Module 2.4

Non Clinical Overview

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List of Abbreviations

ADD	:	After Discharge Duration
ADP	:	Adenosine DiPhosphate
ADT	:	After Discharge Threshold
AED	:	Anti-Epileptic Drug
AS	:	Absence Seizures
ATC	:	Anatomical Therapeutic Chemical Classification System
ATP	:	Adenosine Triphosphate
AUC	:	Area Under Curve
b.i.d	:	Twice a day
BI	:	Benefit Indices
BP	:	British Pharmacopoeia
Ca	:	Calcium
CBMN	:	Cytochalasin-blocked micronucleus
CCB	:	Calcium Channel Blockers
CCI	:	Chronic Constriction Injury
Cmax	:	Maximum Concentration
CMC	:	Carboxymethylcellulose
CNS	:	Central Nervous System
DNA	:	Deoxyribonucleic acid
DRG	:	Dorsal root ganglion
DRRC	:	Dose-Response Relationship Curves
ED	:	Effective Dose
EEG	:	Electroencephalogram
FDA	:	Food and Drug Administration
FPIA	:	Fluorescence Polarization Immunoassay
GABA	:	Gamma-Aminobutyric Acid
GAERS	:	Genetic Absence Epilepsy Rats from Strasbourg
GGE	:	Genetic Generalised Epilepsy

GIRK	:	G protein activated inwardly rectifying K+ channels
GLP	:	Good Laboratory Practices
Hrs	:	hours
ICH	:	International Conference on Harmonization
i.p.	:	Intraperitoneal
i.v.	:	Intravenous
K	:	Potassium
Kg	:	Kilogram
LD	:	Lethal Dose
LGN	:	Lateral Geniculate Nucleus
LEV	:	Levetiracetam
Mfg	:	Manufacturing
mg	:	milligram
Mg	:	Magnesium
mL	:	millilitre
mM	:	Millimetre
mmol/l	:	millimoles per litre
Na	:	Sodium
NA	:	Not Applicable
NCS	:	Nonconvulsive Seizures
NEC	:	Non-Epileptic Control
NMDA	:	N-methyl-D-aspartate
NMT	:	Not More Than
NREM	:	Non Rapid Eye Movement
PBBI	:	Penetrating Ballistic-like Brain Injury
Ph. Eur.	:	European Pharmacopoeia
PI	:	Protective Indices
PTZ	:	Pentylenetetrazole
REM	:	Rapid Eye Movement
RNA	:	Ribonucleic acid
RLD	:	Reference Listed Drug

SC	:	Subcutaneous
SD	:	Standard Deviation
SNL	:	Spinal Nerve Ligation
SPC	:	Summary of Product Characteristics
SS	:	Seizure Severity
SWD	:	Spike Wave Discharges
TC	:	Thalamocortical
TI	:	Teratogenic Index
Tmax	:	Time to achieve concentration
TSE	:	Transmissible Spongiform Encephalopathies
U.K.	:	United Kingdom
USFDA	:	United States Food and Drug Administration
°C	:	Degree Celcius
%	:	Percentage
µM	:	micrometre

2.4 Non-Clinical Overview

Introduction

The product Ethosuximide 250 mg / 5 mL Oral Solution is a generic product of the proprietary medicinal product.

Ethosuximide 250 mg / 5 mL Oral Solution has been filed as a Generic Application, in accordance with 2004/27/EC as an amendment to Article 10.1(a) (iii) of council directive 2001/83/EC based on well established, which is essentially similar to the reference product Zarontin 250 mg/ 5 mL syrup marketing authorization of Essential Pharma Ltd., [redacted] Since the marketing authorization application is being made for a generic version of already existing medicinal product in market, no new non-clinical studies are carried out by the applicant.

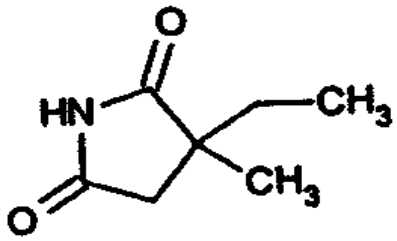
Drug Properties:

Ethosuximide [(R, S)-2-ethyl-2-methylsuccinimide, (R, S)-2-ethyl-2-methylp yrrolidine-2,5-dione] is an antiepileptic drug indicated primarily for the treatment of absence seizures. Ethosuximide is a chiral drug substance containing one asymmetric centre and is used clinically as the racemate. [redacted]

Chemical name: 3-ethyl-3-methyl-2, 5-pyrrolidinedione, 3-ethyl-3-methyl-, (±)-.

Molecular formula: $C_7H_{11}NO_2$

Structure:



Molecular weight: 141.17

[redacted]

2.4.1 OVERVIEW OF THE NON-CLINICAL TESTING STRATEGY

2.4.1.1 General Aspects, Literature and GLP, Formulation and Biopharmaceuticals, Impurities and Degradation products

General Aspects

A Marketing Authorization application for Ethosuximide 250 mg / 5 mL Oral Solution is being filed. The products Ethosuximide 250 mg / 5 mL Oral Solution is generic product of the proprietary medicinal product Zarontin 250 mg/ 5 mL syrup containing ethosuximide marketing authorization of Essential Pharma [redacted] for which the marketing authorization was granted in the European Union.

An abridged dossier is being submitted for grant of marketing authorization in accordance with 2004/27/EC as an amendment to Article 10.1(a) (iii) of council directive 2001/83/EC based on well established.

Therapeutic Indications:

Ethosuximide is indicated in the following conditions:

- Pyknoleptic absences as well as complex and atypical absences.
- Myoclonic-astatic petit mal and myoclonic fits of adolescents (impulsive petit mal), if other medicinal products are not effective and/or are not tolerated.

Literature

This non-clinical overview is based on the data obtained from international published articles, books and internet, typically searching databases of regulatory authorities i.e. approved monographs, pub med databases, science direct databases and cross-references through Google search.

GLP

No new non-clinical study is conducted by the applicant and not required for current applications. Hence, GLP is not applicable.

Formulation and Biopharmaceuticals

The product Ethosuximide 250 mg / 5 mL Oral Solution have been formulated to be essentially similar to reference drug product Zarontin 250 mg/ 5 mL syrup containing ethosuximide of Essential Pharma Ltd., United Kingdom

[redacted]

[redacted]

[redacted]

2.4.1.2 Animal Tests and Related Human Studies

No new non-clinical study is conducted by the applicant and not required for current applications.

The applicant has conducted bioequivalence study in healthy adult human subjects and is been discussed in the clinical overview.

2.4.1.3 Summary and Conclusion

Ethosuximide 250 mg / 5 mL Oral Solution was analyzed for their physical, chemical and microbiological properties by methods described in BP monograph, general chapters of Ph. Eur. and additional in-house procedures. The finished dosage form is found to conform to in-house requirements at release and under stability study conditions.

[redacted]

Drug product impurity [redacted] is in line with ICH Q3B.

2.4.2 PHARMACOLOGY

2.4.2.1 General Aspects, Primary and Secondary Pharmacodynamics

General Aspects

Pharmacotherapeutic group: Anti-epileptics, succinimide derivatives

ATC code: N03AD01

Ethosuximide is an anti-epileptic of the class of succinimides that apparently exerts multiple mechanisms of action. The activity of ethosuximide in absence type epilepsy seems to rely primarily on the inhibition of T-type calcium channels in the thalamus.

[redacted]

Mechanism of action

The structure of ethosuximide contains a five-membered ring with two negatively charged carbonyl oxygen atoms. The anticonvulsant effect of the drug is attributed to the “ring nitrogen” situated between the two groups. The drug exists as a racemic mixture of two separate enantiomers because of a chiral carbon at position two of the succinimide ring. [redacted]

Antiepileptic drugs protect against seizures by modulating neuronal excitability through effects on Na+ and Ca2+ channels, and GABAergic and glutamatergic neurotransmission. Among antiepileptic drugs, ethosuximide is used mainly for its selective effect on absence (petit mal) epilepsy. Partial inhibition of T-type Ca2+ channels by ethosuximide at therapeutically relevant concentrations has been thought to play an important role in its unique antiseizure action. Furthermore, ethosuximide has been shown to inhibit noninactivating Na+ and Ca2+-activated K+ channels and the inhibition of these channels might also be relevant. [redacted]

Ethosuximide blocks voltage-dependent sodium channels and reduces calcium currents through T-type channels. Also, ethosuximide, at therapeutic concentrations, enhances both, dopaminergic and serotonergic neurotransmission and inhibits glutamate-induced excitation. The drug also shares a very unique mechanism among antiepileptic drugs – it apparently reduces the overproduction of nitric oxide and free radicals, which may be related to its anticonvulsant and

neuroprotective effects. However, zonisamide does not seem to interfere with GABA-mediated events. [redacted]
It has been shown by microelectrode recording in brain slices that ethosuximide decreases low-threshold T-type Ca2+ currents in thalamic neurons, and has no effect on the high voltage-activated Ca2+ currents. The drug reduces peak amplitude of the low-threshold T-type Ca2+ current in the neurons of the thalamic ventrobasal complex, as well as in the reticular nucleus of the thalamus. The blockade of voltage-dependent low-threshold T-type Ca2+ current was demonstrated at clinically relevant ethosuximide concentrations in a dose-dependent manner in thalamic neurons isolated from cats, guinea pigs, Wistar, or Sprague Dawley rats. The effects of anticonvulsants and other drugs on the Na+-adenosine triphosphatase and Mg2+-ATPase activities of synaptosomes and their components have also been determined. The Mg2+-ATPase activity of synaptosomes was not affected by the drugs. However, the Na+– K+-ATPase activity was inhibited by ethosuximide, phenytoin, or diazepam. [redacted]

Ethosuximide is a succinimide derivative which has anticonvulsant action and can be used against petit mal epilepsy. Electrophysiologically, petit mal epilepsy is characterized by 3-Hz spike-wave rhythm in the electroencephalogram, the generation of which may be at least partly due to the activation of low-threshold inactivating calcium current in brain neurons. This hypothesis was confirmed by the results obtained on thalamic neurons, in which succinimides specifically depressed the low-threshold (T-type) calcium currents. The data obtained seem to be very important, as until now only a few substances were found with specific action on this type of calcium channels. [redacted]

The mechanism of action underlying the analgesic effects of ethosuximide on chemotherapy-induced pain is unclear. Ethosuximide is a relatively selective antagonist at T-type voltage sensitive calcium channels. The electrophysiological properties of T-type calcium channels show their involvement in neuronal bursting and hyper excitability, although they cannot sustain neurotransmission unaided. Ethosuximide has a higher affinity for inactivated T-type channels in cloned human calcium channels and blocks T-currents at a concentration two-fold less than that required for L-current blockade in rat dorsal root ganglion (DRG) neurons. The three genes (a1G, a1H and a1I) that encode T-type calcium channels are expressed in small and medium DRG neurones, dorsal horn of the spinal cord and thalamic regions. This pattern of expression is in accordance with studies describing T-type currents in small and medium DRG neurons, superficial dorsal horn neurons and thalamic neurones. In thalamic neurons, this current is blocked by ethosuximide. Therefore, inhibitory effects of ethosuximide on chemotherapy-induced pain are elicited via the antagonism of T-type calcium channels, the potential site(s) of action could be peripheral, spinal or supra spinal. However, more recently, ethosuximide has also been shown to possess activity at sodium and potassium currents in thalamic neurones. Thus it is

a possibility that the effects of ethosuximide may occur as a result of a blockade at several types of ion channels[redacted]

Primary Pharmacodynamics

The neuronal process by which ethosuximide, a first choice anti-absence drug, prevents absence seizures is still unresolved. Recent clinical findings have indicated that focal cortical regions are involved during absence seizures. Consistently, it has been shown in genetic models of absence epilepsy that epileptic discharges arise from a delimited region of the cerebral cortex. [redacted] made simultaneous in vivo electrocorticographic and intracellular recordings from the cortical focus of the genetic absence epilepsy rat from Strasbourg and examined the effects of systemic injection of ethosuximide at a therapeutic concentration. The investigators show that the interruption of seizures by ethosuximide is correlated with a recovery, in the hyperactive focus neurons, of physiologic values of membrane potential, firing rate, and pattern, as measured in analogous neurons from nonepileptic rats. This study strongly suggests that the antiepileptic action of ethosuximide is mediated by a specific disruption of the hyperactivity of ictogenic neurons, which recover a physiological activity preventing paroxysmal discharges and absence seizure initiation. [redacted]

The effects of ethosuximide on calcium channels were studied on dorsal root ganglion neurons from one-day old rats using the patch-clamp technique. Bath application of ethosuximide induced dose-dependent and reversible suppression of calcium currents without affecting their time-course. Substantial differences between the effects of ethosuximide on the low-threshold and high-threshold (T- and L-) currents were observed. Ethosuximide reduced the T-current with greater potency than the L-current (K4 for T-current is 7 μM vs 15 μM for L-current). This relative specificity of its action still remained if applied at concentrations up to 1 mM. These data support the hypothesis according to which the anti-epileptic action of ethosuximide is related to reduction of the low-threshold calcium currents in sensory neurons. ([redacted]

