases including cancer, inflammation, cardiovascular disease and hypertension (Hata and Breyer, 2004).

Prostaglandins exert their effects by activating rhodopsin-like seven transmembrane spanning G Protein-Coupled Receptors (GPCRs). The prostanoid receptor subfamily comprises eight members (DP, EP1-4, FP, IP and TP) and recently, a ninth prostaglandin receptor was identified-the chemoattractant receptor homologous molecule expressed on Th2 cells (CRTH2) (Hata and Breyer, 2004).

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The precise roles prostaglandin receptors play in physiologic and pathologic settings are determined by multiple factors including cellular context, receptor expression profile, ligand affinity and differential coupling to signal transduction pathways. This complexity is highlighted by the diverse and often opposing effects of prostaglandins within the immune system. In certain settings, prostaglandins function as pro-inflammatory mediators, but in others, they appear to have anti-inflammatory properties. Prostanoids including various prostaglandins (PGs)2 and thromboxanes (TXs) are cyclooxygenase (COX) metabolites of C-20-unsaturated fatty acids such as arachidonic acid. These substances are synthesized in response to various stimuli in a variety of cells, released immediately after synthesis and act in the vicinity of their synthesis to maintain local homeostasis (Sugimoto and Narumiya, 2007). Two isoforms of cyclooxygenase act on

arachidonic acid to produce constitutive prostaglandin (PGE1) from COX-1 or inflammatory prostaglandin (PGE2) from COX-2. Among prostanoids, the E type PGs, particularly PGE2 derived from arachidonic acid, is most widely produced in the body, most widely found in animal species and exhibits the most versatile actions (Sugimoto and Narumiya, 2007).

The mechanisms whereby PGE2 exerts its pleiotropic effects, once a mystery in physiology, have been clarified through the biochemical identification and cDNA cloning of the four EP subtype receptors. Thus, development of highly selective agonists and antagonists to each EP subtype and information obtained by studies on mice deficient in each EP receptor now provide opportunities to manipulate various PGE2-mediated pathological processes (Sugimoto and Narumiya, 2007).

Prostaglandins have been shown to mediate inflammation neuronally. This induced inflammation of prostaglandin is epileptogenic (Oliveira *et al*., 2008). Recent studies have shown that therapeutic inhibition of prostaglandin reduces onset and latency period of seizures in experimental animals (Oliveira *et al*., 2008; Brandt *et al*., 2004). Thus, prostaglandin inhibition may be likened to killing two birds with a stone; control of seizure as well as antiepileptogenesis.

One compelling challenge in the therapy of epilepsy is to develop anti-epileptogenic drugs with an impact on the disease progression. The search for novel targets has focused recently on brain inflammation since this phenomenon appears to be an integral part of the diseased hyperexcitable brain tissue from which spontaneous and recurrent seizures originate. There are three inflammatory pathways, namely the IL-1 receptor/Toll-like receptor signaling, COX-2 and the TGF-β signalling but, COX-2 appears to be the most relevant (Vezzani *et al*., 2013a).

With interest waning in the use of cyclooxygenase-2 (COX-2) inhibitors for inflammatory disease, prostaglandin receptors provide alternative targets for the treatment of COX-2-mediated pathological conditions in both the periphery and the central nervous system. Activation of prostaglandin E2 receptor (PGE(2)) subtype EP2 promotes inflammation and is just beginning to be explored as a therapeutic target (Jiang *et al*., 2013). Sustained COX-2 activation in the CNS can result in PGE_2-mediated brain inflammation and injury.

For example, the EP1 prostaglandin receptor was reported to mediate COX-2–directed neurotoxicity in ischemic stroke and EP2 accelerates the progression of experimental amyotrophic lateral sclerosis. EP2 is emerging as a principal mediator of brain inflammation after injury (Jiang *et al*., 2013).

This study is therefore aimed at comparing the antiseizure or synergistic seizure inhibitory activity of aspirin a non selective COX inhibiter and celecoxib the selective COX-2 inhibitor. Furthermore, to determine if aspirin which is cheap and readily available is as good as celecoxib in antiseizure activity and whether it could replace celecoxib for this purpose in developing countries and African sub region with poor economic setting.

MATERIALS AND METHODS

Materials consisted of aspirin, Celebrex (Pfizer Nigeria) and valproic acid tablets which were purchased from Madonna University pharmacy. Pentylenetetrazole was bought from University of Ghana, Legon, Ghana. A total of 30 adult Wister rats of both sexes and of an average weight of 100 g were bought from Madonna University animals' house.

Methods: Aspirin, 300 mg was dissolved in 30 mL of distilled water for injection while 20 mg of valproic acid was dissolved in 20 mL separately in the same fluid.

Animals were allowed two weeks acclimatization and then weighed shortly before commencement of drug dosing. Five animals were used for convulsion threshold titration while the balance of 25 was divided into 5 groups (A-E) of 5 animals per group.

