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Implications of oxidative-nitrosative stress (ONS) for astrocytic functions in the central nervous system (CNS) pathology

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Oxidative stress (OS) and nitrosative stress, further collectively referred to as oxidative-nitrosative stress (ONS), is a major causative factor in most if not all pathological conditions of the CNS. ONS is associated with excessive accumulation of reactive oxygen (ROS) and nitrogen species (RNS). Of these, the superoxide anion radical ($O_2^{\bullet-}$), the hydroxyl radical (•OH) and peroxynitrite ($ONOO^-$), a product of conjugation of nitric oxide and superoxide, bear the brunt for most of the cell damage [Valko et al., Int J Biochem Cell Biol, 2007; Dikalov, Free Radical Biol Med, 2011]. While traditional views have considered the neuron as the sole target and victim of ONS, growing evidence implicates of astrocytes in the response. One or any combination of the three scenarios ensuing the reaction of astrocytes to ONS have been envisaged: i) direct transmission of ONS to the neurons; ii) impairment of astrocytic metabolism and function contributing to dysfunction of neurons, iii) onset or/and improvement of neuroprotective responses. While none of the three scenarios occurs exclusively in any of the pathological conditions, multiple examples point to domination of one or the other. Most illustrious manifestations of each, and the hypothetical or proven underlying mechanisms are provided below.

Transmission of the ONS wave from astrocytes to neurons occurs under conditions involving inflammatory response. Cytokines released from microglia activate the inducible form of nitric oxide synthase (iNOS) in astrocytes. Excess of NO produced by iNOS damages astrocytic and neuronal mitochondria alike which leads to excitotoxic neuronal damage which is mediated by excessive release of neuronal and astroglial glutamate followed by overactivation of neuronal NMDA receptors [Bal-Price and Brown, J Neurosci, 2001]. The deleterious effects in mitochondria are due to the activity of NO and its active metabolites: peroxynitrite and S-nitrosothioles (R-S-NO), the latter being derived from the conjugation of NO with a peptide chain, often involving molecules critical for cell metabolism and survival [Brown & Bal-Price, Mol Neurobiol, 2003]. In some instances, such as in multiple sclerosis-affected brain, increased iNOS immunoreactivity may occur exclusively in astrocytes, bypassing microglia [Liu et al., Am J Pathol, 2001].

ONS occurring in astrocytes is directly involved in the pathogenesis of ammonia-induced cerebral edema, which is the major cause of death in patients in acute hepatic encephalopathy (HE) resulting from acute liver failure (ALF). ALF-induced cerebral edema is mainly cytotoxic in nature, resulting from astrocytic swelling which is directly related to ONS. Accumulation of ROS and RNS in ammonia-exposed astrocytes has been shown to result from i) excessive activation of NMDA receptors on astrocytes [Schliess et al., FASEB J, 2002; Zielińska et al., Neurochem Int, 2003; Kruczek et al., Biol Chem, 2011], and/or ii) increased NADPH oxidase activity [Reinehr et al., Glia, 2007; Skowrońska et al., J Neurochem, 2010].

Improvement of astrocytic neuroprotective functions elicited by ONS is mediated by increased astrocytic synthesis of glutathione (GSH), the major antioxidant in mammalian tissues. In the CNS, astrocytes are the main site of GSH synthesis, and degradation products of astrocytic GSH are the metabolic precursors of GSH synthesis in neurons [Dringen et al., J Neurosci, 1999]. Astrocytic GSH synthesis is upregulated in both acute and chronic HE [Hilgier et al., Toxicol Sci, 2010], which is thought to contribute to the scarcity of irreversible neuronal damage observed in this disease as compared to typical neurodegenerative disorders. Increased expression of enzymes involved in GSH synthesis and uptake of its precursors and, hence, the rate of GSH renewal is under control of a specific transcription factor Nrf2 [Lee et al., J Biol Chem, 2003]. Nrf2 is expressed constitutively in the cell cytoplasm which following its synthesis is translocated directly to the nucleus, where it activates the antioxidant response element (ARE) (for details on how the Nrf2-ARE system is regulated the reader is referred to an excellent review by Nguyen et al. [J Biol Chem, 2009]. A number of recent experimental studies in *in vitro* and *in vivo* models of neurode-

generative diseases have proven that increased expression/activity of Nrf2 in glia confers protection to neurons against ONS. In astrocytic-neuronal co-cultures, specific infection of astrocytes with adenovirus carrying Nrf2 message, increased total intracellular GSH and its release into the culture medium, and protected neurons from oxidative glutamate toxicity [Shih et al., J Neurosci, 2003]. In astrocyte/neuronal co-culture derived from Nrf2 knock-out (Nrf2–/–) mice, Nrf2 transfection using the above paradigm decreased the sensitivity of the neurons to MPTP, the toxin modeling Parkinson disease symptoms [Chen et al., Proc Natl Acad Sci USA, 2009]. Nrf2 transfection *in vivo* to astrocytes residing the CNS of SOD1 G93A mice, which constitute a model of amyotrophic lateral sclerosis (ALS) attenuated the most prominent neurological and biochemical symptoms of the disease [Vargas et al., J Neurosci, 2008].

While specific involvement of ONS in the astrocytic dysfunction related to epilepsy remains to be delineated, indirect evidence appears to favor this concept. High levels of ONS markers and low glutathione peroxidase (GP) activity are recorded in patients with drug-resistant epilepsy, and become normalized following resection of the epileptic foci [Lopez et al., Clin Biochem, 2007]. Induction of seizures in epilepsy-prone rats (GEPR-9s) with kainic acid was associated with an array of biochemical manifestations of ONS, including increased GP immunostaining in astrocytes [Shin et al., Neurochem Int, 2008]. Given the well documented role of modifications of astroglial functions (including gliotransmission) in the generation and propagation of seizure activity [Jabs et al., Epilepsia, 2008], further studies on the roles of astrocytic ONS in epilepsy are clearly warranted.

Interactions of 1-methyl-1,2,3,4-tetrahydroisoquinoline with the various antiepileptic drugs in the mouse maximal electroshock- induced seizure model

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1-Methyl-1,2,3,4-tetrahydroisoquinoline (1MeTHIQ) is present in the human and rodent brains as an endogenous parkinsonism-preventing substance and exhibits neuroprotective properties through the regulation of dopaminergic activity [Antkiewicz-Michaluk et al., Neurotox Res, 2011]. 1MeTHIQ was proved to play a key role in neuroprotection against numerous experimental neurotoxins, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 1-methyl-4-phenylpyridinum, β-carbolines, tetrahydroisoquinoline, 1-benzyl-1,2,3,4-tetrahydroisoquinoline and rotenone [Antkiewicz-Michaluk et al., Eur J Pharmacol, 2003; Kotake et al., J Neurochem, 1995; Tasaki et al., Nature, 1991]. 1-MeTHIQ offers a unique and complex mechanism of neuroprotection in which antagonism to the glutamatergic system may play a very important role, suggesting the potential of 1MeTHIQ as a therapeutic agent in various neurodegenerative disorders of the central nervous system [Antkiewicz-Michaluk et al., J Neurochem, 2006]. Additionally, 1-MeTHIQ antagonizes a rise in brain dopamine metabolism, glutamate release in frontal cortex and locomotor hyperactivity produced by MK-801, but not the impairment of working memory [Pietraszek et al., Neurotox Res, 2009]. Moreover, 1MeTHIQ also shares neuroprotective abilities with established uncompetitive NMDA receptor antagonists, which may suggest that the inhibitory effect of 1MeTHIQ on NMDA receptors plays a key role in its anti-excitotoxic activity [Kuszczyk et al., Pharmacol Rep, 2010]. It seems that antagonism of NMDA receptors is a new mechanism of 1MeTIQ-evoked neuroprotection based on the induction of neuronal tolerance to excitotoxicity.

Considering neuroprotective properties of 1MeTHIQ, the endogenous compound has been studied in the maximal electroshock (MES)-induced tonic sei-

zure model in mice. According to the obtained results 1MeTHIQ elevated in a dose-dependent manner the threshold for electroconvulsions in mice [Luszczki et al., Neuropharmacology, 2006], and possessed the anticonvulsant action in mice at various pretreatment times ranging between 5 and 120 min after its systemic (*ip*) administration [Luszczki et al., Eur J Pharmacol, 2009]. Additionally, 1MeTHIQ enhanced the protective action of carbamazepine (CBZ) and valproate (VPA), but not that of phenobarbital (PB) or phenytoin (PHT) against MES-induced seizures in mice [Luszczki et al., Neuropharmacology, 2006]. Moreover, the type I isobolographic analysis revealed that the combination of 1MeTHIQ with PB at the fixed-ratios of 1:3, 1:1 and 3:1 exerted supra-additive (synergistic) interaction in the MES-induced seizure test in mice. In contrast, the combinations of 1MeTHIQ with CBZ, PHT and VPA exerted additive interaction for the fixed-ratio combinations of 1:3, 1:1 and 3:1 in the mouse MES-model [Luszczki et al., Eur J Pharmacol, 2009]. Additionally, the type I isobolographic analysis revealed the existence of supra-additive interaction between 1MeTHIQ and topiramate (TPM) in the mouse MES model [Luszczki et al., Epilepsy Res, 2010].

The aim of the presented study was to characterize the anticonvulsant effects of 1MeTHIQ in combination with various antiepileptic drugs (AEDs), including clonazepam (CZP), ethosuximide (ETS), tiagabine (TGB), levetiracetam (LEV), gabapentin (GBP) and vigabatrin (VGB) in the mouse MES-induced seizure model. The anticonvulsant interaction profile between 1MeTHIQ and VGB, CZP, ETS, TGB, LEV, GBP and CZP in the mouse MES model was determined using a type II isobolographic analysis. This type of isobolography can be applied if one of the tested drugs produces no effects and is considered as virtually ineffective in the proposed experimental model of epilepsy [Luszczki et al., Epilepsy Res, 2012]. Of note, all the tested AEDs were inactive in the mouse MES-induced seizure model. This is why, the type II isobolographic analysis was used in the present study. Acute adverse effects produced by the combinations of 1MeTHIQ with the studied AEDs with respect to motor coordination and muscular strength were assessed in the chimney and grip-strength tests, respectively. Total brain concentrations of 1MeTHIQ and the studied AEDs were measured to determine any pharmacokinetic contribution to the observed anticonvulsant effect.

