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RESEARCH ARTICLE

A Comparative Study on In-Vitro Nitric Oxide Scavenging Activity between Zonegran and Levetiracetram

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Abstract

Objective: Zonegran and Levetiracetram are two new ranges of anti-epileptics available in the market. Studies have been made earlier to demonstrate the antioxidant activity of the drugs Zonegran and Levetiracetam. Both have a good antioxidant property but there was not any comparative study for both the drugs. This study is to compare the anti-epileptic drugs Levetiracetam and Zonegran and demonstrate which drug has higher antioxidant property through Nitric Oxide radical scavenging activity. Methods: Nitric oxide scavenging activity was determined according to GriessIllosvoy reaction, with the help of Griess reagent. Ascorbic acid is used as positive control for the study. Conclusion: The study done suggests that both Zonegran and Levetiracetram possess antioxidant properties. At lower doses, Zonegran shows higher antioxidant potential over Levetiracetram and at higher doses, Levetiracetram shows slightly higher antioxidant property.

Keywords: *Free radicals; Antioxidants; Nitric oxide scavenging assay; Zonegran; Levetiracetram; Griess Illossvoy reaction.*

Introduction

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defence system of antioxidants. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged [1].

Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, sometimes included in this category.

The body cannot manufacture these micronutrients so they must be supplied in the diet. The process by which the free radicals are removed (scavenged) by enzymes or antioxidant is called free radical scavenging activity [2].

There are several methods for the free radical scavenging activity such as,

- DPPH Radical scavenging activity.
- Nitric Oxide radical scavenging activity.
- Hydrogen peroxide Radical scavenging activity.
- Superoxide Radical scavenging activity.

The nitric oxide scavenging activity is the method that is used in this process. Zonegran is a newer anticonvulsant with weak carbonic anhydrase inhibitory action that modifies maximal electroshock seizures and inhibits kindled seizures, but doesn't antagonize PTZ.

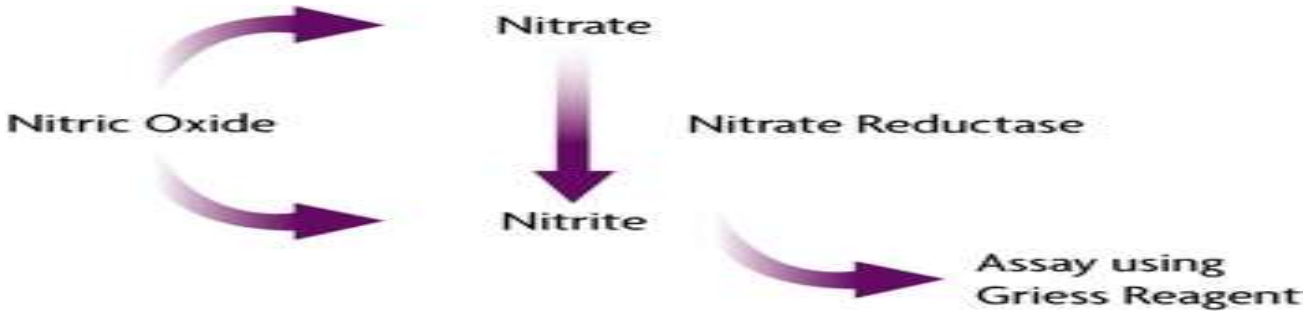
Prolongation of Na^+ channel inactivation, resulting in suppression of repetitive neuronal firing has been observed. It has also been found to suppress T-type of Ca^{2+} currents in certain neurons. It is well absorbed orally and excreted in urine. It has a $t_{1/2}$ of >60 hrs [3]. Levetiracetam is a unique anticonvulsant which suppresses kindled seizures, but is ineffective against maximal electroshock PTZ. Clinical efficacy has been demonstrated both as adjuvant medication as well as monotherapy in refractory partial seizures with or without generalization.

The mechanism of action is not known. It is absorbed orally, excreted in urine and has a $t_{1/2}$of 6-8 hrs [4].Various studies have been made earlier to demonstrate the antioxidant activity of the drugs, Zonegran and Levetiracetam. Both have a good antioxidant property but there was not any comparative study for both the drugs. This study is to compare the anti-epileptic drugs Levetiracetam and Zonegran and demonstrate which drug has higher anti oxidant property.