[redacted] examined whether chronic treatment with ethosuximide (i) possesses antiepileptogenic effects in the GAERS model of GGE, (ii) is associated with a mitigation of behavioural comorbidities, and (iii) influences gene expression in the somatosensory cortex region where seizures are thought to originate. GAERS and Non-Epileptic Control (NEC) rats were chronically treated with ethosuximide (in drinking water) or control (tap water) from 3 to 22 weeks of age. Subsequently, all animals received tap water only for another 12 weeks to assess enduring effects of treatment. Seizure frequency and anxiety-like behaviours were serially assessed throughout the experimental paradigm. Treatment effects on the expression of key components of the epigenetic molecular machinery, the DNA methyltransferase enzymes, were assessed using qPCR. Ethosuximide treatment significantly reduced seizures in GAERS during

the treatment phase, and this effect was maintained during the 12 weeks post-treatment phase. Further, the anxiety-like behaviours present in GAERS were reduced by ethosuximide treatment. Molecular analysis revealed that ethosuximide treatment was associated with increased expression of DNA methyltransferase enzyme mRNA in cortex. Chronic ethosuximide treatment has disease-modifying effects in the GAERS model of GGE, with anti-epileptogenic effects against absence seizures and mitigation of behavioural comorbidities. ([redacted]

[redacted] performed experiments to determine, in the WAG/Rij model of absence epilepsy, if early long-term treatment with some established anti-absence drugs might prevent the development of seizures, and whether such an effect could be sustained. WAG/Rij rats were treated for approximately 3.5 months (starting at 1.5 months of age, before seizure onset) with either ethosuximide or levetiracetam. Investigators demonstrated that both drugs are able to reduce the development of absence seizures, exhibiting anti-epileptogenic effects in this specific animal model. In conclusion, experiments suggest that the WAG/ Rij rat model undergoes an epileptogenic process during early life, which stabilizes around the age of 6 months and that early antiepileptic drug pretreatment can interfere with this process. These findings suggest that absence epilepsy in this strain of rats very likely follows an epileptogenic process during life and that early therapeutic intervention is possible, thereby opening a new area of research for absence epilepsy and antiepileptic drug treatment strategies. [redacted]

[redacted] addressed the anticonvulsant effects of phenobarbital, valproate, and ethosuximide in the amygdale of kindled guinea pigs to further validate this model for the screening of anticonvulsant drugs. Behavioral toxic effects were assessed at 30 min following drug administration using quantitative locomotor tests, as well as scores on sedation and muscle relaxation rating index. The anticonvulsant efficacy of the drugs were evaluated from measurements of after discharge threshold (ADT), after discharge duration (ADD), and behavioral seizure severity(SS) during early and late phases of kindling acquisition, and in kindled guinea pigs. ADD and SS were also measured in response to both threshold and supra threshold kindling stimulation. All drugs exerted slight to moderate sedative effects in guinea pig on both the behavioral tests and rating index. Investigators found that phenobarbital exhibited effective anticonvulsant properties in guinea pigs by consistently reducing ADD and SS in response to both threshold and supra threshold kindling stimulation. Valproate exhibited effective anticonvulsant properties at threshold stimulation and less effective properties at supra threshold stimulation. Lastly, ethosuximide lacked effective anticonvulsant action at either threshold or supra threshold kindling stimulation. Study results indicate that the guinea pig kindling model correctly predicted the actions of phenobarbital, valproate, and ethosuximide in the treatment of partial seizures. ([redacted]

In-Vitro Studies

The action of ethosuximide on Na+, K+, and Ca2+ currents and on tonic and burst-firing patterns was investigated in rat and cat thalamic neurons *in vitro* by using patch and sharp microelectrode recordings. [redacted]

These results indicate an action of ethosuximide on *I*NaP and on the Ca2+-activated K1 current, which explains the decrease in burst firing and the increase in tonic firing. [redacted]

In addition to the effect of ethosuximide on voltage-dependent Ca2+ currents of the thalamus, ethosuximide also has complex actions on the persistent Na+ and sustained K+ currents in cortical and thalamic neurons. Ethosuximide decreases the persistent Na+ and Ca2+ activated K+ currents but has no effect on the transient Na+ current. It has been observed to decrease the persistent Na+ and sustained K+ currents in layer V cortical pyramidal and thalamic relay neurons of rats and cats *in vitro*. Likewise, ethosuximide was shown to produce Na+ and K+ channel block and altered K+ channel gating in squid giant axons. Although the effect on membrane properties occurred only at high concentrations, this suggests a possibility of a direct action of ethosuximide on action potential generation. However, ethosuximide was reported to have no effect on high-frequency sustained repetitive firing of Na+ action potentials and at high concentration (700 μM) to slightly reduce GABA responses in mouse central neurons. In addition to the effects on Na+ and K+ currents, ethosuximide attenuated seizures and enhanced seizure-associated increases in thalamic activator protein 1 DNA-binding activities in lethargic mice, a genetic model of absence seizures. In this model, ethosuximide attenuated seizure behavior and the increased DNA-binding activity. ([redacted]

Secondary Pharmacodynamics

Ethosuximide has also been shown to have neuroprotective, antinociceptive and life-span-extending effects and has the potential for treating several neuropsychiatric disorders, including Parkinson's disease, methamphetamine-induced stereotyped behavior and alcohol dependence. However, the molecular and cellular mechanisms underlying the effects of antiepileptic drugs have not yet been sufficiently clarified. [redacted]

G protein activated inwardly rectifying K+ channels (GIRK, or Kir3) play an important role in regulating neuronal excitability, heart rate and platelet aggregation. In the present study, the effects of various antiepileptic drugs on GIRK channels were examined. Ethosuximide at clinically relevant concentrations inhibited GIRK channels expressed in Xenopus oocytes. The inhibition was concentration-dependent, but voltage-independent, and time-independent during each voltage pulse. However, the other antiepileptic drugs tested: phenytoin, valproic acid, carbamazepine, phenobarbital, gabapentin, topiramate and zonisamide, had no significant effects on GIRK channels even at toxic concentrations. In contrast, Kir1.1 and Kir2.1 channels were insensitive to all of the drugs tested. Ethosuximide also attenuated ethanol-induced GIRK currents. These inhibitory effects of ethosuximide were not observed when ethosuximide was applied intracellularly. In granule cells of cerebellar slices, ethosuximide inhibited GTPγS-activated GIRK currents. Moreover, ADP- and epinephrine-induced platelet aggregation was inhibited by ethosuximide, but not by charybdotoxin, a platelet Ca2+-activated K+ channel blocker. These results suggest that the inhibitory effects of ethosuximide on GIRK channels may affect some of brain, heart and platelet functions. ([redacted]

The effects of the anticonvulsant in rodents involved reducing the frequency of spike wave discharges (SWDs), analgesia and reducing depression. However, the relationship of ethosuximide with anxiety remains unclear. In rodent models, after administration of 300 mg/kg/day ethosuximide in the drinking water from 3 to 22 weeks of age, genetic absence epilepsy rats showed increased exploration and number of entries into the inner arena of the OF, showing an anxiolytic effect. In another study on Long–Evans rats, which had SWDs, rats showed more entries and longer duration in the open arms of the elevated plus maze and the centre arena of the open field test after intraperitoneal administration of 100 mg/kg ethosuximide, also showing anxiolytic properties. However, in a shock-based conditioned emotional response test on adult Sprague-Dawley rats, intraperitoneal administration of 30 or 100 mg/kg ethosuximide had no anxiolytic effect. [redacted]

The effects of ethosuximide have been examined both electrophysiologically and behaviorally in the SNL model of neuropathic pain. Intrathecal administration of ethosuximide (5–1055 μg)

depressed spinal neuronal responses to electrical and natural stimulation evoked by both Ad and C-fibres. Intraperitoneal, but not intraplantar, administration of ethosuximide (50 - 300 mg/kg) elicited a dose-related inhibition of mechanical and thermal hyperalgesias up to 1 h post-administration. These highlight ethosuximide as a potential therapy for the treatment of neuropathic pain. ([redacted]

[redacted] examined potential analgesics on established painful peripheral neuropathies induced by paclitaxel and vincristine, to identify possible mechanisms underlying, and potential clinical treatments for chemotherapy-induced pain. Paclitaxel-induced pain appears to be relatively resistant to opioid therapy i.p. 4 mg/kg morphine was ineffective and i.p. 8 mg/kg morphine only elicited up to a 50% reversal of mechanical allodynia/hyperalgesia. Interestingly, a maximally tolerated dose (i.p. 0.2 mg/kg) of the potent NMDA receptor antagonist MK-801 produced no significant reversal of the mechanical allodynia/hyperalgesia suggesting that NMDA receptors have little role in paclitaxel-induced pain. Ethosuximide (i.p. 450 mg/kg) an anti-epileptic and relatively selective T-type calcium channel blocker elicited a near complete reversal of mechanical allodynia/hyperalgesia. Repetitive dosing with ethosuximide (i.p. 100 or 300 mg/kg daily for 3 days) showed a dose-related consistent reversal of mechanical allodynia/hyperalgesia with no evidence of tolerance. Ethosuximide (i.p. 300 mg/kg) also reversed paclitaxel-induced cold allodynia and vincristine-induced mechanical allodynia/hyperalgesia. These data suggest that T-type calcium channels may play a role in chemotherapy-induced neuropathy and moreover identify ethosuximide as a new potential treatment for chemotherapy-induced pain. [redacted]

The effects of acute ethosuximide exposure on locomotor activity and anxiety-related thigmotaxis behaviours on zebrafish larvae were evaluated. The activity of AB strain zebrafish larvae at 5 and 7 days post fertilization (dpf) was analysed under normal constant illumination and stressful light–dark transitions. Under constant illumination, ethosuximide at concentrations of 2, 5 and 10 mmol/l increased the distances travelled and intensified locomotor responses to a novel environment. In addition, 40 mmol/l ethosuximide decreased the travel distance and attenuated the locomotor response to darkness. The effects were age related. Under constant illumination, ethosuximide at 40 mmol/l reduced thigmotaxis behaviours in larvae at both ages. Under the light–dark transition, 5 mmol/l ethosuximide reduced thigmotaxis behaviours in 7-dpf larvae. Investigators conclude that under constant lighting, ethosuximide at low concentrations (2, 5 and 10 mmol/ l) stimulated the locomotor activity of zebrafish larvae, whereas a high concentration (40 mmol/ l) inhibited the activity. Ethosuximide at a low concentration (5 mmol/ l) showed anxiolytic effects during the stressful light–dark transition in 7-dpf larvae. The effects of ethosuximide were age and concentration related. [redacted]

Ethosuximide is known to have significant wound-healing properties. [redacted] evaluated the wound-healing properties in rats. Accordingly, ethosuximide, phenobarbital, and barbituric acid containing ointments were applied onto full-thickness excision and incision wounds created on the dorso-lumbar region of experimental rats. ethosuximide and PHO-treated incision wounds illustrated significant enhancement in breaking strengths (1380 ± 61 and 1240 ± 42 g, respectively) compared to vehicle controls (1070 ± 18 g) and barbituric acid (1080 ± 45 g). Moreover, biochemical analyses revealed significant increase in hydroxyproline contents in ethosuximide and phenobarbital treated wounds compared to vehicle controls. Histological evaluation revealed that both ethosuximide and phenobarbital promoted collagen synthesis and deposition. Results describe the significant wound healing merits of ethosuximide and phenobarbital as potential clinical agents for treatment of chronic wounds. [redacted]

[redacted] al explored the analgesic effects of the low voltage-activated T-type Ca2+ channel blockers ethosuximide, trimethadione, and mibefradil in persistent and acute nociceptive tests. The anticonvulsant effects of the compounds were also determined in the intravenous pentylenetetrazol seizure model. Following intraperitoneal administration, ethosuximide and trimethadione dose-dependently reversed capsaicin-induced mechanical hyperalgesia. Similarly, the highest dose of mibefradil tested (30 μg, intracisternal) reversed capsaicin-induced mechanical hyperalgesia. Ethosuximide and mibefradil produced statistically significant analgesic effects in both early and late phase formalin induced behaviors and trimethadione reduced late phase behaviors. Additionally, ethosuximide and trimethadione produced antinociceptive effects in the rat-tail flick reflex test. In contrast, following intracisternal administration, mibefradil had no effect in the tail flick reflex test. In addition, the anticonvulsants ethosuximide and trimethadione increased the doses of pentylenetetrazol required to produce both first twitch and clonic seizures. In contrast however, mibefradil had no anticonvulsant effect. The present results demonstrate that the clinically used anticonvulsants ethosuximide and trimethadione provide analgesic effects at doses, which are anticonvulsant. Furthermore, the data further supports the idea that T-type Ca2+ channels may be important targets for treating persistent pain syndromes. [redacted]

A study was performed to examine the effect of ethosuximide and morphine in the behavioral responses following the chronic constriction injury (CCI) model of neuropathic pain. [redacted]