The results from the present study indicate the supra-additive (synergistic) interaction between 1MeTHIQ and ETS (at the fixed-ratio of 1:10), CZP (at the fixed-ratios of 50:1 and 25:1), and GBP (at all the tested fixed-ratios of 1:10, 1:5, 1:2 and 1:1) in the mouse MES model. All the interactions appear to be particularly favorable from a clinical view point. Pharmacokinetic estimation of total brain concentrations of 1MeTHIQ with ETS and CZP showed no changes in the total brain concentrations of the studied drugs. In contrast, the measurement of total brain concentrations of 1MeTHIQ and GBP in combination at the fixed-ratio of 1:10 showed a significant increase in total brain concentrations of 1MeTHIQ. Additional measurement of total brain concentrations of 1MeTHIQ and GBP the fixed-ratio of 1:1 indicated no significant changes in total brain concentrations of 1MeTHIQ and GBP in experimental animals. The remaining combinations of 1MeTHIQ with ETS (at the fixed-ratios of 1:1, 1:2 and 1:5) and CZP (at the fixed-ratios of 200:1 and 100:1), as well as, all combinations of 1MeTHIQ with TGB, LEV and VGB were additive in the mouse MES-induced seizure model. Moreover, all the studied AED combinations produced no acute adverse effects with respect to motor coordination and muscular strength as assessed in the chimney and grip-strength tests, respectively.

In conclusion, the type II isobolographic analysis used in the present study confirmed a strong influence of 1MeTHIQ on the anticonvulsant efficacy of ETS, GBP and CZP in the MES-induced seizure test in mice, even if the tested AEDs are ineffective in this animal model of epilepsy. The estimation of total brain concentrations of 1MeTHIQ and AEDs affirmed no pharmacokinetic interactions between the studied drugs, except for the combination of 1MeTHIQ with GBP at the fixed-ratio of 1:10. The synergistic interaction between 1MeTHIQ and ETS, CZP and GBP as well as the additive interaction of 1MeTHIQ with TGB, LEV and VGB in the mouse MES model are worthy of recommendation for further clinical settings.

Leptin and epilepsy

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Leptin (from the greek word *leptos* meaning "thin") was identified in 1994 by positional cloning of the *ob* gene responsible for obesity in homozygous *ob/ob* mice – the experimental model of obesity characterized in 1950s and extensively used since that time in experimental studies. Leptin, encoded by the *Ob* gene, is the 16 kDa protein synthesized and secreted by white adipose tissue, which acts on hypothalamic neurons to suppress food intake and increase energy expenditure. Homozygous *ob/ob* mice do not produce functional leptin and consequently develop severe obesity as a result of hyperphagia and reduced energy expenditure (decrease in locomotor activity and lower basal metabolic rate as reflected by decrease in body temperature). In addition, *ob/ob* mice exhibit severe insulin resistance, hyperinsulinemia, hyperglycemia, hypercortisolemia and infertility. Administration of recombinant leptin normalizes body weight and corrects all metabolic abnormalities in these animals [Zhang et al., Nature, 2004]. Inherited leptin deficiency is a very rare cause of obesity in humans. Most obese individuals have high plasma leptin concentration, which reflects greater amount of adipose tissue and resistance of hypothalamus to anorectic effect of this hormone. Leptin is responsible for "adipostat" – the negative feedback mechanism which regulates food intake depending on the amount of triglyceride stores in adipose tissue. Currently it is suspected that the main physiological role of leptin is to signal energy deficit. When the amount of adipose tissue decreases as the effect of food restriction, hypoleptinemia results in increase in appetite, decrease in energy expenditure, and inhibition of energy-consuming processes such as inflammatory/immune response and reproduction.

Leptin receptor belongs to a class I cytokine receptors and is a single membrane-spanning protein which consists of extracellular, transmembrane and intracellular domains. Six isoforms of the leptin receptor, Ob-Ra through Ob-Re, have been identified. They are encoded by a single gene and are synthesized by alternative mRNA splicing. All of them (except Ob-Re) share the same extracellular and transmembrane domains but differ in intracellular domain. The longest isoform, Ob-Rb, responsible for anorectic effect of leptin stimulates cytosolic tyrosine kinase Jak2 which phosphorylates Signal Transducer and Activator of Transcription-3 (STAT-3) protein; the latter translocates to the nucleus and stimulates the expression of target genes. In addition, Ob-Rb can signal through other mechanisms such as protein kinases C, mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K) or nitric oxide. Ob-Ra, Ob-Rc and Ob-Rd have shorter intracellular domains and cannot stimulate the Jak2-STAT-3 pathway but are able to trigger other signaling mechanisms. Ob-Re is a truncated receptor which consist only of a part of extracellular domain; it circulates in plasma as a soluble leptin receptor (sLR) – the main leptin-transporting protein [Fruhbeck, Biochem J, 2006].

Leptin receptors are expressed in almost all tissues and chronic hyperleptinemia associated with the metabolic syndrome is implicated in the pathogenesis of many disorders including arterial hypertension, atherosclerosis, heart failure, autoimmune and inflammatory diseases (multiple sclerosis, rheumatoid arthritis, etc.), breast, colon and endometrial cancers, gestational diabetes, polycystic ovarian syndrome and non-alcoholic fatty liver disease. In experimental studies leptin has been demonstrated to have both pro- and anticonvulsant activity. For example, intracerebroventricularly administered leptin increased the frequency of penicillin-induced epileptiform EEG activity in the rat by stimulating neuronal NO synthase [Ayyildiz et al., Brain Res Bull, 2006; Aslan et al., Brain Res, 2010]. In mice, leptin administered intraperitoneally augmented NMDA-induced and, to a lesser extent, AMPA- or kainate-induced clonic and tonic seizures [Lynch et al., Brain Res Bull, 2010]. In contrast, centrally administered leptin reduced the amplitude and frequency of seizures induced by 4-aminopyridine (the antagonist of voltage-sensitive K^+ channels) in the rat. Similarly, intranasally administered leptin exhibited anticonvulsant activity in mouse pentetrazole model [Xu et al., J Clin Invest, 2008]. These effects are most likely associated with the inhibition of AMPA receptor-, but not NMDA receptor-, mediated neurotransmission. Consistently with these data, leptin deficient *ob/ob* mice demonstrate greater

frequency of generalized clonic and clonic-tonic seizures after intraperitoneal pentetrazole injection [Erbayat-Altay et al., Neurosci Lett, 2008]. These data suggested that leptin, (especially administered intranasally) could be a novel antiepileptic drug. However, in the above mentioned studies leptin was administered before inducing acute seizures and it is unclear if it is effective in chronic epilepsy. In addition, chronic elevation of leptin to supraphysiological level often induces leptin resistance. Finally, because leptin was demonstrated to inhibit glutaminergic neurotransmission in hippocampal neurons, the possible adverse effects such as impairment of learning and memory should be taken into account.

Leptin had neuroprotective activity in different experimental models. For example, leptin inhibited apoptosis of SH-SY5Y neuroblastoma cells induced by serum deprivation [Russo et al., Endocrinology, 2004] or MPTP [Lu et al., Neurosci Lett, 2006]. Similarly, leptin protected cortical neurons against hypoxia or glucose deficiency-induced apoptosis [Zhang et al., Stroke, 2007] and hippocampal neurons against NMDA- or oxidative stress-induced apoptosis [Guo et al., J Biol Chem 2008]. *In vivo*, leptin reduced status epilepticus-induced neurodegeneration of hippocampal neurons [Obeid, Epilepsy Behav, 2010].

Many of the currently used antiepileptic medications affect plasma leptin concentration. Valproate, carbamazepine, gabapentin, vigabatrin and pregabalin increase plasma leptin concentration, topiramate and felbamate have the opposite effects, whereas diphenylhydantoin, oxcarbazepine, levetiracetam, lamotrigine and tiagabine do not change leptin level. Changes in plasma leptin in patients treated with antiepileptic drugs usually parallel changes in adiposity and body weight [Hamed, Epilepsy Res, 2007].

Brand and generic drugs in epilepsy

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Among ca. 50 million people suffering from epilepsy in the world, 70% display an improvement in seizure control as a result of pharmacotherapy [Villanueva et al., Epilepsy Behav, 2010]. When AEDs are prescribed for the first time to patients, there is a choice between the original brand of a specific drug or a generic option. Epilepsy usually requires long-term therapy (sometimes lasting even for patients' whole lives), a continuity of treatment is an issue of a crucial meaning for patients [Kramer et al., Epilepsy Behav, 2007].

New and successfully approved drugs produced by pharmaceutical companies are protected legally and commercially from other companies by patents. In the European Union, the patenting process, usually completed by the phase II trial stage, can give a 10-year period of exclusive production and marketing rights to the manufacturer of the "innovator" product. The pricing during this period is calculated and set at the level so as to ensure resources for research and development as well as profit, a certain percentage of which is reinvested in developing new products. When this time period is over, other manufacturers may seek licenses to market forms of the innovative product. Additional clinical trials of these formulations are not required if such manufacturers are able to prove an "essential similarity" in its qualitative and quantitative composition – in terms of its active substances having the same pharmaceutical form and bioequivalence. The formulation can be marketed as "generic" without the need for expensive regulatory clinical trials [Heaney and Sander, Lancet Neurol, 2007].

As mentioned above, generic drugs are expected to be essentially similar to their corresponding brand-name counterparts. This means that they must contain the same active ingredient as the original brand and be available at the same dose and by the same route of administration. They have to demonstrate acceptable bioequivalence with the original brand, although the range considered acceptable is wide and is evaluated only in healthy volunteers. The following section

presents in detail the important question of bioequivalence for generic drugs.

In contrast, the generic drug may differ from the original one in the manufacturing process employed, in the excipients with which the active principle is associated in the final drug product, and in the appearance of the drug product (shape, color, or both). As a result of those variations, dissolution rates in the gastrointestinal tract may be influenced and, in consequence, absorption of the drug substance and overall pharmacokinetics. In addition, generic drugs may have a different shelf life than those of original brand [Rosenbaum et al., Epilepsia, 1994].