Materials & Methods

Principle

Nitric oxide is based on the principle that Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions which can be determined by the use of GriessIllosvoyreaction.



Source: The drugs Levetiracetam and Zonegran are procured from Sun Pharmaceutical Industries Ltd.

Reference antioxidant: Ascorbic Acid

Test drugs: Levetiracetam and Zonegran.

Procedure

Nitric oxide scavenging activity was determined according to GriessIllosvoy reaction. The reaction mixture contained: 10mM SNP (sodium nitroprusside) in 0.5M Phosphate buffer, pH 7.4 and various doses (5-200 µg/ml) of the sample solution in a final volume of 3ml. After incubation for 60min at 37^0 C, Griess reagent (0.1% α-napthyl-ethylenediamine in water and 1% sulphanillic acid in 5% H_2PO_4) was added. The pink chromophore generated during diazotization of nitrate ions with sulphanilamide and subsequent coupling with α-napthyl-ethylenediamine was measured spectrophometrically at 540nm. Ascorbic acid was used a positive control.

Calculation: Nitric oxide scavenging ability (%) was calculated by using the formula:

$$\frac{\text{A 540 nm of control} - \text{A 540 nm of sample} \times 100}{\text{A 540 nm of control}}$$

Results

Table 1: Percentage Inhibition of Nitric Oxide at Various Concentrations of the Drugs

S.No.	Concentration (mg)	% Inhibition of NO		
		Ascorbic Acid	Zonegran	Levetiracetram
1	5	78.57	68.36	65.30
2	25	81.63	70.40	69.38
3	50	87.75	73.46	72.44
4	100	93.87	75.51	76.53
5	200	97.95	-	80.61