[redacted] Results suggest that ethosuximide block tactile and thermal hypersensitivity after the CCI model, also, ethosuximide potentiates the analgesic effects of morphine in neuropathic pain conditions and behavioral responses. ([redacted]

2.4.2.2 Safety Pharmacodynamics and Drug Interactions

[redacted] evaluated the efficacy of ethosuximide and valproate against seizures induced by lindane administration in mice. Ethosuximide afforded complete protection against both clonic and tonic seizures induced by pentylenetetrazol. Rightward shift in the dose response curve indicated that the potency of ethosuximide against tonic seizures was higher that this for the clonic seizures. However, this effect did not reach statistical significance. The ED50 values of ethosuximide for the protection against pentylenetetrazol-induced clonic and tonic seizures were 71.6 and 60.5 mg/kg, respectively. Valproate also fully protected animals from both clonic and tonic seizures induced by pentylenetetrazol, with the respective ED50 values of 55.8 and 45.3 mg/kg. Both ethosuximide and valproate afforded complete and dose-dependent protection against seizures induced by lindane. However, the potencies of these drugs were lower than those obtained against pentylenetetrazol seizures. The present study shows that ethosuximide and valproate exhibit dose-dependent protection against both clonic and tonic seizures evoked by lindane administration in mice. The efficacy of these drugs against pentylenetetrazol-induced seizures was also confirmed in the present study. The protective efficacy of ethosuximide and valproate against experimentally induced lindane seizures may suggest possible efficacy of these drugs against seizures in lindane-poisoned patients. ([redacted]

A study was undertaken to test the effect of two of the standard clinically used antiepileptic drugs on epileptic discharges in this model, ethosuximide and diphenylhydantoin. Epileptic bursts in the electroencephalogram (EEG) were significantly reduced following administration of ethosuximide with plasma levels of 60 µg per milliliter, and there was a linear correlation between the plasma level and antiepileptic effect. Diphenylhydantoin produced a lesser reduction in epileptic activity, and there was no correlation between the plasma level and effect. Study showed that the response of this experimental form of epilepsy to ethosuximide was relatively specific as compared with that to diphenylhydantoin. The response to ethosuximide was often seen at sub toxic plasma levels that were within or just above the usual clinical therapeutic range for this drug. Results tentatively conclude that certain basic physiologic mechanisms common to both feline generalized epilepsy and human generalized corticoreticular epilepsy could account for the effectiveness of ethosuximide in both conditions. On the other hand, a variety of seizure forms with underlying mechanisms different (penty1enetetrazo1 - induced convu1sive seizures, cobalt-induced focal seizures, and penicillin-induced non-convulsive generalized seizures) all

seem to respond to ethosuximide. This would seem to suggest that ethosuximide affects some fundamental epileptogenic mechanism common to a variety of seizure forms. [redacted]

Another study assessed the anti-seizure profile of ethosuximide (12.5–187.5 mg/kg) and phenytoin (5–30 mg/kg) in a spontaneously occurring nonconvulsive seizures (NCS) model associated with penetrating ballistic-like brain injury (PBBI). [redacted] Study results demonstrate that ethosuximide and phenytoin are effective against spontaneously occurring NCS following PBBI, and suggest that these AEDs may be effective at treating post-traumatic NCS. [redacted]

Chronic alcohol abuse depresses the nervous system and, upon cessation, rebound hyperexcitability can result in withdrawal seizure. Withdrawal symptoms, including seizures, may drive individuals to relapse, thus representing a significant barrier to recovery. [redacted] examined whether ethosuximide, a T-channel antagonist, could decrease the severity of ethanol withdrawal seizures by evaluating electrographical and behavioral correlates of seizure activity. DBA/2J mice were exposed to an intermittent ethanol exposure paradigm. Mice were treated with saline or ethosuximide in each withdrawal period, and cortical EEG activity was recorded to determine seizure severity. A progression in seizure activity with each successive withdrawal period was reported. Treatment with ethosuximide reduced ethanol withdrawal-induced spike and wave discharges (SWDs), in terms of absolute number, duration of events, and contribution to EEG power reduction in the 6–10 Hz frequency range. Overall, we observed a decrease in handling induced convulsion severity in mice treated with ethosuximide. These findings suggest that ethosuximide may be a useful pharmacological agent for studies of alcohol withdrawal and treatment of resulting seizures. [redacted]

[redacted] investigated the effects of chronic intermittent ethanol exposures on the rhythmic patterns and relative power of different EEG power bands and the average spectrum during two types of sleep. Investigators also assessed the efficacy of ethosuximide as a pharmacologic intervention during a subsequent week of sustained withdrawal. Two groups of eight-week old

male C57BL/6 mice were implanted with wireless EEG/EMG telemetry and subjected to 4 weeks of chronic, intermittent ethanol vapor exposure and withdrawal. During the week after the final withdrawal, mice were administered ethosuximide (200 mg/kg) or saline. Chronic intermittent ethanol exposure produced changes in the diurnal rhythms of the delta and theta bands that persisted into a subsequent week of sustained withdrawal. These disruptions were restored with the T-channel blocker ethosuximide. Repeated ethanol exposures preferentially increased the relative proportion of lower frequency power (delta and theta), whereas higher frequencies were decreased. The ethanol-induced decreases in relative power for the higher frequencies continued into the sustained withdrawal week for both groups. Increases in absolute delta and theta power were observed in averaged NREM and REM sleep spectral data during withdrawal in ethosuximide-treated animals, suggesting increased sleep intensity. These results suggest that persistent alterations in delta and theta EEG rhythms during withdrawal from chronic intermittent ethanol exposure can be ameliorated with ethosuximide and that this treatment might also increase sleep intensity during withdrawal. ([redacted]

Drug Interactions

An experimental study evaluated the pharmacodynamic interaction between ethosuximide and caffeine sodium benzoate in the pentetrazole induced clonic seizures in mice. Caffeine sodium benzoate at doses of 69.3 and 92.4 mg/kg attenuated the protective action of ethosuximide against pentetrazole induced seizures, by increasing its median effective dose from 127.7 to 182.3 and 198.3 mg/kg respectively. The acute exposure to caffeine sodium benzoate may diminish the antiseizure protection offered by ethosuximide in epileptic patients. Therefore, patients treated with ethosuximide should avoid caffeine sodium benzoate. [redacted]

[redacted]l characterize the interactions between levetiracetam and the conventional antiepileptic drugs (AEDs) clonazepam, ethosuximide, phenobarbital and valproate in suppressing pentylenetetrazole (PTZ)-induced clonic seizures in mice by use of type II isobolographic analysis. [redacted] indicates that levetiracetam in combination

with ethosuximide, clonazepam, phenobarbital and valproate offers potential anticonvulsant synergy. [redacted]

A study characterized the interaction between tiagabine and ethosuximide, two antiepileptic drugs, in pentylenetetrazole (PTZ) induced clonic seizures in mice using isobolographic analysis. The results indicated that both drugs produced clear anticonvulsant effects against PTZ-induced clonic seizures in mice, but that their dose-response relationship curves (DRRCs) were not parallel, consequently necessitating the isobolographic analysis for non-parallel DRRCs. The isobolographic analysis revealed that the combination of tiagabine with ethosuximide at the fixed ratio of 1:1 exerted an additive interaction against PTZ induced clonic seizures in mice. At present, there are no recommendations to use this drug combination in epileptic patients. [redacted]

The combination of ethosuximide and valproate has been assessed experimentally in mice by studying the effect of the pharmacodynamic interactions between the two drugs on the efficacy vs. toxicity ratio. All results were expressed in terms of brain concentrations. A purely additive anticonvulsant interaction was found. However, due to a less than additive neurotoxic interaction, the combination had a better efficacy vs. toxicity score than either drug alone. These results, although not representative for all drug combinations, provide evidence that the dissociation between the anti-convulsant and the neurotoxic interaction of two antiepileptic drugs can result in a better protective index for certain combinations of antiepileptic drugs. ([redacted]

The effect of pretreatment with morphine-like analgesics on the anticonvulsant effect of phenytoin and phenobarbital against electroconvulsions and of phenobarbital and ethosuximide against pentetrazole-induced convulsions was studied in mice. High doses of morphine (4 mg/kg subcutaneously), fentanyl (100 µg/kg subcutaneously), pethidine and pentazocine (4 and 8 mg/kg subcutaneously) increased the anti-electroshock effect of phenytoin; that of phenobarbital was likewise increased by morphine (2 and 4 mg/kg), fentanyl (25-100 µg/kg) and pentazocine (2-8 mg/kg), whereas pethidine had no corresponding effect. There was no consistent effect of pretreatment with the analgesics studied on the anticonvulsant effects of phenobarbital and ethosuximide in the pentetrazole seizure threshold test. [redacted]

[redacted] characterized the interactions between loreclezole and clonazepam, ethosuximide, phenobarbital and valproate in suppressing pentylenetetrazole (PTZ)-induced seizures and in producing acute neurotoxic adverse effects in the chimney test in mice. Moreover, protective indices (PIs) and benefit indices (BIs) were calculated so that a ranking in relation to advantageous combination could be established. All AED combinations comprising loreclezole and clonazepam, ethosuximide, phenobarbital and valproate were additive in their seizure

suppression. [redacted]

[redacted] studied the interaction between valproate and ethosuximide in diminishing the incidence of absence-like spike-wave discharges (SWDs) in the EEG of WAG/Rij rats. The incidence of SWDs in the EEG was determined for the period of 15–75 min after injection and compared to the incidence of SWDs prior to injection. The parameters for diminishing the incidence of the SWDs were: valproate: ED50: 121 mg/kg; ethosuximide: ED50: 21.5 mg/kg; valproate / ethosuximide: ED50: 112/16 mg/kg. Isobolic analysis showed that a higher drug load was needed of the combination than of the individual drugs to achieve a 50% reduction of SWDs: factor 1.67. The interaction between valproate and ethosuximide was shown to be infra-additive in diminishing the incidence of SWDs in WAG/Rij rats. In conclusion, valproate and ethosuximide antagonize each other, both in reducing spontaneous active behaviour and in reducing the incidence of absence-like phenomena in the EEG of WAG/Rij rats. [redacted]

Sodium valproate, ethosuximide, 200 mg/kg ip and flunarizine 5 or 10 mg/kg ip were first administered independently to rats in order to study their effects on behavioural and EEG aspects of spike and wave discharges (SWDs) induced by γ-hydroxybutyrate (GHB, 100 mg/kg ip). GHB treated rats show behavioural changes and concomitant repetitive EEG episodes of 7 to 9 Hz SWDs, mimicking human absence seizures (AS), and can be used as a pharmacological model. The number and duration of SWDs were calculated for 1 hr from the EEG and were parameters for drug evaluation. Sodium valproate and ethosuximide at 200 mg/ kg significantly reduced SWD number and duration/ hr, while flunarizine showed significant reduction only at 10 but not at 5 mg/kg. Combination of flunarizine 10 mg/kg with either Sodium valproate or ethosuximide showed significant reduction of SWD number and duration, suggesting an additive effect of the anti-absence agents with the calcium channel blocker, flunarizine, on experimental absence seizures in rats. In conclusion, the results obtained in this study further confirm the synergistic effect of either Sodium valproate or ethosuximide in combination with flunarizine in another experimental model and sustains previous evidence that flunarizine may be a valuable additive in pharmacoresistant epilepsy. [redacted]

[redacted] characterized the anticonvulsant effects of levetiracetam in combination with ethosuximide in the mouse 6 Hz psychomotor seizure model. The combinations of levetiracetam with ethosuximide for the fixed-ratios of 1:5 and 1:10 appear to be particularly favorable combinations exerting supra-additive interaction in the mouse 6 Hz psychomotor seizure model. It may be concluded that because of the synergistic interactions between levetiracetam and ethosuximide, the combination might be useful in clinical practice. ([redacted]

2.4.2.3 Summary and Conclusion

Pharmacodynamics

Ethosuximide is an anti-epileptic of the class of succinimides that apparently exerts multiple mechanisms of action. The blockade of low-threshold Ca2+ currents is likely to be responsible for its efficacy in primary generalized absence type epilepsy. The anti-epileptic action of ethosuximide is related to reduction of the low-threshold calcium currents in sensory neurons. The activity of ethosuximide in absence type epilepsy seems to rely primarily on the inhibition of T-type calcium channels in the thalamus. Ethosuximide blocks voltage-dependent sodium channels and reduces calcium currents through T-type channels. Also, ethosuximide, at therapeutic concentrations, enhances both, dopaminergic and serotonergic neurotransmission and inhibits glutamate-induced excitation. The drug also shares a very unique mechanism by reducing the overproduction of nitric oxide and free radicals, which may be related to its anticonvulsant and neuroprotective effects.

Collectively the actions of ethosuximide in terms of site, neurotransmitter, and channel types suggest a combination of direct and indirect effects at various levels of the brain. An in vivo study in rats strongly suggests that the antiepileptic action of ethosuximide is mediated by a specific disruption of the hyperactivity of ictogenic neurons, which recover a physiological activity preventing paroxysmal discharges and absence seizure initiation.

