Biochemical Modifications Study of Cerebral Metabolites by Spectroscopy in Epilepsy Treatment

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Through this research, our main focus was: to investigate the biochemical brain metabolites (NAA-N-acetylaspartate, GABA-Gama-Aminobutyric Acid, Asp-Aspartate, CR-Creatine, Gln-Glutamine, GPC-Glicerophosphocholine, PC-Phosphocholine, PCr-Phosphocreatine, Tau-Taurine, N-MDA-N-Metyl-D-Aspartate, Serine, Glicine, Cho-Choline); the neuroimagistic, the brain biochemical and metabolic abnormalities in children and adolescents with epilepsy before and after treatment; to review the main antiepileptic medication administered to these patients; and to make some correlations with the results obtained through Magnetic Resonance (MR) Spectroscopy, for further proper early detection and intervention in children and adolescents with epilepsy. Our research was performed between 2010-2017, involving 45 children and adolescents with epilepsy. Also, the patients were evaluated through MR Spectroscopy at baseline and after pharmacotherapy. Through the MR Spectroscopy, we investigated key aspects of the brain function and metabolism. The antiepileptic medication was chosen according to the guides and the type of epileptic seizures. The efficacy of the chosen therapy was evaluated through the change of the relevant MR spectroscopy biochemical brain metabolites. Our results, showed statistically significant modified values and concentrations of the biochemical cerebral metabolites. Our research was a proof, which the evaluation of the biochemical brain metabolites through MR Spectroscopy is of high clinical utility in prevention, early detection and intervention in epilepsy in children and adolescents.

Keywords: biochemical metabolites, NAA-N-acetylaspartate, GABA-Gama-Aminobutyric Acid, glutamate, epilepsy, antiepileptic medication

Relatively little research has been conducted concerning the investigation of the biochemical brain metabolites through MR Spectroscopy in children and adolescents with epilepsy [1-3].

The main aims of our study were: to illustrate, that these neuro-imagistic measures can offer valuable quantitative biological, biochemical markers of basic pathophysiological, biochemical mechanisms dysfunctions in pediatric epilepsy; that they can be utilized to gain deeper theoretical insights into illness etiology and pathophysiology and may lead to improvements in early detection and more effective and targeted treatment of epilepsy; and to make some neuroimagistic correlations, concerning MR Spectroscopy biochemical metabolic abnormalities and modifications.

The MR Spectroscopy is a versatile, non-invasive instrument, which permits the in vivo identification and quantification of the biochemical substances and neurometabolites in the brain. It is very useful, for the clinical evaluation and longitudinal monitoring [4].

Our main focus was also, to investigate the biochemical brain metabolites through MR Spectroscopy (NAA-N-acetylaspartate, GABA-Gama-Aminobutyric Acid, Asp-Aspartate, CR-Creatine, Gln-Glutamine, GPC-Glicerophosphocholine, PC-Phosphocholine, PCr-Phosphocreatine, Tau-Taurine, N-MDA-N-Metyl-D-Aspartate, Serine, Glicine, Cho-Choline) [5-10].

Glutamate and glutamine, which can be clearly identified and, in part, quantified in MR Spectroscopy of the brain, play important roles in normal and pathological biochemistry. Pathways of glutamate metabolism include transamination, dehydrogenation, deamination and decarboxylation (to GABA). Glutamine is notable in hepatic encephalopathy, but is also a significant metabolic fuel in several other organs and tissues, including neoplasms. *Myo*-inositol is a 6-carbon alcohol which acquires new interest from its detection and quantitation in MR Spectroscopy [11-14].

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One of its roles is the biochemical relationship to messenger-inositol polyphosphates [15-17]. The new perspectives in the field of neuroimagistics and biochemistry give us the opportunity to make some connections between the clinical features, the neurobiological, biochemical and neuroimagistic markers and the further clinical evolution and prognostic in epilepsy [15]. The treatment of election in the management of epilepsy should be chosen in correlation with the biochemical, neurobiological, neuroimagistic and clinical profile of the target patients. When choosing the suitable pharmacotherapy, the neuroimagistic markers should be also carefully analyzed [18].

In our present research, we approach the theme of modern treatment strategies, correlated with the neurobiological, biochemical and neuroimagistic markers, in the management of epilepsy in children and adolescents. Through the MR Spectroscopy, we investigated key aspects of the brain function and metabolism [19-21]. The antiepileptic medication was chosen according to the guides and the type of epileptic seizures.