The Center for Drug Evaluation and Research (CDER), part of the Food and Drug Administration (FDA), is responsible for determining whether a generic formulation is equivalent to the branded counterpart. Created in 1987, CDER's focus became essential to address the increasing volume of new drug applications (NDAs), among other issues [Berg et al., Epilepsy Behav, 2008]. CDER created the Office of Generic Drugs, which assumed responsibility for review of abbreviated new drug applications (ANDAs) and the Generic Drugs Advisory Committee. Key to CDER activity is the publication Approved Drug Products with Therapeutic Equivalence Evaluations, also known as the "Orange Book." As defined in the Orange Book [Berg et al., Epilepsy Behav, 2008], "Drug products are considered to be therapeutic equivalents (...) if they can be expected to have the same clinical effect and safety profile when administered to patients under the conditions specified in the labeling." The Orange Book encourages substitution with generic drugs to contain costs [Berg et al., Epilepsy Behav, 2008].

Generic pharmaceuticals can provide economic benefits at the individual and system levels [Haas et al., Ann Intern Med, 2005]. However, a debate has been present for more than two decades about the advantages of generic substitutes [Schwartz, Am J Med, 1985]. For medications with narrow therapeutic ranges, such as antiepileptic drugs (AEDs), the economic benefits are not obvious. It has been argued that the savings achieved by switching a patient with controlled epilepsy from a brand to a generic AED could be offset, at least in part, by breakthrough seizures, other adverse effects, and the necessity for increased supervision of the patient's condition [Guberman and Corman, Can J Neurol Sci, 2000; Crawford et al., Seizure, 2006].

When generic substitution occurs, it is recommended by many practice guidelines that the plasma levels be monitored to ensure that drug exposure remains unchanged. If necessary, the dose can be adjusted to maintain plasma levels and thus avoid potential problems associated with too low (loss of seizure control) or too high (emergence of side effects) exposure before they arise. Ideally, plasma levels need to be monitored both before and after switching into generics. In practice, this is not always feasible and has obvious cost implications.

What is more, the optimal range of plasma levels are not adequately characterized for some new AEDs. Nonetheless, systematic collection of data on plasma levels during generic switching is very important as it provides an opportunity to assess bioequivalence in routine conditions of care hence, to identify generic forms that may be associated with a particular risk of inappropriate drug exposure [Kramer et al., Epilepsy Behav, 2007].

The process of switching from one drug form to another may bring certain psychological consequences for patients, which must not be neglected. A primary care-based survey in England [Crawford, Seizure, 1996] indicated that 7% of patients prescribed AEDs admitted to have experienced anxiety as a result of changing the form of their medication. The risk of patients reporting side effects may be increased by the expectations following a switch. As presented in the Anti-Epileptic Medication Packaging Survey of 1835 patients with epilepsy in the United Kingdom, 32% of patients whose AED prescription was switched from brand to generic or between generics in the previous year claimed that this was associated with the emergence of more or different side effects [Goodwin, Nurs Times, 2005]. As a result, the need for consulting and thereby a growth in health care cost may be observed [Jumaoas, Epilepsia, 1989]. According to a multinational telephone-based survey, in which 974 patients and 435 physicians were examined, 23% of patients expressed their concern about possible breakthrough seizures as a result of a generic substitution and 27% of physicians believed that they had treated patients in whom this had occurred [Haskins et al., Epilepsy Behav, 2005]. Furthermore, 58% of patients indicated to have been uncomfortable with generic substitution and 31% of physicians were uncomfortable about prescribing a generic AED.

Generic drugs may differ from original ones in shape, color and name and this seemingly trivial issue

may be of a great importance to people with cognitive impairment, which is frequent in epilepsy. Such patients may have concerns about whether the medication is the same [Goodwin, Nurs Times, 2005] or whether mistakes have been made. These patients may have developed habits and routines for taking medication that can be disrupted by unfamiliar drug forms or names [Kramer et al., Epilepsy Behav, 2007].

For patients whose seizures are well controlled on their current therapy, regardless a brand or generic AED is employed, the treatment goal is to minimize the risk of relapse. In such instances, it is recommended by most practice guidelines not to switch to another form of a drug [Kramer et al., Epilepsy Behav, 2007]. Apart from loss of seizure control, other potential issues in switching brands in these patients are the risk of emergence of adverse events, the risk of poor adherence, the risk of mistakes, and psychological factors. It is important to plan a decision of switching from one brand to another with an appropriate time schedule. Patients could be encouraged to keep a diary to record the exact date of the switch as well as any seizures or adverse events before and after the switch has been implemented. There is no information available on the relative benefits of different substitution schedules, although it would not unjustified to think that simple switch from one form to another could only bring negative or unsatisfactory results. As it has been indicated before, monitoring of plasma levels before and after switching is generally necessary, in particular for drugs with a narrow therapeutic index, to ensure the continuity in exposure. Obviously, this could generate certain costs, which should be taken into account along with a convenience of such monitoring [Majkowski et al., Epileptologia, 2004]. In countries, where pharmacists are eligible or even obliged to substitute a cheaper generic for the drug prescribed by the physician, it is important that they be aware of the need to involve the physician in any decision to switch brands. Pharmacists must realize the risks associated with generic substitution and of the possible legal consequences of loss of seizure control in previously well-controlled patients.

The issues associated with generic substitution may be especially pertinent in certain groups of patients with epilepsy. These high-risk groups have not been studied systematically, and there is generally little or no documented evidence that allows the real impact of these issues to be quantified. There are, however, certain hypothetical risks result from the treatment switch and they need to be taken into account by physicians. If patients have already experienced a generic substitution, it may be useful to collect a detailed history of the switch, to identify potential problems in the individual patient and to allow a more precise estimation of the risk-benefit ratio of the intended substitution. The following paragraphs present examples of hypothetical risks in specific patient groups. If generic alternatives are only available as tablets, this could present an issue for children and the elderly, due to possible difficulties in swallowing a drug. Patients with other comorbid diseases may be more sensitive to side effects if drug exposure increases after a switch. In the case of hepatic or renal disease, drug elimination may be affected. Due to pharmacokinetic interactions between oral contraceptive drugs and AEDs, generic substitution may be problematic. If plasma levels change abruptly after switching, contraceptive failure may occur [Crawford, CNS Drugs, 2002].

To manage expectations and optimize adherence in patients, they should receive detailed and adequate explanations from neurologists, pharmacists, or both. As well as the prescribing physician, patient associations could play an important role in patient education concerning generic AEDs.

Another problem may arise in health care systems, where reference price policy is employed. Patients, when using one form of a given AED rather than another, may be expected to cover the difference between the prescribed drugs and the reference price. The issues need to be explained to the patient, and consent for any additional cost must be obtained. Patients for whom generic substitution is not considered appropriate, for example, those who are well controlled on their present drug, should be advised to keep, wherever possible, to the same form if they change physician or pharmacist. Obviously, the patient may have bigger or smaller influence upon decisions of health care institutions, depending on countries, culture and social groups. However, an informed and participative patient is more likely to benefit from the most appropriate care. Providing adequate information to patients is vital when they are prescribed a combination of AEDs. In such instances there is a significant risk of drug interactions if bioequivalence is not absolute. Patients treated with multiple AEDs may obtain information from pharmacists.

From the viewpoint of a health care resources management, generic substitution may be a beneficial and recommended solution in terms of keeping the costs

of therapies at affordable levels. On the other hand, potential issues related to systematic generic substitution of AEDs include inadequate bioequivalence, loss of seizure control, emergence of side effects, and poor adherence, which also carry a considerable costs. Thus, all issues must be balanced adequately and carefully in order to ensure patients a proper treatment and this should be assessed individually for any patient in whom switching to a generic alternative is being considered. In this context, it may be helpful to elaborate European guidelines to help clinicians and pharmacists understand the issues related to generic substitution of antiepileptic drugs.

Influence of carbenoxolone (an inhibitor of gap-junctions) on the protective activity of some antiepileptic drugs in the pentylenetetrazol seizure model in mice

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Epilepsy is one of the most frequent disorders of the central nervous system (CNS). It is estimated that there are over 50 million epileptic patients all over the world, which is approximately 1% of the population [Chrościńska-Krawczyk et al., Pharmacol Rep, 2011]. Carbenoxolone (CBX), previously used as an antiulcer drug, is an inhibitior of gap junctional intercellular communication and inhibits 11β-hydroxysteroid dehydrogenase [Endong et al., J Neuroimmunol, 2011]. It is noteworthy that communication between neurons through gap-junctions is believed to represent an important synchronizing mechanism in the brain [Bennett, J Neurocytol, 1997]. Experimental data provides evidence that gap-junction blockers exhibit some anticonvulsant activity per se and CBX potentiated the anticonvulsant efficacy of a number of antiepileptic drugs (AEDs) against audiogenic seizures in mice [Gareri et al., Eur J Pharmacol, 2004]. Taking what was stated above into consideration, the aim of the present study was to determine the influence of CBX on the protective activity of some AEDs: tiagabine (TGB), valproate (VPA), gabapentin (GBP) and ethosuximide (ETS) in the pentylenetetrazol (PTZ) seizure model in mice.

The experiments were conducted on male Swiss mice. AEDs and CBX were administered intraperitoneally. The threshold for clonic convulsions was determined in control mice by subcutaneous administration of PTZ at doses ranging from 50 to 100 mg/kg. The convulsive action of PTZ was evaluated as the CD_{50} (median convulsive dose, i.e. the dose of PTZ necessary to produce clonic seizures in 50% of the mice tested). The possible anticonvulsant effect of CBX was evaluated in the threshold PTZ test. The anticonvulsant activity of AEDs, administered alone or in combination with CBX, was determined against PTZ at a dose of 100 mg/kg, its predetermined CD_{97} (the dose necessary to induce clonic seizures in 97% of animals). The anticonvulsant activity of the AEDs, alone or combined with CBX, was determined by evaluating their respective ED_{50} values, i.e. the calculated doses required to block clonic seizures in 50% of mice. In this study, CBX at a dose of 150 mg/kg (but not at 75 mg/kg) elevated the threshold in the PTZ seizure model. However, the anticonvulsant activity of VPA, TGB and ETS against the clonic phase of PTZ-induced convulsions was unaffected by CBX at 75 mg/kg.