They were provided with adequate feed, drinking water, comfortable metal cages and housed in a standard animal's house (Oliveira *et al*., 2008).

Group A was given 2 mg kg^{-1} of celecoxib orally. Group B was given 100 mg kg^{-1} of aspirin orally. Group C was given 2 mg kg^{-1} of celecoxib and 10 mg kg^{-1} of valproic acid orally and at intervals of 15 min. Group D was given 100 mg kg^{-1} of aspirin and 10 mg kg^{-1} of valproic acid orally and at intervals of 15 min. Group E was given 2 mL kg^{-1} of pure drinking water orally and acted as negative control.

All animals were then left for 30-45 min with free access to pure drinking water and feeds (*ad libitum*). Pentylenetetrazole (PTZ) 90 m kg^{-1}, used as seizure induction agent was then intraperitoneally, administered to all animals and observed for 0-24 h. Time-onset of myoclonic jerks, righting of hind limbs extension and or death were noted and taken as signs of seizures (Oliveira *et al*., 2008).

Prolongation of onset time of any or all of the signs was deduced as seizure inhibition and absolute seizure inhibition was taken as prolongation of onset time of any or all of the signs beyond 24 h from the time of convulsion induction.

Statistical analysis: Data where possible were analysed using SPSS-version 16.0. Results are presented as

Mean±SD. Comparative statistics was done using student t-Test and ANOVA. P-value less than 0.05 was taken as significant.

RESULTS

In titrating for the seizure inducible dose of PTZ, there were no observed seizure at 50 mg kg^{-1} of PTZ. At 60 m kg^{-1} of PTZ there was seizure after 22.5 min but no death was observed. At 70 m kg^{-1} seizure occurred after 20 min with no death and at 80 m kg^{-1} seizure occurred after 18 min still no death. However, at 90 m kg^{-1} of PTZ, seizure occurred after 8.20 min and death after 10.35 min. (Table 1).

After pre-treatment with celecoxib 2 m kg^{-1} mean seizure onset time was 29.00±0.8 min and mean death time of 32.00±2.1 min after administration of PTZ. Pre-treatment with aspirin 100 m kg^{-1} resulted in mean seizure time of 29.00±1.2 min and mean death time of 32.02±0.72 after administration of PTZ. Celecoxib plus valproic acid and aspirin plus valproic acid treated animals (groups C and D), exhibited no signs of convulsive seizures and remained alive throughout the experimental period (Table 2). The non-treated (negative control) animals (group E) exhibited convulsive signs and died at an average time of 10.4 min post PTZ administration (Table 2).

There was significant elevation in seizure onset time in groups A and B compared to control ($p<0.01$). There was however, no significant difference in seizure onset time between groups A and B ($p>0.79$). There was also a significant difference in mean seizure onset time between groups C and D and group A or B ($p<0.001$ in each case).

Table 1: Determination of seizure induction threshold and activity with PTZ

Dose of PTZ (mg kg^{-1})	No. of rats	Seizure onset time (min)	Death time (min)
50	1	Nil	Nil
60	1	22.5	Nil
70	1	20.00	Nil
80	1	18.00	Nil
90	1	8.20	10.35

Table 2: Mean seizure onset time and death time, following pretreatment with drugs and induction of seizure with PTZ

Groups/dose (mg kg^{-1})	No. of rats	Mean seizure onset time (min)	Mean death time (min)
A-Cele-2	5	29.00±0.8	32.00±2.1
B-Asp-100	5	29.00±1.2	32.02±0.72
C-Cele+Val	5	Nil	Nil
D-Asp+Val	5	Nil	Nil
E-Wat-2 mL kg^{-1}	5	8.280±0.11	10.42±3.2

A-Cele-2: 2 mg kg^{-1} of celecoxib, B-Asp-100: 100 mg kg^{-1} of aspirin, C-Cele+Val: 2 mg kg^{-1} of celecoxib +10 mg kg^{-1} of valproic acid, D-Asp+Val: 100 mg kg^{-1} of aspirin +10 mg kg^{-1} of valproic acid, E-Wat-2 mL kg^{-1}: 2 mL kg^{-1} of distilled water for injection and acted as negative control

DISCUSSION

The present study demonstrated a mild seizure inhibition activity that was similar for both aspirin and celecoxib. The mechanism of action of these agents is blockade of COX, an enzyme that acts on arachidonic acid to produce prostaglandin.

Prostaglandins have been shown to mediate neuronal inflammation. This prostaglandin induced neuronal inflammation is epileptogenic (Oliveira *et al*., 2008; Brandt *et al*., 2004). Recent studies have shown that therapeutic inhibition of prostaglandin reduces onset and latency period of seizures in experimental animals (Oliveira *et al*., 2008).