Ethosuximide treatment significantly reduced seizures in GAERS during the treatment phase and this effect was maintained during the 12 weeks post-treatment phase. Further, the anxiety-like behaviours present in GAERS were reduced by ethosuximide treatment. Molecular analysis revealed that ethosuximide treatment was associated with increased expression of DNA methyltransferase enzyme mRNA in cortex. The action of ethosuximide on *I*NaP and on the Ca2+-activated K1 current explains the decrease in burst firing and the increase in tonic firing in rat studies. Furthermore, ethosuximide attenuated seizure behavior and the increased DNA-binding activity.

Secondary Pharmacology

Ethosuximide has also been shown to have neuroprotective, antinociceptive and life-span-extending effects and has the potential for treating several neuropsychiatric disorders, including Parkinson's disease, methamphetamine-induced stereotyped behavior and alcohol dependence.

Effects of ethosuximide on G protein activated inwardly rectifying K+ channels (GIRK) were examined. Ethosuximide at clinically relevant concentrations inhibited GIRK channels expressed in Xenopus oocytes. The inhibition was concentration-dependent, but voltage-independent, and time-independent during each voltage pulse. The inhibitory effects of ethosuximide on GIRK channels may affect some of brain, heart and platelet functions.

Administration of ethosuximide depressed spinal neuronal responses to electrical and natural stimulation evoked by both Ad and C-fibres in pain models. Ethosuximide elicited a dose-related inhibition of mechanical and thermal hyperalgesias up to 1 hr post-administration. These highlight ethosuximide as a potential therapy for the treatment of neuropathic pain. Ethosuximide also reversed paclitaxel-induced cold allodynia and vincristine-induced mechanical allodynia/hyperalgesia. The study results suggest that T-type calcium channels may play a role in chemotherapy-induced neuropathy and moreover identify ethosuximide as a new potential treatment for chemotherapy-induced pain. Studies in pain models have shown that clinically used ethosuximide provide analgesic effects at doses, which are anticonvulsant and supports the idea that T-type Ca2+ channels may be important targets for treating persistent pain syndromes. Ethosuximide block tactile and thermal hypersensitivity in chronic constriction injury model, also, ethosuximide potentiates the analgesic effects of morphine in neuropathic pain conditions and behavioral responses.

At low concentrations, ethosuximide showed anxiolytic effects during the stressful light–dark transition in zebrafish larvae. Investigators revealed that under constant lighting, ethosuximide at low concentrations (2, 5 and 10 mmol/ l) stimulated the locomotor activity of zebrafish larvae, whereas a high concentration (40 mmol/ l) inhibited the activity. Ethosuximide is also known to have significant wound-healing properties.

Safety Pharmacology

The efficacy of ethosuximide against pentylenetetrazol-induced seizures in mice is confirmed in experimental non-clinical studies. Ethosuximide afforded complete protection against both clonic and tonic seizures induced by pentylenetetrazol. The ED50 values of ethosuximide for protection against pentylenetetrazol-induced clonic and tonic seizures were 71.6 and 60.5 mg/kg, respectively. The protective efficacy of ethosuximide against experimentally induced lindane

seizures may suggest possible efficacy of ethosuximide against seizures in lindane-poisoned patients.

A pre-clinical study comparing ethosuximide and diphenylhydantoin in mice model conclude that certain basic physiologic mechanisms common to both feline generalized epilepsy and human generalized corticoreticular epilepsy could account for the effectiveness of ethosuximide in both conditions. Another study conducted in rats demonstrates that ethosuximide is effective against spontaneously occurring nonconvulsive seizures following penetrating ballistic-like brain injury and suggest that these anti-epileptic drug may be effective at treating post-traumatic nonconvulsive seizures in humans.
Chronic alcohol abuse depresses the nervous system and, upon cessation, rebound hyperexcitability can result in withdrawal seizure. Studies in mice reported that ethosuximide may be a useful pharmacological agent for studies of alcohol withdrawal and treatment of resulting seizures. Overall, we observed a decrease in handling induced convulsion severity in mice treated with ethosuximide. Alterations in delta and theta EEG rhythms during withdrawal from chronic intermittent ethanol exposure can be ameliorated with ethosuximide and that this treatment might also increase sleep intensity during withdrawal.

Pharmacodynamic Drug Interactions

In pentetrazole induced clonic seizures in mice, caffeine sodium benzoate at doses of 69.3 and 92.4 mg/kg attenuated the protective action of ethosuximide against pentetrazole induced seizures, by increasing its median effective dose from 127.7 to 182.3 and 198.3 mg/kg respectively. Levetiracetam in combination with ethosuximide offers potential anticonvulsant synergy. The isobolographic analysis revealed that the combination of tiagabine with ethosuximide at the fixed ratio of 1:1 exerted an additive interaction against PTZ induced clonic seizures in mice. At present, there are no recommendations to use this drug combination in epileptic patients. Because of the synergistic interactions between levetiracetam and ethosuximide, the combination might be useful in clinical practice.

The combination of ethosuximide and valproate has been assessed experimentally in mice by studying the effect of the pharmacodynamic interactions between the two drugs on the efficacy vs. toxicity ratio. The combination had a better efficacy vs. toxicity score than either drug alone suggesting that dissociation between the anti-convulsant and the neurotoxic interaction of two antiepileptic drugs can result in a better protective index for certain combinations of antiepileptic drugs. There was no consistent effect of pretreatment with morphine-like analgesics studied on the anticonvulsant effects of phenobarbital and ethosuximide in the pentetrazole seizure threshold test.

Coadministration of valproate with ethosuximide antagonizes each other, both in reducing spontaneous active behaviour and in reducing the incidence of absence-like phenomena in the EEG of WAG/Rij rats. Another study in rats confirmed the synergistic effect of either valproate or ethosuximide in combination with flunarizine.

2.4.3 PHARMACOKINETICS

2.4.3.1 General Aspects, Absorption, Distribution and Bioavailability, Metabolism and Excretion

General Aspects

Pharmacokinetics of ethosuximide enantiomers were studied in rats. Ethosuximide, both as a racemic mixture and as the individual enantiomers, was administered to conscious rats by the intravenous (i.v.) and intraperitoneal (i.p.) routes. Pharmacokinetic parameters were estimated using standard non-compartmental methods and tabulated below.

[redacted]

Table 02: Mean (±S.D.) pharmacokinetic parameters estimated following the administration of ethosuximide as individual enantiomers (10 mg i.p.)

[redacted]

[redacted] This indicates that, at the doses studied, the preferential faster elimination of (R)-ethosuximide is not dependent upon the presence of the (S)-enantiomer. Also, for each enantiomer, the lack of any significant difference between estimates of clearance when administered as part of a racemic mixture and when administered separately indicates that neither enantiomer affects the clearance of the other.

[redacted]

Pharmacokinetics of ethosuximide was evaluated in rhesus monkeys to characterize the diurnal oscillations in steady-state plasma levels. Morning steady-state (9 am) plasma levels were significantly higher than the corresponding evening (5 pm) plasma levels during a 3-week zero-order infusion of ethosuximide to six monkeys. Circadian periodicity in steady-state plasma

levels was investigated in three monkeys over 4 months under controlled experimental conditions: blood sampling at 2-hr intervals for 26 hrs, 1 day/week; fixed lighting, feeding, and noise schedules; and electroencephalogram monitoring. The plasma concentration-time curves showed two minima in the 12 noon-2 pm and 8 pm-12 midnight periods and the later involved the largest percent change in plasma levels (4% - 8%). The plasma concentration-time data were subjected to cross-correlation analysis, which indicated a circadian rhythm in steady-state plasma levels with a period of 24-26 hr. Study revealed diurnal fluctuations (circadian periodicity) in steady-state plasma levels of ethosuximide in monkeys. [redacted]

[redacted] demonstrated the systemic availability of Ethosuximide after rectal administration in rabbits and the effect of the suppository base on its absorption. Ethosuximide was formulated in different suppository bases. Male white Boskat strain rabbits (1.5 - 2.0 kg) were randomly divided into 5 groups of 3 animals each. Rectal administration in polyethylene glycol 400: 4000 and 400: 6000 to rabbits gave high plasma levels where Cmax was 45.66 and 42.66 µg/ml, respectively; while, in the presence of Witepsol fatty base E76 and W35 it was 34.00 and 28.33 µg/ml, respectively. The systemic availability was 89.39%, 82.72%, 58.80% and 47.45% when the bases were polyethylene glycol 400: 4000, polyethylene glycol 400: 6000, Witepsol E76 fatty base and Witepsol W35 fatty base, respectively. [redacted]

Pharmacokinetic parameters of ethosuximide were determined Monkeys. Three types of chronic-dosing experiments were implemented in a group of chronically catheterized male rhesus monkeys: Study I, constant rate infusion; Study II, infusion at three consecutive rates with priming doses; and Study III, oral multiple dosing at two consecutive levels with priming doses. Zero-order infusion rates and priming and maintenance doses were calculated for each animal using individual single-dose pharmacokinetic parameters. Ethosuximide was measured in plasma and urine. Experimental and predicted plasma and urine levels were in good agreement for all three studies. Total body clearance (Studies I and II) and elimination half-life (Studies I, II, and III) were not significantly different from their respective single-dose values. Excretion rate was proportional to infusion rate (Study II) and maintenance dose (Study III). It was established that in the case of ethosuximide in monkey, pharmacokinetic parameters derived from single-dose studies can be used to predict multiple-dosing schedules. [redacted]

Pharmacokinetics of ethosuximide were studied in dogs and compared with the data known for man. After intravenous injection of 40 mg/kg to 9 dogs, the concentration-time curve in serum could be described by open one-compartment model. Serum half-lives were between 11 and 25 hr, the volume of distribution corresponded to between 44% and 66% of body weight. After oral administration of the same dose, maximal serum concentrations were reached within 01 - 04 hrs. and the absorption amounted to 88% - 95%. By treatment with three daily doses, a plateau in serum concentrations could be reached and maintained. At serum concentrations above 100 µl/

ml, half-lives seemed prolonged when compared to the values determined previously with a dose of 40 mg/kg. Ethosuximide rapidly enters the cerebrospinal fluid; equilibrium was established after 20-30 min. Plasma protein binding of the drug was negligible. Comparison of the pharmacokinetic data for dog and man reveals, in spite of the somewhat shorter half-life in the dog, a rather good agreement. In female rats, ethosuximide had an average half-life of 10 hrs, in mice of hardly 1 hrs. Concentrations in serum and brain of mice equilibrated within 10 mins. [redacted]

Absorption

Absorption and distribution following i.p. administration of rac-ethosuximide in rats appeared to be rapid. Data relating to Cp max (the maximum observed plasma concentration of ethosuximide) and Tmax (the time at which Cp max was recorded) are presented in table below. Tmax values following the 40 mg i.p. dose ranged from 0.5 to 2 h. After the 10 mg i.p. doses, Tmax values were all within 1 h. Mean Cp max values for (S)-ethosuximide and (R)-ethosuximide were not significantly different from each other for any of the dose levels or routes of administration studied. Mean Cp max values increased by a factor of approximately 4 (i.e. 33.3–121.5 mg/ml and 33.5–116.8 mg/ml for (S)-ethosuximide and (R)-ethosuximide, respectively) when the i.p. dose was increased from 10 to 40 mg. The areas under the curve (AUC), normalized for body weight, also increased by a factor of approximately 4 (i.e. 95.46 – 336.51 mg/l h/kg and 77.78 – 284.46 mg/l h/kg for (S)-ethosuximide) and (R)-ethosuximide), respectively) when the i.p. dose was increased from 10 to 40 mg. [redacted]

Table 03: Cmax (mean ± S.D.) and Tmax (median {range}) values obtained after ethosuximide (ESM) administration [redacted]



Figure 01 below shows drug concentration observed in rat plasma at various times following chronic intraperitoneal administration of ethosuximide (b.i.d. for 4.5 days). Animals of Groups I, II, III and IV were administered 20, 40, 60, and 80 mg/kg doses of ethosuximide, respectively. The nonlinear least squares fit of post absorption phase to a monoexponential function gave a half-life value of 9.2 hrs at 80 mg/kg dose. A linear relationship between area under the plasma concentration-time curve during a dosing interval and dose was observed. [redacted]