Interestingly, CBX (75 mg/kg) reduced the protective potency of GBP against PTZ. A similar situation was observed with the calcium channel inhibitor, niguldipine, and its combinations with carbamazepine or phenobarbital. Whilst niguldipine alone was capable of increasing the threshold for electroconvulsions or reducing the afterdischarge duration in amygdala-kindled seizures in rats, its combinations with carba-

mazepine or phenobarbital against maximal electroshock-induced convulsions in mice or amygdala-kindled seizures in rats resulted in the reduced anticonvulsant activity of these antiepileptic drugs [Borowicz et al., Eur J Pharmacol, 1997; Borowicz et al., Eur Neuropsychopharmacol, 2002]. Finally, it may be concluded that CBX is not capable of enhancing the protectie activity of AEDs against PTZ.

Retigabine – an unique mechanism of action and new horizons

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Currently used antiepileptic drugs (AEDs) utilize a number of mechanisms involved in their anticonvulsant activity, as for instance potentiation of GABA-mediated events or direct positive effects on $GABA_A$ receptors, inhibition of sodium or calcium voltage-operated channels, and blockade of glutamate-mediated excitation. Some of the AEDs (lamotrigine, topiramate, valproate) exert multiple mechanism and some are quite specific in affecting a particular mechanism, as for example tiagabine or vigabatrin [Czapinski et al., Curr Top Med Chem, 2005]. Despite the existing mono- or polytherapies, still circa 30% of epileptic patients are not properly controlled although there are approximately 30 AEDs available [Czuczwar and Patsalos, CNS Drugs, 2001; Lasoń et al., Pharmacol Rep, 2011; Löscher and Schmidt, Epilepsia, 2011]. However, a possibility arises that the combined treatment of epilepsy would result in better efficacy if based upon rational polytherapy [Czuczwar et al., Expert Opin Drug Metab Toxicol, 2009; Lasoń et al., Pharmacol Rep, 2011].

A continuous search for novel AEDs resulted in the discovery of a drug, representing a completely new mechanism of action – retigabine. This AED is an potassium channel opener and this particular action is exerted at a very low concentration of 0.1 μmol and mainly concerns Kv.7.2–7.3 potassium channels. Apart from this specific novel mechanism of action, retigabine is also a GABA enhancer but at much higher effective concentration of 10 μmol [Czuczwar et al., Pharmacol Rep, 2010; Czuczwar et al., Ther Clin Risk Manag, 2012]. Initial isobolographic evaluation of the interactions of retigabine with AEDs against maximal electroshock-induced seizures in mice has revealed that there was an anticonvulsant synergy for the combination of retigabine with valproate, in cases of combinations with either carbamazepine or lamotrigine additive interactions were evident [Luszczki et al., Naunyn-Schmiedebergs Arch Pharmacol, 2009]. Further studies on the interactions of retigabine with more AEDs in this animal model of seizures are in progress. Clinical data indicate that when used at a daily dose of 1,200 mg daily (an 8-week prospective baseline phase and an 18-week double-blind treatment period with 6-week initial dose titration phase), retigabine effectively increased the responder rate (defined as an at least 50% reduction in partial seizure frequency) in 44.4% of patients, compared to 17.8% in the placebo group. At lower daily doses of 600 and 900 mg this AED was also considerably effective. Dizziness, somnolence, fatigue, confusion, dysarthria, urinary tract infection, ataxia, and blurred vision were the most frequently observed adverse effects. However, retigabine in all cases was used as an add-on therapy [Czuczwar et al., Pharmacol Rep, 2010; Czuczwar et al., Ther Clin Risk Manag, 2012].

To conclude, retigabine offers a completely novel mechanism of action, which has not been shared by the existing AEDs. Apart from its high efficacy in patients with drug-resistant partial epilepsy, this drug has also a therapeutic potential for the management of neuropathic pain, mania, bipolar disorder, stroke, and Alzheimer's disease. All these potential indications have been suggested by the results of experimental studies and only, in the case of Alzheimer's disease, some positive case reports are available [Czuczwar et al., Pharmacol Rep, 2010; Czuczwar et al., Ther Clin Risk Manag, 2012].

The influence of neurogenesis inhibiton in the early stages of life on epileptogenesis in rats

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Antiepileptic drugs (AEDs) are known to produce profound alternations in the developing brain by influencing various processes including neurogenesis, apoptosis, synaptogenesis, cell proliferation and synaptic plasticity. Adverse effects of AEDs on brain growth and behavior have been studied extensively and it is now clear that certain AEDs produce impaired performance in various spatial learning and behavioral tasks [for review see Ikonomidou and Turski, Epilepsy Res, 2010]. We have reported that phenobarbital administered to infant rats at 50 mg/kg not only reduced number of newly formed neurons in the dentate gyrus, but also impaired the performance of the 6 month-old animals in water maze learning and memory task [Stefovska et al., Ann Neurol, 2008]. The impact of neonatal exposure to AEDs on epileptogenesis was recently assessed by Forcelli et al. [Epilepsia, 2011], who reported that exposure to lamotrigine in the second postnatal week resulted in decreased pentylenetetrazole seizure threshold assessed in adult rats [Forcelli et al., Epilepsia, 2011]. Therefore the aim of this study was to evaluate the influence of neonatal exposure to phenobarbital and phenytoin on kindling development and pilocarpine-induced seizures in adult rats.

All animal experiments were approved by the Local Ethics Committee. Experimental groups consisted of 8–10 animals. Briefly, beginning at day 3 after parturition (P3) male Wistar rats were injected intraperitoneally with phenobarbital (50 mg/kg), phenytoin (50 mg/kg) or saline and subsequently returned to their mothers. The whole procedure was repeated on P5, P7, and P9. Animal weights were documented for each experiment. Pups were separated from their mothers on P28 into cages of four littermates. Rats were challenged against pilocarpine- and pentylenetetrazole-indued seizures on P60, whilst corneal kindling was initiated on P45.

The postnatal treatment of rat pups with phenytoin, but not phenobarbital resulted in a decreased number of stimulations to reach stages 4 and 5 of corneal kindling in the adult life. Similarly, animals exposed to phenytoin as pups displayed a significantly higher susceptibility to both pilocarpine- and pentylenetetrazole-induced seizures in adulthood as compared to phenobarbital treated pups and controls.

Our results further support the hypothesis that some AEDs administered in the neonatal period may produce profound functional impairment in the adulthood and that further research on the effects of neonatal exposure to AEDs on epileptogenesis is validated.

A comparison of efficacy two forms valproic acid (syrup and granules) in children with epilepsy

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Epilepsy is a chronic syndrome, one of the most common in neuropediatrics. 50% of patients are taken with epilepsy before the tenth year of age, 65% before the twentieth year of age. In regard of frequency of its occurrence in children (0.5–1%), chronicity and necessity of long and systematic treatment, it can be

treated as a social disease. Activity and course of developmental age epilepsy is different from epilepsy occurring in adults. It is caused by the maturing of systems, especially the central nervous system. It results in individuality and specificity of treatment, its results, drugs side effects and their interactions [Sobaniec, Progress in the diagnostic and therapy of neurological disorders in children, Biofolium eds, 2000 (in Polish)]. The results of anticonvulsant drugs activity in children are dependent on pharamcokinetics which is change able and age dependent [Steinborn, Neurol Dziec, 2006]. There are significant differences concerning drug distribution in tissues in different ages. Pharmacokinetics of anticonvulsant drugs in children depends on their absorption, protein binding, distribution, metabolism and secretion. The analysis of drugs presence in blood and monitoring of their concentration is important especially in children due to the possibility of evaluation of the treatment safety [Artemowicz and Sobaniec, Epileptologia, 2008]. In case of chronic diseases treatment, including epilepsy, it is important to maintain stable, constant in time drug concentration in the serum. Introduction of retard form with prolonged release time, enabled optimisation of epilepsy treatment [Majkowski, Terapia i Leki, 1992; Sendrowski and Sobaniec, Biał Bibliot Pad, 1996].

Valproate (VPA) is an anticonvulsant drug used as a first choice drug in the treatment of epilepsy and epilepsy syndromes both with seizures originally generalized as well as partial ones with or without secondary generalization [Davis et al., Drugs, 1994]. This drug takes a special place in the treatment of developmental age epilepsy. The mechanism of VPA activity in epilepsy and other neurological conditions is still ambiguous and unexplained. Valproic acid and its salts operate by means of more than one mechanism. Valproic acid influences the activity of cells dependent on neurotransmitters, as well as non-dependent [Löscher, Antiepileptic drugs 5th edn, 2002]. Valproate, is in the group of drugs with a broad spectrum of anticonvulsant activity, showing high effectiveness [Covanis, Epilepsia, 1982; Lasoń, Farm Pol. 2005]. It mainly inhibits spreading epileptic discharges, having lesser influence on the foci [Śmigielska-Kuzia and Sobaniec, Biał Bibl Pad, 1997]. In this respect it is the most effective in the treatment of originally generalized seizures, both convulsive and non-convulsive. Presently, it is also, used, with good results, in the treatment of focal seizures of all types [Bergman, Aktuelle Neur, 1999; Guzeva, Zh Nevrol Psikhiatr, 2007].

Up to now, there were VPA forms for parenteral and oral administration in the form of pills, forms of prolonged release time being destined for older children, and syrup for infants and small children. Medication destined for children should be above all effective and well tolerated. However, in case of small patients the important factor is also pleasant or at least neutral flavor. Another aim to be reached is also finding a proper pharmaceutical form that would provide the possibility of dynamic and divided in time absorption from the digestive system, and the maintenance of the stable and therapeutical level in blood and brain.

Due to specific flavor of the syrup and variability of VPA concentration during the day, new pharmaceutical form of the drug with the prolonged release time in the form of microgranules was developed – Depakine Chronosphere preparation. It takes the form of granules with the diameter lower than 400 μm, of no taste and smell. Granules can be added to semi-fluid foods of room temperature what makes swallowing easier. New form of valproic acid is mainly destined for small children as well as older ones having problems with pills swallowing.

The aim of this study was to compare valproic acid concentration, its clinical effectiveness and evaluation of the treatment acceptance in children with seizures originally generalized as well as partial ones with or without secondary generalization, depending on the form of the drug administered (syrup and microgranules).