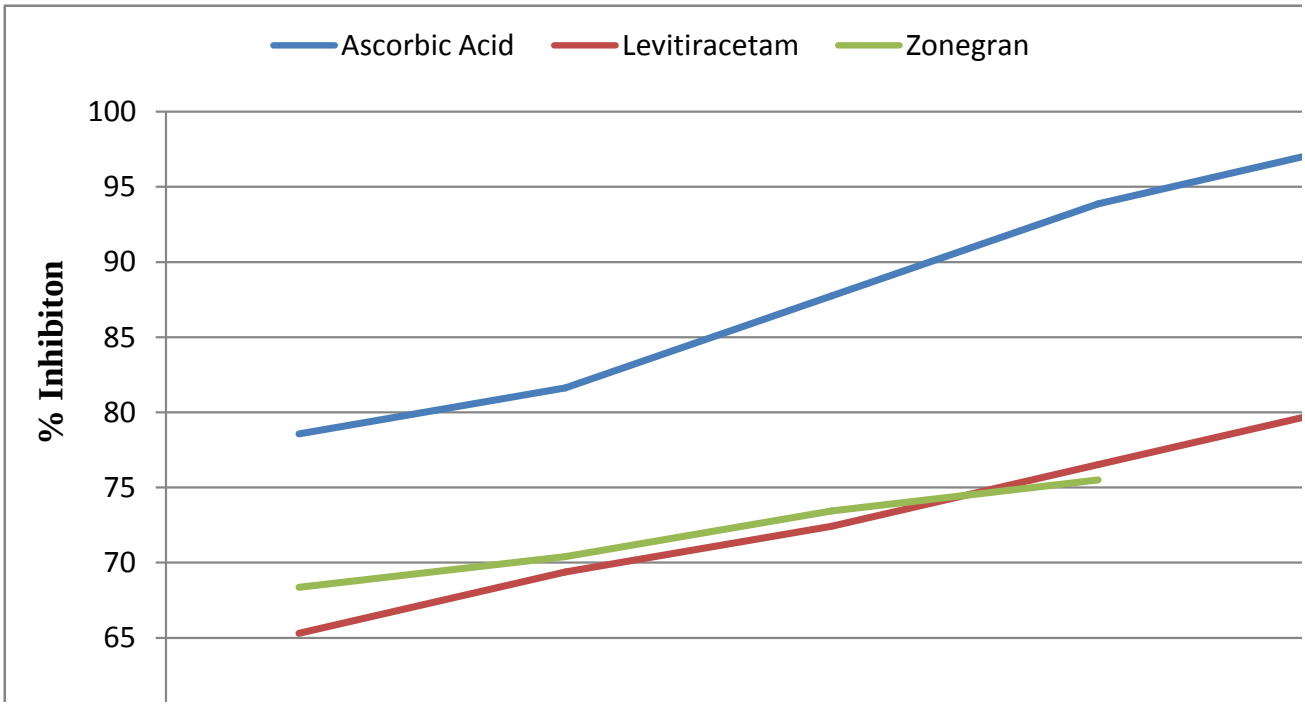
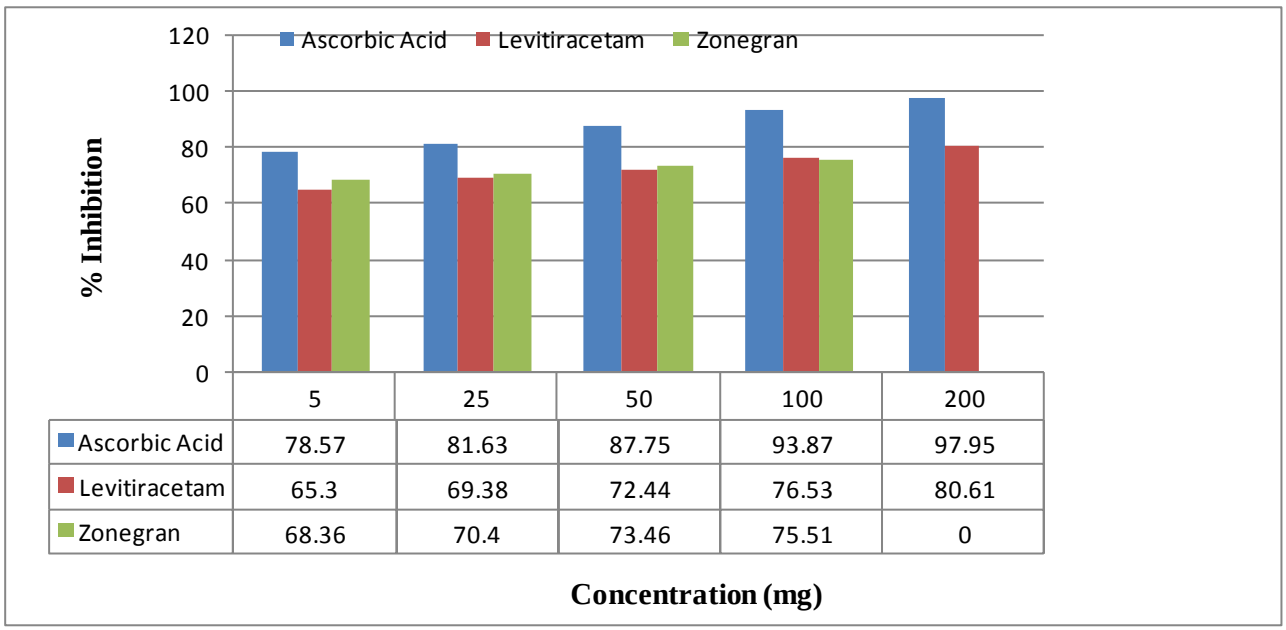


Figure 1: The line graph showing the Differences in the levels of % Inhibition of drugs



	5	25	50	100	200
Ascorbic Acid	78.57	81.63	87.75	93.87	97.95
Levitiracetam	65.3	69.38	72.44	76.53	80.61
Zonegran	68.36	70.4	73.46	75.51	0

Figure 2: Bar Graph depicting the %Inhibition of drugs at various concentrations

Discussion

In a normal cell, there is an appropriate oxidant: antioxidant balance. However, this balance can be shifted, when the production species is increased or when the levels of the antioxidants are diminished. This is called as oxidative stress. An antioxidant is a chemical substance that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of the free radicals which are normal by products of cell metabolism. Free radicals are formed when oxygen is metabolised or formed in the body and are chemical species that posses an unpaired electron in the outer shell of a molecule. These free radicals attack the nearest stable molecules, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. NO is generated in biological tissues by specific nitric oxide synthase, which metabolises arginine to citrulline with formation of NO through a five electron oxidative reaction. The compound sodium nirtoprusside is known to decompose in aqueous solution at a physiological pH (7.2) producing NO. Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite) the quantities of which can be determined using Griess reagent [5].