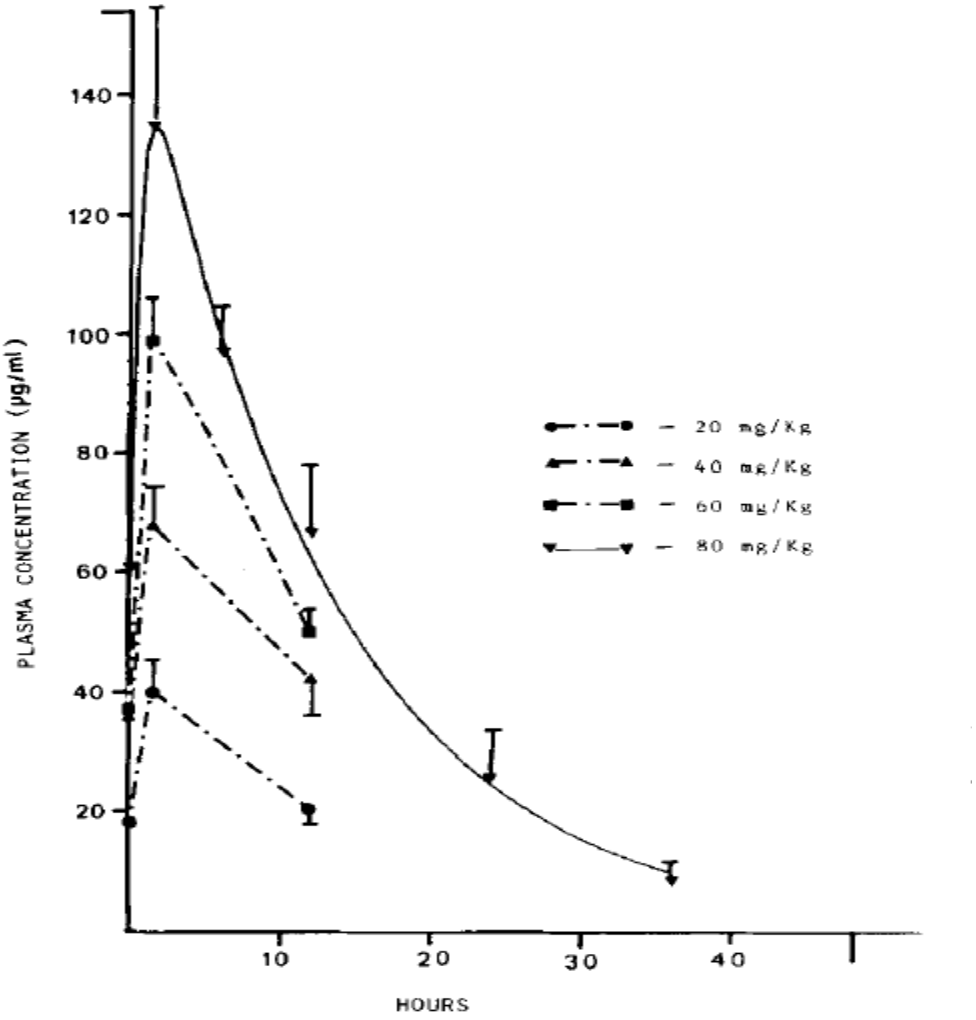


Figure 01: Plots of plasma concentrations vs time following chronic administration of ethosuximide to rats. [redacted]

Distribution and Bioavailability

[redacted] reported apparent volume of distribution of rac-ethosuximide to be to be 0.70+0.031 l/kg. [redacted]

Single and multiple dose disposition kinetics of ethosuximide were studied in male Sprague-Dawley rats following intravenous administration. The plasma disappearance of a single 35 mg/kg dose followed for 75 h was not linear. A dose-ranging study suggested that the apparent non-linearity of ethosuximide's plasma disappearance might be due to enzyme induction over time with the drug exhibiting a faster elimination from 24 h onward. Ethosuximide, after only two daily doses of 35 mg/kg/day, shortened pentobarbital induced sleep in rats. The clearance of ethosuximide was also significantly faster after four daily 35 mg/kg doses than after a single 35

mg/kg dose. Single sample clearance estimates calculated from ethosuximide concentrations prior to enzyme induction viz up to 24 h post-dose, were practically identical to multiple sample clearance values. Both single and multiple sample clearances were calculated assuming linear rather than non-linear elimination. ([redacted]8)

The distribution properties of ethosuximide in four regions of the rat brain-cortex, midbrain, cerebellum, and pons-medulla-were investigated following chronic intraperitoneal administration at four dose levels (20, 40, 60, and 80 mg/kg). Elimination half-life was determined for each tissue from a least squares regression analysis of the drug levels observed at 80 mg/kg. The half-life values obtained, namely, 9.1 hr in cortex and in midbrain, 9.3 hr in cerebellum, and 9.4 hr in pons-medulla, were all quite close to the corresponding value obtained in plasma. Area distribution ratios were calculated for each tissue and ranged from 0.899 to 0.928 in cortex, 0.922 to 0.965 in midbrain, 0.890 to 0.910 in cerebellum, and 0.888 to 0.940 in pons-medulla. A two way analysis of variance showed that the area distribution ratios were not significantly different between doses. However, they were significantly different between tissues at the 1% level of significance. Midbrain area distribution ratio was significantly higher than that for the cerebellum. However, no significant difference could be found between any other two pairs. The effect of dose on the distribution of ethosuximide in the various brain tissues was also examined in terms of a plot of tissue concentrations versus the corresponding plasma concentrations. In each tissue, a linear relationship was observed supporting the earlier finding of no dose dependency in the tissue/ plasma area distribution ratio. The slopes of the regression lines were 0.91 for cortex, 0.94 for midbrain, and 0.90 for cerebellum and pons medulla. [redacted]

Metabolism

Figure 02 shows the structure of ethosuximide and four hydroxymetabolites that have been identified in the rat. [redacted]

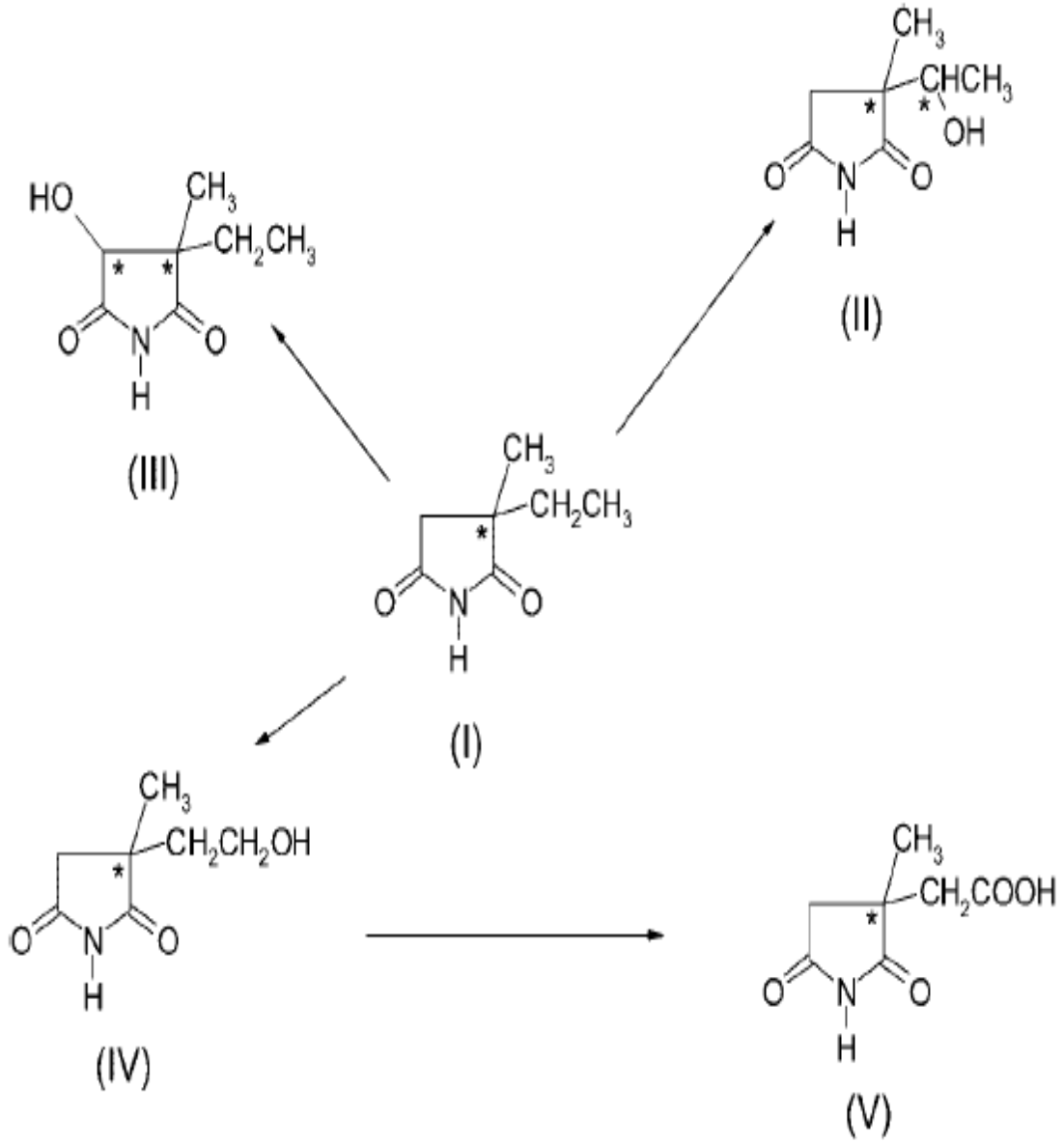


Figure 02: Structures of ethosuximide and its metabolities: (I) Ethosuximide, (II) 2-(1-hydroxyethyl)-2-methylsuccinimide, (III) 2-ethyl-3-hydroxy-2-methylsuccinimide, (IV) 2-(2-hydroxyethyl)-2-methylsuccinimide, (V) 2-carboxymethyl-2-methylsuccinimide [redacted]

The metabolism of ethosuximide in animals and in humans has been extensively investigated. In all studies unchanged ethosuximide was found in the urine. The major metabolic route for ethosuximide in animals is hydroxylation, whilst in humans hydroxylation and extensive conjugation (glucuronide formation) have been reported. In various investigations of the

metabolism of ethosuximide in the rat four hydroxymetabolites have been identified: the two stereoisomeric 2-(1-hydroxyethyl)-2-methyhylsuccinimides (II), 2-ethyl-3-hydroxy2- methyl succinimide (III), 2-(2-hydroxyethyl)-2-methylsuccinimide (IV). The two stereoisomeric 2-(1-hydroxyethy1)-2-methyl succinimides (II) have been variously reported as the isomers of 2-(1-hydroxyethyl)-2-methylsuccinimide and the diastereoisomers of 2-(l-hydroxyethyl)-2-methylsuccinimide. [redacted]

[redacted] reported the identification of 2-carboxymethyl-2-methylsuccinimide (V) as a metabolite of ethosuximide. [redacted] also reported the presence of two peaks that gave identical mass spectra, identified as diastereoisomers of 2-ethyl-3-hydroxy-2-methylsuccinimide (III). [redacted]

Ethosuximide is metabolized by the rat to monohydroxyethosuximides which are excreted in urine, both free and as glucuronic acid conjugates. Four monohydroxymetabolites of ethosuximide have been identified in the urine of rat and man. A dihydroxyethosuximide was present in small quantities in rat urine. Several glucuronides of the monohydroxyethosuximides were also detected in the urine samples. In rat plasma, ethosuximide was present in higher concentration than the metabolites; in human adult and infant plasma, the monohydroxyethosuximides were present in larger quantities than the unchanged drug. [redacted]

The urinary metabolites of ethosuximide (following i.p. administration of the racemate or individual enantiomers to rats) have been studied using chiral gas chromatography and gas chromatography mass spectroscopy. [redacted] Results obtained following the administration of individual enantiomers of ethosuximide indicate that the 2-(1-hydroxyethyl)-2-methylsuccinimide diastereoisomers derived from (R)-ethosuximide are produced in approximately equal proportions [redacted] whilst those from 6)-ethosuximide are produced in unequal proportions [redacted]

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Excretion

The plasma elimination half-life of ethosuximide was found to be 9.0 hr in rats. [redacted]

In a study designed to test for time dependency in elimination kinetics of ethosuximide in monkeys, the drug was chronically infused for 22 days to six animals. Morning (9 am) plasma concentrations were consistently higher than evening (5 pm) concentrations and the presence of circadian rhythm in steady-state plasma levels was suspected. [redacted]

[redacted] estimated the clearance of rac-ethosuximide to be around 0.022 l/h/kg after a 35 mg/kg dose and 0.027 l/h/kg after a 5 mg/kg dose using an achiral analytical procedure. [redacted]

The half-life for (R)-ethosuximide is significantly shorter than that of (S)-ethosuximide; conversely the clearance of (R)-ethosuximide is significantly larger than that of (S)-ethosuximide in rats. Since no significant differences are observed between volumes of distribution, the shorter elimination half-life for (R)-ethosuximide indicates preferential stereoselective elimination of this enantiomer in the rat. [redacted]