The group of 32 patients with diagnosed epilepsy with seizures originally generalized as well as partial ones with or without secondary generalization underwent the study and was treated with VPA. Initially, the drug was administered in the form of syrup, then microgranules. In the treated group there were 17 girls (53%) and 15 boys (47%). Patients were aged 2 – 12.5 (mean 5.9 ± 2.8). Twelve (37.5%) patients were diagnosed with epilepsy with originally generalized seizures, and 20 (62.5%) patients were diagnosed with epilepsy with partial seizures with secondary generalization. Patients were treated with valproate in the syrup form for the period of 1.5 months (4 weeks with the full dose) up to 3 years (mean 14.1 ± 11.6 months). All patients had imaging studies of central nervous system conducted. In 12 children (37.5%) central nervous system structures image was normal. In 20 children (62.5%) some lesions in central nerv-

ous system image were noticed. Up to now, psychomotoric development in 13 children (41%) was normal, in 19 (59%) psychomotoric development retardation was observed, including 3 children (9%) in which autism was diagnosed. All patients were treated with valproic acid in syrup in a day dose of 15.4 mg/kg/day – 54.6 mg/kg/day (mean 35.9 ± 8.9 mg/kg/day), in 3 divided doses. After at least 4 weeks treatment, the form of syrup was changed into microgranules, maintaining previous per day dose. Valproic acid concentration was analyzed twice, during administering valproic acid in syrup and after the change into microgranules (steady state), always before administering the morning dose of the drug. All patients had laboratory study conducted before and after the change of drug form, aiming at evaluation of blood count including blood platelets level and liver functions. During the study, seizures control was conducted, as well as side effects of valproic acid treatment and treatment acceptance. EEG trace was also analyzed, both during treatment with syrup form as well as after reaching the steady state, 6 months after the change. The results of VPA level assay in blood serum were compared during administering both forms of valproic acid, evaluating at the same time the number of seizures and side effects. The opinions on treatment acceptance on the basis of parents observations were gathered (difficulties with administering the drug to children).

In the examined group of 32 patients, after 6 months treatment with valproic acid in micro granules form, in 27 cases (84%) there was an improvement (decrease) in the number of seizures. In 15 (47%) children seizures receded totally. In 5 children (16%) seizures reduced by 50 – 75%, what can be considered a good result. In 7 children (22%) seizures number decreased by 25 – 50 % – an average result. In 5 patients (16%) there was a significant change in seizures frequency – no improvement. There wasn't a patient with worsening condition, meaning the increase in seizures frequency. Good and very good result were obtained in 57% of children with originally generalized seizures and in 43% of children with partial seizures with secondary generalization.

For both drug forms there was a statistically significant increase of VPA concentration in serum, parallel to the increase of drug dose ($p < 0.001$). In both cases, strong positive correlation was obtained ($R = 0.56$). Simultaneously, when comparing both correlations (difference test between both correlation factors, $p = 0.98$), no statistically significant differences between compared factors were observed. At the level of $p < 0.001$ there were statistically significant differences in VPA concentration in serum between measures taken after administering the drug in the form of syrup and measures taken after administering the drug in the form of microgranules. Values of VPA concentration in serum, with the same dose for both forms of the drug, were at the higher level during microgranules treatment. Qualitative analysis of EEG trace (during syrup treatment – VPA and after 6 months of micro granules treatment – VPA), presented an improvement (the decrease in the number of seizures localized and generalized) in 25 (78%) patients. Those were the children with very good and good treatment results, in which the decrease in seizures number correlated with EEG trace improvement. In 5 (18%) children EEG trace became normalized.

In all children, meeting the criteria of inclusion into the study, there was a good tolerance of the treatment with microgranules (VPA) observed and better acceptance of this treatment than the syrup form treatment (VPA). The evaluation of treatment acceptance was performed on the basis of parents opinion (difficulties with drug administration, refusal of taking the drug by a child). Additionally, the number of per day doses was reduced from three while administering syrup to two with microgranules treatment. In our group there were no side effects of the valproic acid treatment, both with syrup and microgranules treatment. The results of the study conducted for the comparison of clinical effectiveness of two forms of valproic acid forms (syrup and microgranules) in children with epilepsy led to the following conclusions:

1. In children with epilepsy, after the change of the valproic acid form from syrup to microgranules, within the 6-months period of observation, significant reduction of epileptic seizures was obtained.
2. The decrease in seizures number correlated with positive changes in the EEG trace. In qualitative analysis of EEG trace, seizures activity was reduced.
3. The change of VPA form from syrup to microgranules enabled reaching significantly higher VPA concentration in the serum, maintaining the same per day dose of the drug.
4. Using VPA form – microgranules provided good tolerance of this form of the drug and better treatment acceptance than VPA in the syrup form.

New concepts of epileptogenesis

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Advances in molecular biology, electrophysiological and immunohistochemical methods had a significant impact on the understanding of the basic mechanism of epileptogenesis [O'Dell et al., J Neurosci Res, 2012]. Epileptogenesis is thought to reflect time-dependent progressive anatomical and functional changes in neuronal networks, which ultimately lead to recurrent seizure occurrence. Important steps in epileptogenesis include: neuronal cell damage, neurogenesis, gliosis, synaptic reorganization, dendritic plasticity, blood-brain dysfunction, neuroimmunological processes and reorganization of extracellular matrix. Functional mutations or polymorphisms of genes coding for neuronal sodium, calcium, potassium and chloride channels are known to be the main cause of some epilepsies. Epileptogenic mutations affect the number of receptors and ion channels, their activation/inactivation kinetics, ion selectivity and affinity for ligands. Analysis of the whole transcriptome has linked epileptogenesis with gene groups involved not only in ion channels and neurotransmitter metabolism or receptor function, but also in neuroplasticity, inflammation and immune response [Pitkanen and Łukasiuk, Lancet Neurol, 2011; Vezzani et al., Epilepsia, 2011]. Correspondingly, it has been demonstrated that immune-related agents, toll-like receptor 4 and high mobility group box-1 induce and perpetuate experimental temporal lobe epilepsy [Maroso et al., J Intern Med, 2011]. Epigenetic regulation of transcription by alterations in DNA methylation or histone modification are thought to play a role in epileptogenesis. This is based on the assumption that epileptogenic insult is able to induce time-dependent changes in waves of genes in the brain, and the gene transcription is regulated by epigenetic mechanisms, i.e., changes in DNA methylation and histone modification. Such changes may have functional consequences, e.g., HDAC4 knockout mice showed spontaneous seizures [Rajan et al., PLoS One, 2009]. Pilocarpine-induced limbic seizures in rats led to a decrease in H4 acetylation on GluR2 promoter and increase histone acetylation on P2 promoter of BDNF gene [Huang et al., J Neurosci, 2002] as well as to enhanced histone H3 phosphorylation [Crosio et al., J Cell Sci, 2003]. In the kainate model of temporal lobe epilepsy, an increase in H4 acetylation and H3 histone phosphorylation has been reported [Sng et al, Eur J Neurosci, 2006; Crosio et al, J Cell Sci, 2003]. Repeated electroconvulsive shock in rats elevated H4 acetylation on c-fos and BDNF promoters, but decreased this parameter in the postseizure period [Tsankova et al., J Neurosci, 2004]. On the other hand, valproate, an antiepileptic drug and inhibitor of histone deacetylase, inhibited abnormal neurogenesis and cognitive deficits in the kainate-induced seizure model [Jessberger et al., J Neurosci, 2007]. Valproate was also reported to provide protection against seizure-related neuronal damage and neurobehavioral changes, however, it did not prevent the secondary epileptogenesis [Brandt et al., Neuropharmacology, 2006]. Epileptogenesis shares many common features with developmental neuronal plasticity, e.g., the imbalance in excitatory/inhibitory amino acid transmission, enhanced expression of some NMDA receptor subunits and neurotrophic factors, decreased expression of GluR2 receptors and GLT-1 and GLAST transporters. Therefore, some concepts emphasize importance of the interactions between neurons, glia and extracellular matrix in epileptogenesis [Eid et al., Epilepsia, 2008]. It has been proposed that glutamate-induced high-frequency calcium waves in astrocyte syncytiums lead to pathological synchronization of neuronal discharges [White et al. Prog Brain Res, 1992]. Epileptic brain tissue from patients with temporal lobe epilepsy or animal models of this disorder showed changes in expression, localization and function of astroglia Kir potassium channels and aquaporins, as well as dysfunction of glutamate transporter, glutamate converting enzyme and glutamine synthetase [Steinhauser et al., Glia, 2012]. The loss of the glutamine synthetase and reduced adenosine level in astrocytes due to the increase in adenosine kinase activity can participate in epileptogenesis. Adenosine, which is regarded as an endogenous anticonvulsant is metabolized by adenosine kinase and overexpression of this enzyme during epileptogenesis may decrease seizure threshold. Moreover, transgenic mice with overexpression of the enzyme are more susceptible to