Zonegran is one of the new antiepileptic drugs. It is a sulphonamide derivative exhibiting a broad spectrum of antiepileptic activity and effective in the treatment of refractory seizures. It prevents the repetitive neuronal firing by blocking the voltage dependent Na^+ channels. It inhibits also T-type Ca^{2+} channels, which is demonstrated in the rat cerebral cortex. Another postulated mechanism is associated with the blockade of K^+ evoked glutamate-mediated synaptic excitation.

Other potential considered mechanism of zonesamide action comprises its inhibitory effect on the excessive nitric oxide production and free radical generation, including hydroxyl and NO radicals. By scavenging excess NO the drug can modulate cGMP formation which is known to be related to initiation and propagation of seizures. Zonegran is rapidly absorbed with a T_{max} ranging between 2.0 and 6.0 hours and the bioavailability is best with oral mode of intake of the drug.

The half-life of the drug varies between 52 to 66 hours. Zonesamide is partly excreted by renal route and partly metabolised by cytochrome P450 mediated reduction [3]. According to Akitane Mori et al, the free radical scavenging activities of ZNS were analyzed by using electron spin resonance. ZNS in the mmolar range, scavenged hydroxyl and nitric oxide radicals in a dose dependant manner, which suggests that the mechanism of anti epileptic effect of ZNS may be involved in the protection of neurons from free radical damage and stabilization of neuronal membranes [6].

Levetiracetram is a broad spectrum anti epileptic that was approved by the US Food and Drug Administration in 1999 and has become one of the most commonly prescribed drugs for treatment of general and partial seizures.

The mechanism of action of the drug is not clearly known, but there are few hypotheses that are found related to the mechanism of action of the drug. The synaptic vesicle protein2 is a transmembrane integral protein that is present at all synaptic sites. It has 3 isoforms 2A, 2B and 2C. SV2A is the most common form. It has been shown to interact with the pre-synaptic protein, synaptotagmin, which is considered the Ca^{2+} sensor for regulation of Ca^{2+} dependant exocytosis of synaptic vesicles.

SV2A is involved in controlling exocytosis neurotransmitter vesicles. SV2A is not essential for synaptic transmission, SV2A knockout mice exhibit seizures. Thus, SV2A ligands could protect against seizures and this is the likely target of levetiracetram. Disturbances in the Ca^{2+} homeostatic mechanisms resulting in elevated intracellular Ca^{2+} levels have been reported in multiple neurological disorders including, stroke, and movement disorder.

Constant Ca^{2+} entry into the neurons via the NMDA receptors during SE and persistent leak of Ca^{2+} from intracellular Ca^{2+} stores have now been firmly established in SE induced epilepsy. Research has shown that blocking the ryanodine receptor mediated Ca^{2+} leak from the endoplasmic reticulum using dantrolene, lowers the elevated Ca^{2+} post SE and prevents the development of epileptiform discharges. Levetiracetram reduces the intra neuronal Ca^{2+} levels by inhibiting ryanodine and IP3 receptor dependant Ca^{2+} release from endoplasmic reticulum.

In addition the drug has shown to inhibit Ca^{2+} entry by blocking the L-type Ca^{2+} channels in the hippocampal neurons of spontaneously epileptic rats. The half life of levetiracetram is 7±1 hours. The efficacy is established with twice daily dosing. The major route of elimination for the (95%) drug is urine [4].

According to Aline de Albuquerque Oliveira et al, it was demonstrated by an in-vitro model, the anti oxidant ability of the anti epileptic drug, is mediated by at least in part by nitrite-nitrate contents, preservation of catalase activity at control parameters. This infers the participation of this effect in the neuroprotective mechanism of these drugs, and reinforces, even indirectly, the hypothesis of oxidative damage in the pathophysiology of epilepsy [7].

Conclusion

The In-vitro study suggests that both Zonegran and Levetiracetram possess antioxidant properties. At lower doses, Zonegran shows higher antioxidant potential over Levetiracetram and at higher doses, Levetiracetram shows slightly higher

antioxidant property. However, the preference of selecting one drug over the other depends on the indication and the risk benefit ratio. Since the study made is an in-vitro study, more related studies in-vivo is required to understand more beneficial effects of the drugs.

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