Following administration of rac-ethosuximide by i.v., i.p. and oral routes in rats, unchanged ethosuximide was detected in urine up to 72 h after drug administration. The appearance of all detected metabolites occurred within 24 h of drug administration. Significantly more (S)-ethosuximide was excreted unchanged than (R)-ethosuximide with all three routes studied. Furthermore, there is a progressive daily trend in the ratio of (S)-ethosuximide to (R)-ethosuximide excreted, the largest trend being observed following administration of the drug via the i.v. route. A substantial amount of the drug was eliminated as the 2-(1-hydroxyethyl)-2-methylsuccinimide (2 pairs of diastereoisomers). Much less drug was eliminated as the 2-ethyl-3-hydroxy-2-methylsuccinimide with only one diastereoisomer observed. Examination of the one pair of diastereoisomers of 2-(1-hydroxyethyl)-2-methylsuccinimide that was resolved showed preferential excretion of one isomer. Comparison of both pairs of diastereoisomers showed that one pair was formed in preference to the other with a ratio of approximately 0.8:1. It is concluded that stereoselective metabolism of ethosuximide occurs. [redacted]

Pharmacokinetic Drug Interactions

A study characterized the interaction between tiagabine and ethosuximide, two antiepileptic drugs, in pentylenetetrazole (PTZ) induced clonic seizures in mice using isobolographic analysis. The nature of the interaction between the drugs administered in combination was ascertained by

estimating plasma and brain concentrations of ethosuximide and tiagabine using fluorescence polarization immunoassay (FPIA) and high performance liquid chromatography. FPIA documented that tiagabine significantly elevated brain ethosuximide concentrations (by 64%), while having no effect on plasma ethosuximide concentrations in experimental animals. In contrast, ethosuximide had no significant impact on plasma and brain concentrations of tiagabine in mice. It can be concluded that the additive interaction between tiagabine and ethosuximide at the fixed-ratio of 1:1 in the PTZ test was complicated by a significant pharmacokinetic increase in total brain ethosuximide concentrations. [redacted]

[redacted] characterized the interactions between loreclezole and clonazepam, ethosuximide, phenobarbital, and valproate in mice. Co-administration of loreclezole with ethosuximide resulted in a significant 42% elevation in total brain ethosuximide concentration. In contrast, higher doses of loreclezole had no effect on brain clonazepam, phenobarbital and valproate concentrations when administered in combination. Only combinations of loreclezole with clonazepam and phenobarbital were completely free of any pharmacokinetic interaction. [redacted]

[redacted] studied the pharmacokinetic interaction between valproate and ethosuximide in rats. No differences between the single drugs (either ethosuximide or valproate) and the combinations were found nor an interaction. This indicates that there is no pharmacokinetic interaction between valproate and ethosuximide in WAG/Rij rats 40 min after a single dose. ([redacted]

A study in mice characterized the interaction between stiripentol and clonazepam, ethosuximide, phenobarbital, and valproate. All antiepileptic drugs (AEDs) combinations comprising of stiripentol and clonazepam, ethosuximide, phenobarbital and valproate were additive in terms of clonic seizure suppression in the PTZ test. However, these interactions were complicated by changes in brain AED concentrations consequent to pharmacokinetic interactions. Stiripentol significantly increased total brain ethosuximide and phenobarbital concentrations, and decreased valproate concentrations, but was without effect on clonazepam concentrations. In contrast, phenobarbital significantly decreased and valproate increased total brain stiripentol concentrations while clonazepam and ethosuximide were without effect. [redacted]w J. [redacted]

2.4.3.2 Summary and Conclusion

Pharmacokinetics of ethosuximide was evaluated in rhesus monkeys to characterize the diurnal oscillations in steady-state plasma levels. The plasma concentration-time data were subjected to cross-correlation analysis, which indicated a circadian rhythm in steady-state plasma levels with

a period of 24-26 hr. Study revealed diurnal fluctuations (circadian periodicity) in steady-state plasma levels of ethosuximide in monkeys. Studies in monkeys showed that ethosuximide pharmacokinetic parameters derived from single-dose studies can be used to predict multiple-dosing schedules. Total body clearance and elimination half-life were not significantly different from their respective single-dose values following intravenous and oral dosing.

Ethosuximide is rapidly absorbed in rats following i.p. administration. Time to reach maximum concentration (Tmax) following the 40 mg i.p. dose ranged from 0.5 to 2 h where after the 10 mg i.p. doses, Tmax values were all within 1 h. A linear relationship between area under the plasma concentration-time curve during a dosing interval and dose was observed. In dogs, following intravenous injection of 40 mg/kg, serum half-lives were between 11 and 25 hrs, the volume of distribution corresponded to between 44% and 66% of body weight. After oral administration of the same dose, maximal serum concentrations were reached within 01 - 04 hrs and the absorption amounted to 88% - 95%.

Ethosuximide is uniformly distributed in the cerebral cortex, midbrain, cerebellum, pons, and medulla of rats. Plasma protein binding of the drug was negligible. Apparent volume of distribution of [redacted]ethosuximide was found to be 0.70 + 0.031 l/kg. Elimination half-life was determined for each tissue from a least squares regression analysis of the drug levels observed at 80 mg/kg. The half-life values obtained, namely, 9.1 hr in cortex and in midbrain, 9.3 hr in cerebellum, and 9.4 hr in pons-medulla, were all quite close to the corresponding value obtained in plasma. Area distribution ratios were calculated for each tissue and ranged from 0.899 to 0.928 in cortex, 0.922 to 0.965 in midbrain, 0.890 to 0.910 in cerebellum, and 0.888 to 0.940 in pons-medulla.

The metabolism of ethosuximide in animals has been extensively investigated with major metabolic route being hydroxylation. Studies reported that unchanged ethosuximide was found in the urine. Ethosuximide is metabolized by the rat to monohydroxyethosuximides which are excreted in urine, both free and as glucuronic acid conjugates. A stereoselective metabolism of ethosuximide is reported. In various investigations of the metabolism of ethosuximide in the rat four hydroxymetabolites have been identified: the two stereoisomeric 2-(1-hydroxyethyl)-2-methyhylsuccinimides (II), 2-ethyl-3-hydroxy2- methyl succinimide (III), 2-(2-hydroxyethyl)-2-methylsuccinimide (IV). The two stereoisomeric 2-(1-hydroxyethy1)-2-methyl succinimides (II) have been variously reported as the isomers of 2-(1-hydroxyethyl)-2-methylsuccinimide and the diastereoisomers of 2-(l-hydroxyethyl)-2-methylsuccinimide.

The plasma elimination half-life of ethosuximide was found to be 9.0 hr in rats. The half-life for (R)-ethosuximide is significantly shorter than that of (S)-ethosuximide; conversely the clearance of (R)-ethosuximide is significantly larger than that of (S)-ethosuximide in rats. Since no

significant differences are observed between volumes of distribution, the shorter elimination half-life for (R)-ethosuximide indicates preferential stereoselective elimination of this enantiomer in the rat.

Pharmacokinetic Drug Interactions

Ethosuximide had no significant impact on plasma and brain concentrations of tiagabine in mice. The additive interaction between tiagabine and ethosuximide at the fixed-ratio of 1:1 in the PTZ test was complicated by a significant pharmacokinetic increase in total brain ethosuximide concentrations.

Co-administration of loreclezole with ethosuximide in mice resulted in a significant 42% elevation in total brain ethosuximide concentration. No pharmacokinetic interaction between valproate and ethosuximide was reported in rats whereas stiripentol significantly increased total brain ethosuximide concentrations in mice.

2.4.4 TOXICOLGY

2.4.4.1 General Aspects

The published literature on the toxicology of ethosuximide is summarised and reviewed to ascertain whether the studies conducted since the original marketing authorisation for the first ethosuximide containing product indicate any significant change in safety assessment for the non-clinical (animal) information. The detail description of various studies undertaken to investigate toxicity of ethosuximide on various animal species is provided as below.

2.4.4.2 Single Dose Toxicity

Non-clinical data reveal no special hazard for humans based on conventional studies of acute dose toxicity. [redacted]

The LD50 values for ethosuximide in mouse at different route of administration are tabulated below:

[redacted]

(ETHOSUXIMIDE - [redacted]

The effects of chronic administration of gradually increasing doses of the antipetit mal agents, ethosuximide and valproic acid, on the performance of “incremental repeated acquisition” and “incremental fixed-ratio tasks” were studied in the epileptic baboon. At approximately equipotent anticonvulsant doses, ethosuximide was reported to be more behaviorally toxic than valproic acid. The behavioral deficits induced by ethosuximide were still present at 8 weeks after cessation of drug treatment in two of the four animals, suggesting that cognitive processes can be disrupted by chronic administration of ethosuximide. ([redacted]

2.4.4.3 Repeat Dose Toxicity

Non-clinical data reveal no special hazard for humans based on conventional studies of repeated dose toxicity. (Ethosuximide Aristo 250 mg/5 ml oral solution, SPC, Aristo Pharma Limited, U.K., 2018)

2.4.4.4 General Aspects, Genotoxicity, Carcinogenicity, Immunotoxicity and Antigenicity

There have been no adequate, well-controlled studies on the carcinogenicity, mutagenicity, or impairment of fertility of this product. [redacted]

Long-term studies of the carcinogenetic potential in animals have not been performed. [redacted]

Ethosuximide did not reveal a potential for mutagenicity or chromosome aberrations when studied *in vitro*. [redacted]

A study by [redacted] addressed the involvement of calcium channel-blockers (CCBs) in inducing male infertility. Thirty-six male mice were orally treated by therapeutic dose of nifedipine and ethosuximide for 20 days followed by 10 days without treatment for drug withdrawal. Chromosome aberrations assay, sperm analysis and testicular expression level of biomarker steroidogenic acute regulatory (StAR) mRNA were measured. Also, histological structure of the testis was investigated to the process of spermatogenesis. Study results revealed that, CCBs significantly increased the percentage of chromosome aberration and sperm shape change. StAR-mRNA expression was significantly down regulated. Sperm count and motility were significantly decreased. However, slight improvement was observed in all tested parameters after drug withdrawal. Seminiferous tubules displayed total atrophy, disruption, severe damage and elongation of tubules with disorganization of germinal epithelium that detached from the basement membrane. The lumen of seminiferous tubules showed complete absence of sperm cells. Both nifedipine and ethosuximide significantly increase chromosome abnormalities, decrease sperm function, and down regulate StAR-mRNA expression. All these side effects may lead to irreversible male sterility. ([redacted]

Genotoxicity

Genotoxicity of nifedipine and ethosuximide was evaluated in 36 male mice following oral therapeutic doses. The chromosome aberration test is a widely accepted genotoxic assay. Study

results revealed that, tested doses of nifedipine and ethosuximide significantly increased the percentage of aberrant cells without any improvement of genotoxic damage after drug withdrawal. These observations are in line with some previous studies regarding CCB genotoxicity. Study provided evidence that, nifedipine and ethosuximide treatment induced genotoxicity. [redacted]

2.4.4.5 Reproductive Toxicity, Teratogenicity, Pregnancy and Nursing

Embryotoxicity studies in rats and mice revealed a higher incidence rate of malformation and changes in behaviour. [redacted]

Teratogenicity of ethosuximide was compared with that of phenytoin in Swiss CD-1 mice. The teratogenic potency was estimated using the minimum teratogenic dose (tD05). In this study, tD05 for phenytoin was 0.17 mmol/kg/day and for ethosuximide, 5.2 mmol/kg/day. The "Relative Teratogenic Index" (LD01/tD05), which reflects the teratogenic hazard of a test agent, was also computed in this study. The indices were 1.2 and 1.6 for ethosuximide and phenytoin, respectively. ([redacted]

Ethosuximide (60, 180 & 360 mg/kg) given to rats on 6^{th} - 16^{th} day of pregnancy produced cleft palate & enlarged cerebral ventricles in offspring. Less frequent exencephaly, exomphalos, phocomelia, open eyes, undescended testes. When ethosuximide given to pregnant rats (days 6-14), hamsters (days 6-10) & rabbits (days 6-18), rats & hamsters showed skeletal malformations; in rabbits eye, limb & tail malformations were also found. [redacted]

Pregnant NMRI mice were given single subcutaneous (SC) injections of 0 or 600 mg/kg ethosuximide in water on day 8 of gestation. On day 18 of gestation, the dams were killed and the fetuses examined. Weight retardation was observed in the ethosuximide exposed fetuses compared to controls. For the plasma and tissue concentration studies, a group of pregnant mice was given a single dose of 400 mg/kg ethosuximide on day 8 of gestation. The animals were sacrificed 30 min later and the embryos (total implantation sites) and maternal blood were sampled and analyzed. Ethosuximide was not teratogenic in this study. [redacted]

Juvenile rats 4-6 weeks old exposed prenatally at days 5-20 of gestation to ethosuximide at 10 mg/kg/dams' body weights per day were examined for behavioral abnormalities. Pinning behavior in the pups aged 4-5 weeks was significantly more frequent than that in age-matched controls. However, basal activity of open-field behavior and activity inhibited by diazepam

administration in the pups aged 5-6 weeks showed no difference from the controls. The intensity of stereotyped behavior induced by apomorphine (1 mg/kg, SC) was significantly greater in the pups aged 5-6 weeks than in the controls. These results indicate that prenatal exposure to ethosuximide may cause changes in the dopaminergic neurons in the central nervous system. [redacted]