seizures [Li et al., Glia, 2012]. On the contrary, inhibitors of adenosine kinase and intrahippocampal implantation of the enzyme-devoid stem cells prevent epileptogenesis [Boison et al., Prog Neurobiol, 2008]. An increasing body of evidence indicates that alterations in the synaptic pool of matrix metalloproteinase 9, mTOR signaling pathway, integrins and synapsins can be critical for development of epilepsy, although there are also some controversies [Yin et al., Med Hypotheses, 2011; Mizoguchi et al., Biochem Res Int, 2011]. Metaloproteinases (MMPs) and tissue inhibitors of MMPs are known to participate in remodeling of extracellular matrix. Deletion and overexpression of the MMP9 gene decreases and increases susceptibility of mice to pentetrazole-induced kindling, respectively. Moreover, deficit of MMP9 inhibits reactive synaptogenesis in the kainate model of temporal lobe epilepsy [Wilczyński et al., J Cell Biol, 2008]. Results of several studies point to the involvement of mTOR kinase in pathomechanism of seizures. The serine/threonine mTOR kinase modulates growth, metabolism and proliferation of cells as well as viability, apoptosis and autophagy and immune processes. Inhibitors of mTOR kinase are potent immunosuppressants and they show antiaging and anticancer activity. Furthermore, mTOR inhibitors prevent seizures in patients with tuberous sclerosis [Kim et. al., Korean J Pediatr, 2011]. The mTOR inhibitor, rapamycine was shown to affect experimental epileptogenesis. It inhibited synaptic reorganization and recurrent excitation in neuronal circuits of dentate gyrus in a model of temporal lobe epilepsy in mice [Tang et al., Biochem Biophys Res Commun, 2012], but it did not influence the frequency of nonprovoked seizures and did not prevent the seizure-related cell damage in the hilus of the dentate gyrus [Buckmaster et al., J Neurosci, 2009]. In the other model of temporal lobe epilepsy, namely, that evoked by electric stimulation of the amygdala in rats, rapamycine had no effect on epileptogenesis [Łukasiuk et al., Neurosci Lett, 2012]. Integrins are heterodimeric transmembrane receptors, which are responsible for cell-cell and cell – extracellular matrix adhesion processes. These interactions are considered to play an important role in neuroplasticity and epileptogenesis [Wu and Reddy, Pharmacol Ther, 2012]. Synapsins form a protein family consisting of 10 isoforms encoded by 3 genes. These proteins participate mainly in functioning of synaptic vesicles and neuronal plasticity. Deletion of the synapsin-coding gene in mice, except for the synapsin III, results in seizures, which are not accompanied by morphological changes in the brain. Moreover, synapsins differentially regulate presynaptic processess in inhibitory and excitatory synapses [Fassio et al., Semin Cell Dev Biol, 2011]. Despite advances in our knowledge of molecular mechanism of epilepsy and neuronal plasticity, no antiepileptogenic drug has been marketed so far [Sloviter and Bumanglag, Neuropharmacology, 2012]. Nonetheless, anti-inflammatory agents, immunosuppressants, inhibitors of leukocyte adhesion, some antiepileptic drugs, CB1 cannabinoid receptor and $\alpha 2$ noradrenergic receptor antagonists, as well as agents representing other pharmacological groups are being extensively studied in the search for an antiepileptogenic agent [Pitkanen and Łukasiuk, Lancet Neurol, 2011].

Perspectives for application of cannabinoids in epilepsy

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Cannabis is one of the oldest psychotropic drugs known to humanity, because according to archeological discoveries in China, it was cultivated and consumed at least since the Neolithic period around 4000 BC [Ben Amar, J Ethnopharmacol, 2006]. There are several species of cannabis, however, the most relevant are *Cannabis sativa*, *Cannabis indica* and *Cannabis ruderalis*. The two main preparations derived from cannabis are marijuana and hashish. Cannabis contains more than 460 known chemicals, more than 60 of which are grouped under the name cannabinoids [Ben Amar, J Ethnopharmacol, 2006]. The major psy-

choactive ingredient of cannabis is Δ-9-tetrahydrocannabinol, commonly known as THC. Other cannabinoids present in *Canabis sativa* include Δ-8-tetrahydrocannabinol, cannabinol, cannabidiol, cannabicyclol, cannabichromene and cannabigerol, but they are present in small quantities and have no significant psychotropic effects compared to THC [Smith, J Psychoactive Drugs, 1998]. Cannabinoids exert their actions by binding to specific receptors: the CB_1 and CB_2 cannabinoid receptors, which are part of the G-protein coupled class and their activation results in inhibition of adenylate cyclase activity. The identification of agonists (i.e., anandamide and 2-arachidonylglycerol – the most studied endogenous cannabinoids) and antagonists of these receptors has stimulated interest in the medical uses of cannabis [Iversen, Brain, 2003; Di Marzo et al., Nature Rev, 2004].

Epilepsy affects about 1% of the world's population. It is estimated that 20–30% of people with epilepsy are not adequately controlled with currently available antiepileptic drugs [Kwan et al., N Eng J Med, 2011]. Several anecdotal reports suggest that cannabis has anticonvulsant properties and would be effective in treating partial epilepsies and generalized tonic-clonic seizures in humans [Consroe et al., JAMA, 1975; Cunha et al., Pharmacology, 1980]. Extensive preclinical studies have documented that R(+)-WIN 55,212-2 mesylate (WIN – a non-selective CB_1 and CB_2 receptor agonist) significantly enhanced the anticonvulsant action of carbamazepine (CBZ), phenytoin (PHT), phenobarbital (PB) and valproate (VPA) in the mouse maximal electroshock (MES)-induced tonic seizure model [Luszczki et al., Pharmacol Biochem Behav, 2011; Tab. 1]. Additionally, WIN potentiated the anticonvulsant action of ethosuximide (ETS), PB and VPA, but not that of clonazepam (CZP) in the mouse pentylenetetrazole (PTZ)-induced clonic seizure model [Luszczki et al., Prog Neuropsychopharmacol Biol Psychiatry, 2011; Tab. 1]. In contrast, ACEA (a highly potent CB_1 receptor agonist) enhanced the anticonvulsant action of PB and VPA, but not that of CBZ, lamotrigine (LTG), oxcarbazepine (OXC), PHT or topiramate (TPM) in the mouse MES model [Luszczki et al., Eur J Pharmacol, 2006; Luszczki et al., Prog Neuropsychopharmacol Biol Psychiatry, 2010]. ACEA potentiated the anticonvulsant action of ETS, PB and VPA, but not that of CZP in the mouse PTZ model [Luszczki et al., Prog Neuropsychopharmacol Biol Psychiatry, in press]. Quite recently, it was found that WIN significantly enhanced the anticonvulsant action of CZP, levetiracetam (LEV), PB, tiagabine (TGB) and VPA, but not that of gabapentin (GBP), pregabalin (PGB) or clobazam (CLB) in the mouse 6Hz (psychomotor) limbic seizure model [unpublished data, Tab. 1].

Considering the results from our preclinical studies, one can conclude that some cannabinoid ligands may play an important role in alleviating seizure attacks and in enhancing the anticonvulsant action of some selected antiepileptic drugs, including PB and VPA (Table 1). If the results from our preclinical studies could be extrapolated into clinical settings, a new therapeutic option would be created for patients with refractory epilepsy inadequately medicated with currently available antiepileptic drugs used in monotherapy.

Tab. 1. Influence of WIN on the anticonvulsant action of the various antiepileptic drugs

	Experimental model of epilepsy		
Drug #	MES	PTZ	6Hz
PB	↑	↑	↑
VPA	↑	↑	↑
CBZ	↑	N.D.	N.D.
PHT	↑	N.D.	N.D.
CZP	N.D.	0	↑
ETS	N.D.	↑	N.D.
LEV	N.D.	N.D.	↑

MES – maximal electroshock-induced tonic seizure model; PTZ – pentylenetetrazole-induced clonic seizure model; 6Hz – psychomotor (limbic) seizure model; PB – phenobarbital; VPA – valproate; CBZ – carbamazepine; PHT – phenytoin; CZP – clonazepam; ETS – ethosuximide; LEV – levetiracetam; ↑ – enhancement of the anticonvulsant action of a tested antiepileptic drug; 0 – no effect; N.D. – not determined; # – experimental studies supported by grants: MISTRZ (from the Foundation for Polish Science, Warszawa, Poland) and NN401797640 (from National Science Centre, Kraków, Poland)

Inhibition of glycolysis as a novel and promising anticonvulsant strategy

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Among the main challenges in modern epileptology is the discovery of new therapies effective in refractory epilepsy and modulating the natural course of the disease. There is increasing awareness that currently available drugs have not fully met expectations as the proportion of patients with uncontrolled seizures reaches up to 30%, which does not differ from historical data [Löscher and Schmidt, Epilepsia, 2011]. Thus, there is a need for looking for new therapeutic targets for effective treatment of refractory epilepsy. Accumulating evidence suggests that metabolic intervention might be alternative therapeutic strategy to control seizures. 2-Deoxy-D-glucose (2DG) displayed anticonvulsant activity in selected models of epileptic seizures [Stafstrom et al., Ann Neurol, 2009] and influenced the progression of epileptogenesis induced by kindling [Garriga-Canut et al., Nat Neurosci, 2006]. This certainly confirmed and extended our previous study [Rejdak et al., Epilepsy Res, 2001], which, to the best of our knowledge, was the first published in the literature, using 2DG in the context of anticonvulsant effects. We found that chronic 2DG treatment in control mice resulted in a moderate but significant decrease in mortality rate after status epilepticus evoked by toxic doses of bicuculline and a tendency toward a lower seizure score. Mechanisms of such protective effects of 2DG were not clear. 2-DG is avidly taken up into cells by hexose transporters and is phosphorylated by hexokinase to form 2-DG-6-phosphate (2-DG-6-P). 2-DG-6-P cannot be further metabolized because it competitively inhibits the next step in glycolysis, which is catalyzed by phosphoglucose isomerase. 2-DG-6-P, therefore, builds to high intracellular levels. These high levels of 2-DG-6-P have been found to allosterically inhibit hexokinase, thus preventing entry of glucose into the glycolytic pathway. Nevertheless, glucose-6-phosphate can still enter the pentose phosphate pathway to generate NADPH but not ATP as in glycolysis. Therefore, as a result of the antimetabolic actions of its downstream product 2-DG-6-P, 2-DG is a potent inhibitor of glycolysis, which shunts glucose metabolism to the pentose phosphate pathway. Therefore, it is plausible that seizure protection conferred by 2-DG results specifically from inhibition of glycolysis [Gasior et al., Epilepsia, 2010].

We also proposed it could be additionally related to metabolic stress (glucose deprivation)-evoked chemical preconditioning with subsequent induction of the brain tolerance. It is of note that in our experiments protein synthesis inhibitor cycloheximide attenuated the protective effects of 2-DG [Rejdak et al., Epilepsy Res, 2001]. Our study [Rejdak et al., Epilepsy Res, 2001] along with later works of other authors [Garriga-Canut et al., Nat Neurosci, 2006; Stafstrom et al., Ann Neurol, 2009] strongly support potential utility of 2DG for treatment of epilepsy but also other neurological conditions where induction of the brain tolerance might be of importance to protect the central nervous system tissues against acute or chronic insults [Rejdak et al., Pol J Pharmacol, 2001; Cadet et al., Mol Neurobiol, 2009], in particular those involving excitotoxicity. There is an ongoing project testing the efficacy of 2DG in combinations with classical and newer AEDs in MES model in order to evaluate its value in the potential treatment armamentarium of epilepsy.