Epileptogenesis, the process leading to epilepsy of a hitherto non-epileptic neuron, is a presumed consequence of brain insults including head trauma, stroke, infections, tumors, Status Epilepticus (SE) and complex febrile seizures. Typically, brain insults produce morphological and functional alterations in the hippocampal formation, including neurodegeneration in CA1, CA3 and, most consistently, the dentate hilus (Langer *et al*., 2011). A more recent finding suggest that the prostaglandin receptor EP2 is critically involved in neuroinflammation and neurodegeneration. EP2 receptor antagonism is therefore a target for adjunct therapeutic strategy in status epilepticus (Jiang *et al*., 2013). Results following pilocarpine-induced status epilepticus indicate that neuronal COX-2 promotes early neuroprotection and then delayed neurodegeneration of CA1 pyramidal neurons. Neuronal COX-2 also promotes neurodegeneration of nearby somatostatin interneurons in the CA1 stratum oriens and dentate hilus (which themselves do not express COX-2). In addition neuronal COX-2 intensifies a broad inflammatory reaction involving numerous cytokines and other inflammatory mediators in the hippocampus and is essential for development of a leaky blood-brain barrier after seizures (Serrano *et al*., 2011). These findings point to a profound role of seizure-induced neuronal COX-2 expression in neuropathologies that accompany epileptogenesis (Serrano *et al*., 2011).

In their recent study on the effect of anti-inflammatory treatment on anticonvulsant actions in experimental models, Vezzani *et al*. (2013b), highlighted EP2 blockade as a possible therapeutic option for treating drug-resistant seizures and for prevention of epileptogenesis.

Prostaglandin inhibition may therefore be beneficial in controlling seizure as well as antiepileptogenesis. This appears contraditary as COX-2 blockade reduced only the severity of spontaneous seizures and indicated a disease-modifying effect. Some researched results suggests that COX-2 contributes to neuronal injury developing after SE, but, inhibition of COX-2 is not an effective means to modify epileptogenesis (Polascheck *et al*., 2010).

Control of epilepsy has primarily focused on suppressing seizure activity by antiepileptic drugs (AEDs) after epilepsy has developed. AEDs have greatly improved the lives of people with epilepsy. However, the belief that AEDs, in addition to suppressing seizures, alter the underlying epileptogenic process and, in doing so, the course of the disease and its prognosis, is not supported by the current clinical and experimental data. An intriguing possibility is to control acquired epilepsy by preventing epileptogenesis, the process by which the brain becomes epileptic (Loscher, 2002). A number of AEDs have been evaluated in clinical trials to test whether they prevent epileptogenesis in humans but to date no drug has been shown to be effective in such trials. Thus, there is a pressing need for drugs that are truly antiepileptogenic to either prevent epilepsy or alter its natural course (Loscher, 2002; Klitgaard and Pitkanen, 2003; Holtman *et al*., 2009).

A biomarker is defined as an objectively measured characteristic of a normal or pathologic biologic process. Identification and proper validation of biomarkers of epileptogenesis (the development of epilepsy) and ictogenesis (the propensity to generate spontaneous seizures) might predict the development of an epilepsy condition; identify the presence and severity of tissues capable of generating spontaneous seizures; measure progression after the condition is established and determine pharmacoresistance. Such biomarkers could be used to create animal models for more cost-effective screening of potential antiepileptogenic and antiseizure drugs and devices and to reduce the cost of clinical trials by enriching the trial population and acting as surrogate markers to shorten trial duration (Engel *et al*., 2013). Most experimental works are thus in animals but, extrapolated to man (Engel *et al*., 2013; Sequeira *et al*., 2013; Mazzuferi *et al*., 2012; Zamecnik, 2012).

The most profound discovery of this study is the synergistic absolute inhibition of seizures and death by combination of valproic acid with either aspirin or celecoxib. The study of Vezzani *et al*. (2013b) demonstrated that inhibition of EP2 molecules will aid drug treatment of drug resistant epilepsy. The present study has buttressed this with the synergistic finding.

Low dose aspirin is already established as effective in the prevention of stroke and better management of cardiovascular diseases; an effect that is also based on non-selective blockade of COX. Celecoxib may have selective COX-2 blockade and perhaps an advantage over aspirin which may cause bleeding and peptic ulcer diseases on prolonged administration but epilepsy occurs five times more commonly in developing nations. Celecoxib costs over 100 times more than aspirin and will not be readily available to most rural and even urban health facilities. Many researchers believe that at 75-300 mg day^{-1} for an average adult along with standard antiepileptics like valproic acid will not be associated with any adverse effects but will have better therapeutic goal in both drug-resistant and non drug-resistant chronic epilepsy/convulsive seizures as well as epileptogenesis.

CONCLUSION

It can be concluded that cyclooxygenase inhibition can reduce the effects of seizures/epilepsy. Celecoxib, a selective COX2 inhibitor, does not exhibit a superior inhibitory activity over aspirin, a non selective COX1 and 2 inhibitor. Cyclooxygenase inhibitor based combination therapy abolishes seizures. The study is though limited to animals, may be extrapolated to man. Thus, clinical trials involving low dose aspirin plus antiseizure agent is recommended especially in chronic epilepsy or drug-resistant seizures.

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