The teratogenic activity of six commonly used antiepileptic drugs, carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, and primidone, was studied at three dose levels (3, 9, and 18 times the human therapeutic dose) in CD1 mice. The drugs, suspended in 1% carboxymethylcellulose, were administered by gastric intubation on Days 6-16 of pregnancy, which covers the period from implantation to the end of structural development. The dams were killed 1 day before term and the fetuses were examined. Full-length cleft palates were observed in all treated groups with the exception of the clonazepam treated group. This defect was not observed in any of the control groups. Other defects such as exencephaly, enlarged cerebral ventricles, exomphalos, open eyes, undescended testes, abnormal palatal bones other than cleft palate, and fused ribs were seen less frequently. If these results are assessed by ranking the drugs in order of teratogenicity, assessed on either a pooled fetus or a pooled litter basis, the drugs fall into three categories: Phenytoin produced the highest incidence of teratogenic effects; carbamazepine, phenobarbital, and primidone produced a lower but still significant incidence of defects; and clonazepam and ethosuximide were the least teratogenic. Nevertheless, there was at least a two-fold increase in defects in these latter two treatment groups compared with the controls. [redacted]

Juvenile rats 4-6 weeks old exposed prenatally at days 5-20 of gestation to ethosuximide at 10 mg/kg/dams' body weights per day were examined for behavioral abnormalities. On day 5 of gestation, the mean body weights of the pregnant rats in the two groups were not significantly different between ethosuximide and controls. The gains in weight from days 5 to 20 of gestation in the two groups also were not significantly different. The length of gestation, number of offspring delivered, and the sex ratio within litters were not significantly affected by the administration of ethosuximide. Pinning behavior in the pups aged 4-5 weeks was significantly more frequent than that in age-matched controls. However, basal activity of open-field behavior and activity inhibited by diazepam administration in the pups aged 5-6 weeks showed no difference from the controls. The intensity of stereotyped behavior induced by apomorphine (1 mg/kg, SC) was significantly greater in the pups aged 5-6 weeks than in the controls. These findings suggest that prolonged exposure to ethosuximide in utero may increase the sensitivity of the dopaminergic system in the CNS of the developing rats, and that play-fighting (pinning behavior) is a sensitive index of functional abnormalities in the CNS in juvenile rats. [redacted]

[redacted] evaluated 06 commonly used antiepileptic drugs in pregnant CDI mice to see if they were teratogenic, and if so, whether they could be placed in some order of teratogenicity. The drugs used were all given at 3, 9 and 18 times the therapeutic dose and were carbamazepine, clonazepam, ethosuximide, phenobarbitone, phenytoin and primidone together with carboxymethylcellulose (CMC) and absolute control groups. The main defects observed in the treated groups were cleft palate (with the exception of clonazepam) and enlarged cerebral ventricles. Other major defects such as exencephaly, exomphalos, phocomelia, open eyes, and undescended testes were seen less frequently. If the results obtained for each drug are pooled on either a per foetus or a per litter basis, the drugs can be divided into three main categories of teratogenicity. On a per foetus basis, phenytoin is in a category of its own in that it produced the highest incidence of foetal defects (20.9%). Primidone, carbamazepine and phenobarbitone fall into the next category, the percentage of foetal defects being 4.8%, 4.7% and 4.7% respectively. In the third category are clonazepam (2.7%) and ethosuximide (3.2%) which produced the lowest incidence of malformations, which are not clearly shown in these experiments to be significantly different from the spontaneous control values of 1.3%. These results indicate that some anticonvulsants are safer than others in the mouse, and this may be true for man also. [redacted]

A study evaluated the teratogenic potential of seven antiepileptic drugs (AEDs) (carbamazepine, ethosuximide, valproic acid, lamotrigine, lacosamide, levetiracetam and topiramate in the zebrafish model. The lethality and teratogenic index (TI) of AEDs were determined and the TI values of each drug were compared with the US FDA human pregnancy categories. The lethality and teratogenicity of antiepileptic drugs were determined and calculated as TI at 72 hpf. TI values were ranked as follows: valproic acid > TPM > lacosamide > carbamazepine > lamotrigine > levetiracetam ≅ ethosuximide. Lacosamide and lamotrigine induced several kinds of malformations and showed significant difference between dose levels. The main malformation of valproic acid was growth retardation, and topiramate induced multi malformation included heart edema, yolk deformity, and scoliosis. The calculated TI values of the compounds were compared with the US FDA human pregnancy category. Three drugs carbamazepine, topiramate and valproic acid, which were classified as pregnancy category D by FDA and showed T1 values greater than 2, while the four drugs, ethosuximide, lamotrigine, lacosamide and levetiracetam, which were classified as category C by FDA, showed a wide range of T1 values between 0.76 and 2.3. [redacted]

2.4.4.6 Miscellaneous

Cytotoxicity

[redacted] investigated ethosuximide treated human lymphocytes with Fenech's CBMN (cytochalasin-blocked micronucleus cytome) assay and single-cell gel electrophoresis (comet assay). The tests allowed examining micronucleus formation, the proliferative status of the viable cells, nuclear blebbing, nucleoplasmic bridge formation, cell death (CBMN assay) and DNA damage (comet assay). The lymphocytes were treated for 24 h with 25, 50 or 100 mg/ml ethosuximide. The cells used for the CBMN assay were examined either immediately or 24 h after the cytochalasin blockade. For the comet assay, cells were examined immediately or 22 h after 2 h ethosuximide treatment. The results indicate that 25 and 50 mg/ml ethosuximide did not induce micronucleus formation, nuclear abnormalities, cell death or DNA damage, nor did they affect cell proliferation, suggesting no cytotoxic or genotoxic effects under such experimental conditions. However, under treatment with 100 mg/ml ethosuximide, an increase in micronucleus formation and nuclear abnormalities, but a decrease in cell proliferation and in DNA damage, and no change in the cell death ratio, were detected. Although apparently contradictory, the data obtained with the 100 mg/ml concentration may indicate that induction of cytotoxicity and genotoxicity are not to be disregarded when considering this drug concentration. [redacted]

[redacted] performed *in vitro* studies to understand the effect of ethosuximide on rat hippocampal neural stem cell's proliferation and differentiation. Investigators reported that therapeutic concentrations of ethosuximide (<100 μM) are non-cytotoxic, whereas higher concentrations adversely affect neural stem cell's viability. Neurospheres growth kinetics assay suggested that non-cytotoxic concentration of ethosuximide enhances the number and size of neurospheres. Low concentration of ethosuximide induces proliferation of neural stem cell, while higher concentrations of ethosuximide are cytotoxic. ([redacted]

A study was aimed to examine the ability of ethosuximide, an anticonvulsant drug, to promote neurogenesis in 3-day-old rat forebrain cortex stem cells. Most of the cells were found to be nestin positive undifferentiated neural stem cells prior to their exposure to ethosuximide. It was noted that the number and percentage of tubulin β-III immunopositive neurons were increased after 6 days treatment with ethosuximide. Upon bFGF withdrawal, exposure to ethosuximide differentiated the stem cells to MAP2 positive neural cells. GABA immunofluorescence images illustrated that ethosuximide increased GABAergic neurons. To determine cell viability, cytotoxicity of ethosuximide treatment was tested by utilizing MTT. Results indicated that ethosuximide produced significant cytotoxic effect at concentrations higher than 1 μM. The present study showed that clinically relevant concentrations of ethosuximide resulted in increased number of GABAergic neurons generated from cortical stem cells without causing any significant cytotoxic effects. [redacted]

Impurity Toxicity

[redacted] reported that derivatives of 2-ethyl-2-methylsuccinic acid had no potential of mutagenicity against the test strains of V. harveyi: BB7, BB7X, BB7M and BB7XM. [redacted]

2.4.4.7 Summary and Conclusion

Non-clinical data reveal no special hazard for humans based on conventional studies of acute and repeated dose toxicity. The LD50 values for ethosuximide in mouse were reported to be 780 mg/kg, 1810 mg/kg, 1752 mg/kg and 1530 mg/kg following intravenous, subcutaneous, intraperitoneal and oral route of administration.

Long-term studies of the carcinogenetic potential in animals have not been performed. Ethosuximide did not reveal a potential for mutagenicity or chromosome aberrations when studied *in vitro.* Ethosuximide significantly increase chromosome abnormalities, decrease sperm function, and down regulate StAR-mRNA expression in male mice which may lead to irreversible male sterility.

Embryotoxicity studies in rats and mice revealed a higher incidence rate of malformation and changes in behaviour. The "Relative Teratogenic Index" (LD01/tD05), which reflects the teratogenic hazard of a test agent, was reported to be 1.2 for ethosuximide in Swiss CD-1 mice. Ethosuximide is classified as category C by FDA with wide range of teratogenic index values between 0.76 and 2.3. Ethosuximide (60, 180 & 360 mg/kg) given to rats on 6^{th} - 16^{th} day of pregnancy produced cleft palate & enlarged cerebral ventricles in offspring. Less frequent observed effects included exencephaly, exomphalos, phocomelia, open eyes, undescended testes, enlarged cerebral ventricles, abnormal palatal bones other than cleft palate, and fused ribs. When ethosuximide given to pregnant rats (days 6-14), hamsters (days 6-10) & rabbits (days 6-18), rats & hamsters showed skeletal malformations; in rabbits eye, limb & tail malformations were also found.

Prenatal exposure to ethosuximide may cause changes in the dopaminergic neurons in the central nervous system in juvenile rats. Prolonged exposure to ethosuximide in utero may increase the sensitivity of the dopaminergic system in the CNS of the developing rats, and that play-fighting (pinning behavior) is a sensitive index of functional abnormalities in the CNS in juvenile rats. As compared to other anti-epileptic drugs, ethosuximide produced the lowest incidence of malformations, which are not clearly shown in these experiments to be significantly different from the spontaneous control values of 1.3%. These suggest that ethosuximide is safer than others in the mouse, and this may be true for man also.

Study in male mice with ethosuximide provided evidence that drug seems to induce genotoxicity. In vitro studies indicate that 25 and 50 mg/ml ethosuximide did not induce micronucleus formation, nuclear abnormalities, cell death or DNA damage, nor did they affect cell proliferation, suggesting no cytotoxic or genotoxic effects under such experimental conditions. However, under treatment with 100 mg/ml ethosuximide, an increase in micronucleus formation and nuclear abnormalities, but a decrease in cell proliferation and in DNA damage, and no change in the cell death ratio, were detected. Low concentration of ethosuximide induces proliferation of neural stem cell, while higher concentrations of ethosuximide are cytotoxic.

2.4.5 INTEGRATED OVERVIEW AND CONCLUSIONS

Introduction

A marketing authorization application for Ethosuximide 250 mg / 5 mL Oral Solution is a generic product of the proprietary medicinal product.

The products Ethosuximide 250 mg / 5 mL Oral Solution is generic product of the proprietary medicinal product Zarontin 250 mg/ 5 mL syrup containing ethosuximide marketing authorization of Essential Pharma Ltd. [redacted]

An abridged dossier is being submitted for grant of marketing authorization in accordance with 2004/27/EC as an amendment to Article 10.1(a) (iii) of council directive 2001/83/EC based on well established

Specific non-clinical studies have not been performed, which is acceptable considering that this is an application for a generic version of a product that has been licensed for many years. The non-clinical overview provides a satisfactory review of the pharmacodynamic, pharmacokinetic, and toxicological properties of ethosuximide, a widely used and well-known active substance.

Ethosuximide is indicated in the following conditions:

- Pyknoleptic absences as well as complex and atypical absences
- Myoclonic-astatic petit mal and myoclonic fits of adolescents (impulsive petit mal), if other medicinal products are not effective and/or are not tolerated.

Pharmacodynamics

Ethosuximide is an anti-epileptic of the class of succinimides that apparently exerts multiple mechanisms of action. The blockade of low-threshold Ca2+ currents is likely to be responsible for its efficacy in primary generalized absence type epilepsy. The anti-epileptic action of ethosuximide is related to reduction of the low-threshold calcium currents in sensory neurons. The activity of ethosuximide in absence type epilepsy seems to rely primarily on the inhibition of T-type calcium channels in the thalamus. Ethosuximide blocks voltage-dependent sodium channels and reduces calcium currents through T-type channels. Also, ethosuximide, at therapeutic concentrations, enhances both, dopaminergic and serotonergic neurotransmission and

inhibits glutamate-induced excitation. The drug also shares a very unique mechanism by reducing the overproduction of nitric oxide and free radicals, which may be related to its anticonvulsant and neuroprotective effects.

Collectively the actions of ethosuximide in terms of site, neurotransmitter, and channel types suggest a combination of direct and indirect effects at various levels of the brain. An in vivo study in rats strongly suggests that the antiepileptic action of ethosuximide is mediated by a specific disruption of the hyperactivity of ictogenic neurons, which recover a physiological activity preventing paroxysmal discharges and absence seizure initiation.