Neuroprotective effect of chosen antiepileptic drugs in cultured hippocampal neurons

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Scientific reports from recent years indicate that many different mechanisms are involved in the neurodegenerative processes. Disturbances to the function of the central nervous system, occurring both acutely and chronically, activate the cascade of complex biochemical processes, which lead to degeneration and finally to the death of neurons [Yakovlev and Faden, NeuroRx, 2004]. There are known to be two pathways of neural death: necrosis and apoptosis. Current studies indicate that both of these processes coincide and can be termed as aponecrosis [Formigli et al., J Cell Physiol, 2000]. Results of numerous investigations have demonstrated the leading role of glutamate in damage to neurons [Liu et al., J Neurosci, 2007]. Overstimulation of glutamate receptors due to excessive exposure to the neurotransmitter glutamate has been implicated as the most important factor contributing to neuronal injury and has been referred to as excitotoxicity [Choi, J Neurobiol, 1992]. Therefore, pharmacological antagonizing excitotoxicity by inhibition of glutamate receptors and Ca^{2+} channel function in neurons is a particularly attractive target for neuroprotection. Some of the antiepileptic drugs, because of their multidirectional mechanism of action including glutamate receptors blockade and Ca^{2+} channel inhibition, have been suggested as promising neuroprotectants.

Neuronal cultures are often used as an *in vitro* model for the assessment of the neuroprotective effects of various drugs against varied cell death-inducible factors [Sendrowski et al., Child Neurol, 2008]. Hippocampal neurons are usually preferred in such experiments. High expression of glutamate receptors on their cellular membrane results in a particular vulnerability of these neurons to the effects of this excessive neurotransmitter [Dong et al., Acta Pharmacol Sin, 2009].

In our centre we decided to perform two different experimental studies targeting on neuroprotection. In the first study we assessed putative protective effects of two novel antiepileptic drugs: levetiracetam and gabapentin on hypoxia-injured cultures of hippocampal neurons. The aim of the second study was to assess a neuroprotective potential of three Ca^{2+} channel inhibitors: cinnarizine, nimodipine and flunarizine, the drugs widely used in the treatment of various neurological disorders, including epilepsy. In this experiment, hippocampal neurons were injured by glutamate.

Primary cultures of hippocampal neurons were prepared from embryonic day 18 Sprague-Dawley rats following Brewer [Brewer, J Neurosci Res, 1993]. Dissected hippocampi were purchased commercially and delivered in B27/Hibernate E from Brain Bits (BrainBits, USA). Both experiments were performed after 7 days in culture. Hypoxia was maintained by flushing the incubator with 20% CO_2 for 24 h. Excitotoxic insult was performed by exposure of cultured neurons to 125 μM glutamate for 15 min. Quantitative assessments of neuronal injury were done by light-microscopic morphometry, measuring the lactate dehydrogenase (LDH) activity in the media and by counting and establishing the cells in flow cytometry with annexin V/PI staining. The results of our study indicate that none of the used concentrations of the drugs exerted a toxic effect *per se* on cultured neurons.

In the first experiment, hypoxia caused death of 25% of the population of neurons in control cultures without antiepileptic drugs. Both levetiracetam and gabapentin exerted promising neuroprotective effect. In cultures with higher concentrations of the drugs, two-fold higher number of neurons remained viable as compared with control cultures without drugs [Sendrowski et al., Folia Histochem Cytobiol, 2011; Sendrowski et al., Child Neurol, 2011]. In the second study with glutamate, neuroprotective effect of Ca^{2+} channel inhibitors were controversial. In contrast to the beneficial anti-apoptotic effect of flunarizine, cinnarizine was ineffective in such neuroprotection. Addition of nimodipine to the culture media caused in unfavorable pro-apoptotic effect on hippocampal neurons. On the other hand, nimodipine and cinnarizine have promising anti-necrotic properties, whereas flunarizine has not [Sendrowski et al., Pharmacol Rep, 2013]. Summarizing, novel antiepileptic drugs: levetiracetam and gabapentin exert beneficial neuroprotective effect on cultured hippocampal in hypoxic conditions. From the studied Ca^{2+} channel inhibitors, flunarizine possesses the most promising neuroprotective potential against excitotoxic damage *in vitro*.

The neuroprotective effects of topiramate in the experimental model of febrile seizures in rats

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Febrile seizures (FS) occur between 6 months and 5 years of age at body temperature above 38.5°C, usually during infection with fever [Knudsen, Epilepsia, 2000]. FS are the most common form of convulsions in childhood and have been associated with an increased risk of epilepsy in the future life. Temporal lobe epilepsy (TLE) due to its frequent drug-resistance remains a challenge for the epileptologist. TLE is the most prevalent type of epilepsy, but its origin is still not well understood. Intractable TLE is often associated with specific hippocampal cell loss termed mesial temporal sclerosis. This pathology is characterized by neuronal loss and gliosis, most prominent in the hippocampal CA1 and CA3 sectors A number of studies have shown a significant relationship between a history of FS, particularly of the complex type, in childhood and the presence of mesial temporal sclerosis, as identified on magnetic resonance imaging [Cendes et al., Neurology, 1993; Scott et al., Brain, 2000].

The aim of the study was to estimate potentially neuroprotective effects of TP M in the experimental model of febrile convulsions.

The experiment used 24 young Wistar rats aged 22–30 days. The degree of brain maturity in such rats corresponds to that of 1- or 2-year-old children. The animals were divided into 4 groups, 6 rats in each. Hyperthermia was induced by placing the animals in a 30 × 30 × 60 cm water bath filled with 45°C warm water to such a depth that a rat standing on its hind legs and leaning against a container wall had its head above water surface. Water temperature was maintained at the same level. Rats were put into water for 4 minutes or until convulsions appeared and then moved to a separate container lined with lignin [Jiang et al., Epilepsia, 1999]. The rats (except for control) were placed in water for four consecutive days. Topiramate (80 mg/kg b.m. dissolved in 2 ml normal saline) was administered with an intragastric tube, 90 min before the animals were placed in the water bath (group TPM+FS). In the FS+TPM group, the drug was administered in the same way and at the same dose, immediately after the each convulsion episode. Control rats and FS group received only normal saline. The dose of the drug was chosen according to literature references [Niebauer and Gruenthal, Brain Res, 1999; Edmonds et al., Life Sci, 2001].

Morphometric investigations of the hippocampus sections were conducted routinely, by assessing the number of neurons in the high power field in the CA1 and CA3 sectors separately in the control group and in each experimental group. Experimentally induced febrile convulsions resulted in the death of 60% of the neurons in this Ammonal cortex area as compared to the control group. In the rats receiving TPM before FS, the number of survival neurons was markedly higher (the death of only 22% of neurons was observed). In the CA3 sector, febrile convulsions led to the death of 50% of neurons as compared to the control group. The loss of 28–43% of neurons was observed in the other two experimental groups.

Our findings indicate a very strong unfavorable effect of FS on the hippocampal neurons in young rats. Induced FS caused advanced neurodegenerative changes in the pyramidal neurons of the hippocampal CA1 and CA3 sectors, with more than 50% neuronal loss. We also showed a beneficial effect of TPM on this process. TPM exerts a neuroprotective effect on the Ammonal cortex neurons in the rat, especially when administered before FS. Its beneficial action is mainly reflected in a higher number of survival neurons as compared to the untreated animals. Our results are very promising and may have clinical implications. Topiramate could be applied to prevent the effects of long-lasting and recurrent FS in children, thus extending the list of currently used preparations.

Interactions between levetiracetam and various antiepileptic drugs in the mouse 6 Hz psychomotor seizure model – an isobolographic analysis

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Approximately 70% of patients with epilepsy can be satisfactorily treated with a single antiepileptic drug (AED) [Kwan and Brodie, N Engl J Med, 2000]. Despite the major progress in discovering new drugs that demonstrate a high antiepileptic potential, there is still a group of about 30% of patients who are refractory. These patients need to be treated by two or more AEDs in an attempt to control their seizure attacks. In some cases, combining two AEDs with different mechanisms of action may have advantages [Deckers et al., Epilepsia, 2000]. Hence, it is of pivotal importance to search for the drug combinations that would comprise rational polytherapy. From a theoretical point of view, the most advantageous AED combination is that between two AEDs that are synergistic with respect to their therapeutic activities and with concomitant infra-additivity (antagonism) in relation to their adverse effects.

Group of patients with uncontrolled seizures has not changed substantially in recent years [Löscher and Schmidt, Epilepsia, 2011]. There are many reasons for this situation. One of them may be the lack of new animal models that could be used to screen new compounds with antiepileptic potential. With few exceptions, all AEDs have been discovered by using conventional animal models, e.g., the maximal electroshock (MES)- and pentylenetetrazole (PTZ)-induced seizures.

One of few new animal models that was successfully introduced into AED screening was 6 Hz seizure model. In this model seizures are evoked by low-frequency (6 Hz) and long duration (3 s) of electrical stimulation via corneal electrodes. Whereas the MES or PTZ tests induce tonic-clonic and myoclonic seizures, respectively, the 6 Hz model is a model of partial psychomotor (limbic) seizures. These differences in clinical seizure types may explain why 6 Hz model is currently becoming a model of pharmacoresistance that allows mass-screening of novel compounds. Despite being discovered in the years fifties of the 20th century, a 6 Hz model was abandoned for many years because of the lack of phenytoin sensitivity. Subsequently, it appeared that there may be drugs that do not exhibit efficacy in classic animal models, but they can be effective in clinical practice. The most important example is levetiracetam, which was found to be inactive against the MES- and PTZ-induced seizures [Löscher and Hönack, Eur J Pharmacol, 1993]. However, in the 6 Hz model, levetiracetam appeared to be highly effective in blocking psychomotor seizures [Barton et al., Epilepsy Res, 2001]. The case of levetiracetam exemplifies how important is to search for new screening models of epilepsy to minimize the risk of missing a potential AED.

Levetiracetam, an *S*-enantiomer pyrrolidine derivative, is a second-generation AED that is used in monotherapy and adjunctive treatment of patients with partial-onset seizures with or without secondary generalization [Cereghino et al., Neurology, 2000] and for adjunctive therapy of myoclonic seizures [Greenhill et al., Epilepsia, 2001] and primarily generalized tonic-clonic seizures [Kumar and Smith, Seizure, 2004]. The exact mechanism of action of levetiracetam is still unknown. However, molecular studies involving transgenic mice suggest that there is a specific binding site for this drug. Levetiracetam binds to a synaptic vesicle protein 2A (SV2A), which is involved in vesicle neurotransmitter exocytosis [Lynch et al., Proc Natl Acad Sci USA, 2004]. Interestingly, there may be more mechanisms that may account for antiepileptic action of levetiracetam. For example, it has been reported that levetiracetam reduces voltage-operated K^+ current and inhibits the delayed-rectifier K^+ current in neurons [Madeja et al., Neuropharmacology, 2003], reduces N-type and partially P/Q-type high-voltage-activated Ca^{2+} currents [Lukyanetz et al., Epilepsia, 2002], but not low-voltage-activated Ca^{2+} currents [Zona et al., Seizure, 2001]. In experimental models of epilepsy, levetiracetam has increased the threshold for electroconvulsions and suppressed seizures in kindled and genetically epileptic animals [Gower at al., Eur J Pharmacol, 1992; Klit-

gaard et al., Eur J Pharmacol, 1998]. Moreover, the drug attenuates spike-and-wave discharges in DBA/2J mice (an animal model of absence epilepsy) [Marrosu et al., Epilepsy Res, 2007], and it demonstrates potent anticonvulsant effects against audiogenic seizures in Krushinsky–Molodkina rats (a strain of rats selected for susceptibility to audiogenic seizures) [Vinogradova and van Rijn, Epilepsia, 2008].

Considering the fact that levetiracetam is virtually ineffective in routinely used models of acutely evoked seizures except for the 6 Hz psychomotor seizures, it was of crucial importance to determine the interaction profile for levetiracetam in combination with some selected AEDs that were ineffective against 6 Hz-induced psychomotor seizures in mice. The objective of our study was to evaluate interactions of levetiracetam in combination with carbamazepine, phenytoin, topiramate and vigabatrin in 6 Hz psychomotor seizure model and to use the type II isobolographic analysis to determine pharmacodynamic interactions between these drugs.

Our study revealed that the combinations of levetiracetam with carbamazepine and phenytoin at the fixed-ratios of 1:5 and 1:10 were supra-additive (synergistic) in the 6 Hz psychomotor seizure test. In contrast, the AED combinations at the fixed-ratios of 1:1 and 1:2 exerted additive interaction. Moreover, the combination of levetiracetam with topiramate and vigabatrin at the fixed-ratio of 1:10 exerted supra-additive (synergistic) interaction, whereas the AED combinations at the fixed-ratios of 1:1, 1:2 and 1:5 displayed additive interaction in the 6 Hz psychomotor seizure test in mice.

In conclusion, our data have shown that levetiracetam administered singly and in combination with various AEDs displayed protective activity against seizures evoked by 6 Hz corneal electrical stimulation. Moreover, the isobolographic analysis revealed that levetiracetam in combinations with some selected AEDs (i.e., carbamazepine, phenytoin, topiramate and vigabatrin) produced supra-additive (synergistic) interactions at the particular fixed-ratio combinations. Based on this study, one can conclude that the combinations of levetiracetam with carbamazepine, phenytoin, topiramate and vigabatrin, would be clinically favorable, if the results from this preclinical study could be extrapolated to clinical settings.

Characterization of rodent model of tetramethylenedisulfotetramine (TETS)-induced seizures and potential treatment options

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Tetramethylenedisulfotetramine (tetramine, TETS) is a highly lethal neurotoxic rodenticide that was first synthesized in 1933 as a condensation product of sulfamide and formaldehyde [Wood and Battye, J Soc Chem Ind (Lond), 1933; Zhang et al., Forensic Sci Int, 2011]. TETS is an odorless and tasteless, extremely hazardous crystalline powder that is 100 times more toxic than cyanide [Xiang et al., Forensic Sci Int, 2001; Wu and Sun, Toxicology, 2004; Whitlow et al., Ann Emerg Med, 2005]. Toxicological investigations found that TETS is a highly toxic convulsant poison [Hecht and Henecka, Angew Chem, 1949] with LD_{50} of 0.1–0.2 mg/kg when administered parenterally to mice or rats [Haskell and Voss, Pharm Assoc Am Pharm Assoc (Baltim.), 1957; Voss et al., J Pharm Sci, 1961; Casida et al., Toxicol Appl Pharmacol, 1976]. Although its production and sale has been banned in Mainland China since 1991, TETS is still available on the black market in rural regions [Li et al., Neurotoxicology, 2012]. Sold illegally in China, TETS is responsible for numerous intentional and unintentional human poisonings [Zhu et al., Fa Yi Xue Za Zhi, 2004; Whitlow et al., Ann Emerg Med, 2005; Zhang et al., Forensic Sci Int, 2011; Li et al., Neuro-

toxicology, 2012]. The majority of TETS poisonings takes place in China. In 2002, after illicit importation, the first documented case of TETS human poisoning in the United States occurred in New York City [Barrueto et al., Morb Mortal Wkly Rep, 2003]. Illegal importation into other countries is especially dangerous since illnesses caused by TETS may be difficult to diagnose when not suspected [Poon et al., Hong Kong Med J, 2005]. Moreover, TETS in considered a chemical threat nerve agent that if released accidentally or used intentionally as an act of terrorism could result in mass casualties [Jett and Yeung, Proc Am Thorac Soc, 2010].

TETS is stable chemically and is slowly metabolized [Poon et al., Hong Kong Med J, 2005]. Although ingestion appears to be the most common route of exposure, occupational poisoning through inhalation has been reported [Whitlow et al., Ann Emerg Med, 2005]. The onset of TETS poisoning symptoms is rapid and occurs within 30 min to a few hours after exposure but may take up to 13 h [Chau, Hong Kong Med J, 2005]. While mild to moderate poisoning may produce symptoms like headache, dizziness, nausea, vomiting and agitation, in severe cases patients suffer generalized clonic-tonic convulsions that may lead to refractory status epilepticus followed by coma and death [Barrueto et al., J Toxicol Clin Toxicol, 2003; Croddy, Arch Toxicol, 2004]. Oral LD_{50} in humans is believed to be as low as 0.1 mg/kg which is similar to that in rodents [Guan et al., J Anal Toxicol, 1993].

It is believed that TETS acts as a noncompetitive, reversible blocker of $GABA_A$ receptors [Bowery et al., Br J Pharmacol, 1975]. Excitation of the central nervous system with absence of evident effects on peripheral neuromuscular junctions, skeletal muscles and autonomic transmission suggests that the central nervous system is the main target of TETS toxicity [Haskell and Voss, Pharm Assoc Am Pharm Assoc, 1957]. Recent animal studies have confirmed that TETS is a potent convulsant in rats and mice with LD_{50} of 0.12 mg/kg and 0.22 mg/kg when administered intraperitoneally and orally, respectively [Zolkowska et al., JPET, 2012]. Moreover, intraventricular application of TETS caused similar behavioral responses and lethality as systemic administration. This is in agreement with previous findings that TETS seizure activity is attributed to its exclusive action on the brain [Haskell and Voss, Pharm Assoc Am Pharm Assoc, 1957; Zolkowska at al., JPET, 2012]. TETS is one of the most potent chemical convulsants and induces seizure activity that is similar to that of other $GABA_A$ antagonists like PTZ and picrotoxin [Dhir et al., JPET, 2011; Zolkowska at al., JPET, 2012]. TETS activity as a $GABA_A$ receptor antagonist is comparable to that of picrotoxin, although animal studies showed that its convulsant potency is 40-times stronger that picrotoxin [Squires et al., Life Sci, 1984; Ratra et al., Toxicol Appl Pharmacol, 2001, Zolkowska et al., JPET, 2012]. TETS administered intraperitoneally or orally has comparable potency to that acquired by intravenous delivery. Additionally, great parental and oral bioavailability of TETS resulted in rapid seizure onset when administered in higher doses [Zolkowska at al., JPET, 2012]. Furthermore, TETS was found to displace specific [^{35}S]*t*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to rat brain membranes [Squires et al., Mol. Pharmacol, 1983; Esser et al., Chem Res Toxicol, 1991; Ratra et al., Toxicol Appl Pharmacol, 2001]. Noteworthy, TBPS is significantly more potent as a functional antagonist of $GABA_A$ receptor Cl^- flux when compared to TETS [Obata et al., JPET, 1988; Van Renterghem et al., Brain Res, 1987] but surprisingly TBPS is less potent as a convulsant in mice [Holland et al., Epilepsia, 1992]. Summarized, TETS is a significantly more potent convulsant *in vivo* than expected based on its known activity as a $GABA_A$ receptor antagonist. A possible explanation for such great systemic potency of TETS may be peripheral metabolism to a more potent convulsant substance, although currently there is no evidence supporting this. It is well known that chemical kindling with one of the inhibitors of central $GABA_A$ receptor function results in permanent decrease in the threshold of excitability for other $GABA_A$ antagonist like PTZ [Grecksch et al., Neuropharmacology, 1990; Corda et al., Pharmacol Biochem Behav, 1991]. However, repeated administration of low doses of TETS in mice failed to induce clearly progressive and sustain increased in seizure threshold [Zolkowska et al., JPET, 2012]. This further underlines the important difference from other $GABA_A$ receptor antagonists.

There is no known antidote for TETS poisoning and treatment is mostly supportive [Whitlow et al., Ann Emerg Med, 2005]. Currently available medical treatments for acute TETS intoxication include dimercaptopropanesulfonate, high doses of vitaminum B and diazepam [Chau et al., Hong Kong Med J, 2005]. Plasma exchange, hemodialysis and hemoperfusion are frequently used as for toxin removal from the blood stream [Chau et al., Hong Kong Med J, 2005;

Whitlow et al., Ann Emerg Med, 2005]. Unfortunately, in many cases even aggressive anticonvulsat treatment fails to deliver positive outcome. Urgently needed novel therapeutic approaches for acute TETS poisoning are currently under investigation.

Recent reports suggest that non-competitive AMPA-R antagonists may be especially useful in treatment of TETS poisoning. Perampanel, high potency selective noncompetitive AMPA receptor antagonist, presently being researched for epilepsy treatment showed promising activity when administered as a pre treatment to TETS administration [Zolkowska et al., Epilepsy Curr, 2012]. Long half life of perampanel when compared to diazepam may provide sustained seizure protection and block seizures when administered at later times when there is refractoriness to diazepam.