Ethosuximide treatment significantly reduced seizures in GAERS during the treatment phase and this effect was maintained during the 12 weeks post-treatment phase. Further, the anxiety-like behaviours present in GAERS were reduced by ethosuximide treatment. Molecular analysis revealed that ethosuximide treatment was associated with increased expression of DNA methyltransferase enzyme mRNA in cortex. The action of ethosuximide on *I*NaP and on the Ca2+-activated K1 current explains the decrease in burst firing and the increase in tonic firing in rat studies. Furthermore, ethosuximide attenuated seizure behavior and the increased DNA-binding activity.

Secondary Pharmacology

Ethosuximide has also been shown to have neuroprotective, antinociceptive and life-span-extending effects and has the potential for treating several neuropsychiatric disorders, including Parkinson's disease, methamphetamine-induced stereotyped behavior and alcohol dependence.

Effects of ethosuximide on G protein activated inwardly rectifying K+ channels (GIRK) were examined. Ethosuximide at clinically relevant concentrations inhibited GIRK channels expressed in Xenopus oocytes. The inhibition was concentration-dependent, but voltage-independent, and time-independent during each voltage pulse. The inhibitory effects of ethosuximide on GIRK channels may affect some of brain, heart and platelet functions.

Administration of ethosuximide depressed spinal neuronal responses to electrical and natural stimulation evoked by both Ad and C-fibres in pain models. Ethosuximide elicited a dose-related inhibition of mechanical and thermal hyperalgesias up to 1 h post-administration. These highlight ethosuximide as a potential therapy for the treatment of neuropathic pain. Ethosuximide also reversed paclitaxel-induced cold allodynia and vincristine-induced mechanical allodynia/hyperalgesia. The study results suggest that T-type calcium channels may play a role in chemotherapy-induced neuropathy and moreover identify ethosuximide as a new potential treatment for chemotherapy-induced pain. Studies in pain models have shown that

clinically used ethosuximide provide analgesic effects at doses, which are anticonvulsant and supports the idea that T-type Ca2+ channels may be important targets for treating persistent pain syndromes. Ethosuximide block tactile and thermal hypersensitivity in chronic constriction injury model, also, ethosuximide potentiates the analgesic effects of morphine in neuropathic pain conditions and behavioral responses.

At low concentrations, ethosuximide showed anxiolytic effects during the stressful light–dark transition in zebrafish larvae. Investigators revealed that under constant lighting, ethosuximide at low concentrations (2, 5 and 10 mmol/ l) stimulated the locomotor activity of zebrafish larvae, whereas a high concentration (40 mmol/ l) inhibited the activity. Ethosuximide is also known to have significant wound-healing properties.

Safety Pharmacology

The efficacy of ethosuximide against pentylenetetrazol-induced seizures in mice is confirmed in experimental non-clinical studies. Ethosuximide afforded complete protection against both clonic and tonic seizures induced by pentylenetetrazol. The ED50 values of ethosuximide for protection against pentylenetetrazol-induced clonic and tonic seizures were 71.6 and 60.5 mg/kg, respectively. The protective efficacy of ethosuximide against experimentally induced lindane seizures may suggest possible efficacy of ethosuximide against seizures in lindane-poisoned patients.

A pre-clinical study comparing ethosuximide and diphenylhydantoin in mice model conclude that certain basic physiologic mechanisms common to both feline generalized epilepsy and human generalized corticoreticular epilepsy could account for the effectiveness of ethosuximide in both conditions. Another study conducted in rats demonstrates that ethosuximide is effective against spontaneously occurring nonconvulsive seizures following penetrating ballistic-like brain injury and suggest that these anti-epileptic drug may be effective at treating post-traumatic nonconvulsive seizures in humans.

Chronic alcohol abuse depresses the nervous system and, upon cessation, rebound hyperexcitability can result in withdrawal seizure. Studies in mice reported that ethosuximide may be a useful pharmacological agent for studies of alcohol withdrawal and treatment of resulting seizures. Overall, we observed a decrease in handling induced convulsion severity in mice treated with ethosuximide. Alterations in delta and theta EEG rhythms during withdrawal from chronic intermittent ethanol exposure can be ameliorated with ethosuximide and that this treatment might also increase sleep intensity during withdrawal.

Pharmacodynamic Drug Interactions

In pentetrazole induced clonic seizures in mice, caffeine sodium benzoate at doses of 69.3 and 92.4 mg/kg attenuated the protective action of ethosuximide against pentetrazole induced seizures, by increasing its median effective dose from 127.7 to 182.3 and 198.3 mg/kg respectively. Levetiracetam in combination with ethosuximide offers potential anticonvulsant synergy. The isobolographic analysis revealed that the combination of tiagabine with ethosuximide at the fixed ratio of 1:1 exerted an additive interaction against PTZ induced clonic seizures in mice. At present, there are no recommendations to use this drug combination in epileptic patients. Because of the synergistic interactions between levetiracetam and ethosuximide, the combination might be useful in clinical practice.

The combination of ethosuximide and valproate has been assessed experimentally in mice by studying the effect of the pharmacodynamic interactions between the two drugs on the efficacy vs. toxicity ratio. The combination had a better efficacy vs. toxicity score than either drug alone suggesting that dissociation between the anti-convulsant and the neurotoxic interaction of two antiepileptic drugs can result in a better protective index for certain combinations of antiepileptic drugs. There was no consistent effect of pretreatment with morphine-like analgesics studied on the anticonvulsant effects of phenobarbital and ethosuximide in the pentetrazole seizure threshold test.

Coadministration of valproate with ethosuximide antagonizes each other, both in reducing spontaneous active behaviour and in reducing the incidence of absence-like phenomena in the EEG of WAG/Rij rats. Another study in rats confirmed the synergistic effect of either valproate or ethosuximide in combination with flunarizine.

Pharmacokinetics

Pharmacokinetics of ethosuximide was evaluated in rhesus monkeys to characterize the diurnal oscillations in steady-state plasma levels. The plasma concentration-time data were subjected to cross-correlation analysis, which indicated a circadian rhythm in steady-state plasma levels with a period of 24-26 hr. Study revealed diurnal fluctuations (circadian periodicity) in steady-state plasma levels of ethosuximide in monkeys. Studies in monkeys showed that ethosuximide pharmacokinetic parameters derived from single-dose studies can be used to predict multiple-dosing schedules. Total body clearance and elimination half-life were not significantly different from their respective single-dose values following intravenous and oral dosing.

Ethosuximide is rapidly absorbed in rats following i.p. administration. Time to reach maximum concentration (Tmax) following the 40 mg i.p. dose ranged from 0.5 to 2 h where after the 10 mg i.p. doses, Tmax values were all within 1 h. A linear relationship between area under the plasma

concentration-time curve during a dosing interval and dose was observed. In dogs, following intravenous injection of 40 mg/kg, serum half-lives were between 11 and 25 hrs, the volume of distribution corresponded to between 44% and 66% of body weight. After oral administration of the same dose, maximal serum concentrations were reached within 01 - 04 hrs and the absorption amounted to 88% - 95%.

Ethosuximide is uniformly distributed in the cerebral cortex, midbrain, cerebellum, pons, and medulla of rats. Plasma protein binding of the drug was negligible. Apparent volume of distribution of rac-ethosuximide was found to be 0.70 + 0.031 l/kg. Elimination half-life was determined for each tissue from a least squares regression analysis of the drug levels observed at 80 mg/kg. The half-life values obtained, namely, 9.1 hr in cortex and in midbrain, 9.3 hr in cerebellum, and 9.4 hr in pons-medulla, were all quite close to the corresponding value obtained in plasma. Area distribution ratios were calculated for each tissue and ranged from 0.899 to 0.928 in cortex, 0.922 to 0.965 in midbrain, 0.890 to 0.910 in cerebellum, and 0.888 to 0.940 in pons-medulla.

The metabolism of ethosuximide in animals has been extensively investigated with major metabolic route being hydroxylation. Studies reported that unchanged ethosuximide was found in the urine. Ethosuximide is metabolized by the rat to monohydroxyethosuximides which are excreted in urine, both free and as glucuronic acid conjugates. A stereoselective metabolism of ethosuximide is reported. In various investigations of the metabolism of ethosuximide in the rat four hydroxymetabolites have been identified: the two stereoisomeric 2-(1-hydroxyethyl)-2-methyhylsuccinimides (II), 2-ethyl-3-hydroxy2- methyl succinimide (III), 2-(2-hydroxyethyl)-2-methylsuccinimide (IV). The two stereoisomeric 2-(1-hydroxyethy1)-2-methyl succinimides (II) have been variously reported as the isomers of 2-(1-hydroxyethyl)-2-methylsuccinimide and the diastereoisomers of 2-(l-hydroxyethyl)-2-methylsuccinimide.

The plasma elimination half-life of ethosuximide was found to be 9.0 hr in rats. The half-life for (R)-ethosuximide is significantly shorter than that of (S)-ethosuximide; conversely the clearance of (R)-ethosuximide is significantly larger than that of (S)-ethosuximide in rats. Since no significant differences are observed between volumes of distribution, the shorter elimination half-life for (R)-ethosuximide indicates preferential stereoselective elimination of this enantiomer in the rat.

Pharmacokinetic Drug Interactions

Ethosuximide had no significant impact on plasma and brain concentrations of tiagabine in mice. The additive interaction between tiagabine and ethosuximide at the fixed-ratio of 1:1 in the PTZ

test was complicated by a significant pharmacokinetic increase in total brain ethosuximide concentrations.

Co-administration of loreclezole with ethosuximide in mice resulted in a significant 42% elevation in total brain ethosuximide concentration. No pharmacokinetic interaction between valproate and ethosuximide was reported in rats whereas stiripentol significantly increased total brain ethosuximide concentrations in mice.

Toxicity

Non-clinical data reveal no special hazard for humans based on conventional studies of acute and repeated dose toxicity. The LD50 values for ethosuximide in mouse were reported to be 780 mg/kg, 1810 mg/kg, 1752 mg/kg and 1530 mg/kg following intravenous, subcutaneous, intraperitoneal and oral route of administration.

Long-term studies of the carcinogenetic potential in animals have not been performed. Ethosuximide did not reveal a potential for mutagenicity or chromosome aberrations when studied *in vitro.* Ethosuximide significantly increase chromosome abnormalities, decrease sperm function, and down regulate StAR-mRNA expression in male mice which may lead to irreversible male sterility.

Embryotoxicity studies in rats and mice revealed a higher incidence rate of malformation and changes in behaviour. The "Relative Teratogenic Index" (LD01/tD05), which reflects the teratogenic hazard of a test agent, was reported to be 1.2 for ethosuximide in Swiss CD-1 mice. Ethosuximide is classified as category C by FDA with wide range of teratogenic index values between 0.76 and 2.3. Ethosuximide (60, 180 & 360 mg/kg) given to rats on 6^{th} - 16^{th} day of pregnancy produced cleft palate & enlarged cerebral ventricles in offspring. Less frequent observed effects included exencephaly, exomphalos, phocomelia, open eyes, undescended testes, enlarged cerebral ventricles, abnormal palatal bones other than cleft palate, and fused ribs. When ethosuximide given to pregnant rats (days 6-14), hamsters (days 6-10) & rabbits (days 6-18), rats & hamsters showed skeletal malformations; in rabbits eye, limb & tail malformations were also found.

Prenatal exposure to ethosuximide may cause changes in the dopaminergic neurons in the central nervous system in juvenile rats. Prolonged exposure to ethosuximide in utero may increase the sensitivity of the dopaminergic system in the CNS of the developing rats, and that play-fighting (pinning behavior) is a sensitive index of functional abnormalities in the CNS in juvenile rats. As compared to other anti-epileptic drugs, ethosuximide produced the lowest incidence of malformations, which are not clearly shown in these experiments to be significantly different

from the spontaneous control values of 1.3%. These suggest that ethosuximide is safer than others in the mouse, and this may be true for man also.

Study in male mice with ethosuximide provided evidence that drug seems to induce genotoxicity. In vitro studies indicate that 25 and 50 mg/ml ethosuximide did not induce micronucleus formation, nuclear abnormalities, cell death or DNA damage, nor did they affect cell proliferation, suggesting no cytotoxic or genotoxic effects under such experimental conditions. However, under treatment with 100 mg/ml ethosuximide, an increase in micronucleus formation and nuclear abnormalities, but a decrease in cell proliferation and in DNA damage, and no change in the cell death ratio, were detected. Low concentration of ethosuximide induces proliferation of neural stem cell, while higher concentrations of ethosuximide are cytotoxic.

Conclusion

The available preclinical information suggests that ethosuximide has a safety pharmacology and toxicity profile that will not preclude its clinical use according to the restrictions addressed in the SmPC. The characteristics of ethosuximide are adequately reflected in the SmPC and the indications and precautions for the use of this drug are justified by its pharmacological properties. The benefit risk profile of ethosuximide is considered to be favourable, provided that the product is used according to the SmPC. Overall, it can be stated that the overview prepared for ethosuximide covers all aspects of safety assessment required and can therefore demonstrate acceptable level of safety for ethosuximide under the conditions stipulated in the SmPC and marketing authorisation should be granted for ethosuximide.

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