The studies and guides mention, as antiepileptic/ anticonvulsivant treatments:

- for tonic-clonic seizures-Valproat, Levetiracetam, Lamotrigine, Lacosamide, Phenobarbital;
- for absence seizures-Valproat, Ethosuximide, Lamotrigine;
- for partial seizures-Levetiracetam, Topiramat, Carbamazepine, Oxcarbazepine, Lacosamide;
- also benzodiazepines (Diazepam, Clonazepam, Lorazepam, Nitrazepam) for status epilepticus and myoclonic seizures.

Experimental part

Patients, Material and Methods

Our present research was performed in the University Hospital of Psychiatry and Neurology for Children and Adolescents, Timi'oara, between 2010-2017, involving 45 children and adolescents with epilepsy.

Our actual study is focusing especially on biochemical, neurobiological, neuroimagistic, respectively clinica land treatment aspects and on specific correlations in epilepsy.

The study sample consisted of 45 patients, children and adolescents with epilepsy. We obtained for each patient the informed assent and the informed consent from the parents/legal guardians. Our study was done in accordance with the Ethical Committee regulations of the Victor Babes University of Medicine and Pharmacy, Timi'oara, with the ICH-GCP (Good Clinical Practice) regulations and guidelines and in accordance to some published models [22-24].

Also, the patients were evaluated through MR Spectroscopy at baseline and after pharmacotherapy. Through the MR Spectroscopy, we investigated key aspects of the brain function and metabolism. The antiepileptic medication was chosen according to the guides and the type of epileptic seizures. We also performed metabolic abnormalities investigations because some of the antiepileptic medication need follow-up concerning these aspects.

Biochemical metabolites' and neuroimagistic investigations (MR Spectroscopy)

For the correlation of clinical data with the cerebral biochemical, biological changes, we performed the neuroimagistic investigations. The patients have been evaluated through MR Spectroscopy. Through the MR Spectroscopy, we investigated key aspects of the brain function and metabolism.

We quantified the following neurometabolites: NAA-N-Acetyl Aspartat, GABA-Gama-Aminobutiric Acid, Asp-Aspartat, CR-Creatine, Gln-Glutamine, Glx = Glutamat + Glutamine, GPC-Glicerophosphocoline, PC-Phosphocoline, PCr-Phosphocreatine, Tau-Taurine, N-MDA-N-Metil-D-Aspartat, Ino-Inositol, Serine, Glicine, Cho-Coline.

We used the MR Spectroscopy Software Package for the MR spectral quantification, which automatically calculates a matrix of the correlation quotients of the cerebral metabolites.

Metabolic abnormalities investigations

We investigated the metabolic parameters – lipid profiles (blood cholesterol levels, triglycerides), insulin blood levels, blood sugar levels, blood pressure, BMI (body mass index) change, weight gain. For every patient treated with antiepileptic medication, a clinical examination, a set of hematological, biochemical, lipid, coagulation blood tests and EEG (electroencephalogram), were performed. Blood pressure, heart rate and vital signs were also monitored. Also glucose blood levels were monitored. Glucose (molecular formula: $C_6H_{12}O_6$) is a mono-sacharide existing in nature only as D-isomer form.

The lipid profiles of the patients were assessed through the high-density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, total cholesterol and triglycerides.

HDL is the smallest and most dense of lipoprotein particles and plays an anti-atherogenic role by removing the fat molecules from cells. The non-HDL cholesterol is considered to cause atheroma and it is a good predictor of cardiovascular events. LDL has a larger diameter than normal cholesterol and represents a high cardiovascular risk.

Cholesterol = [(3beta)-cholest-5-en-3-ol], an organic molecule with 256 stereo-isomers biosynthesized by all cells, being a crucial component of cell membranes and a precursor for steroid hormones, vitamin D and bile acids.

Triglycerides are esters derived from the combination of glycerol and three fatty acids (RCO_2H, $R'CO_2H$ and $R"CO_2H$), according to the formula:

$$HOCH_2CH(OH)CH_2OH + RCO_2H + R'CO_2H + R"CO_2H \rightarrow RCO_2CH_2CH(O_2CR')CH_2CO_2R" + 3H_2O$$

Statistical analysis

All analyses were carried out using SPSS software (version 17.0, Chicago, IL, USA) and Microsoft Excel.We also applied the Pearson test for the correlation of the obtained results. For the comparison of the values between the different groups, we applied the Friedman nonparametric test for pair values.

Results and discussions

MR Spectroscopy Results

Through the MR spectroscopy, we found modified values and concentrations of the cerebral biochemical metabolites for the patients with epilepsy, convulsive seizures:

- high: GABA values, especially in the hippocampus and thalamus, in temporal lobe epilepsy and in the frontal lobe in idiopathic generalized epilepsy, glutamate values especially in the frontal cortex, identifying brain lesions and GABA being a key component for abnormal hyperexcitability in epilepsy;

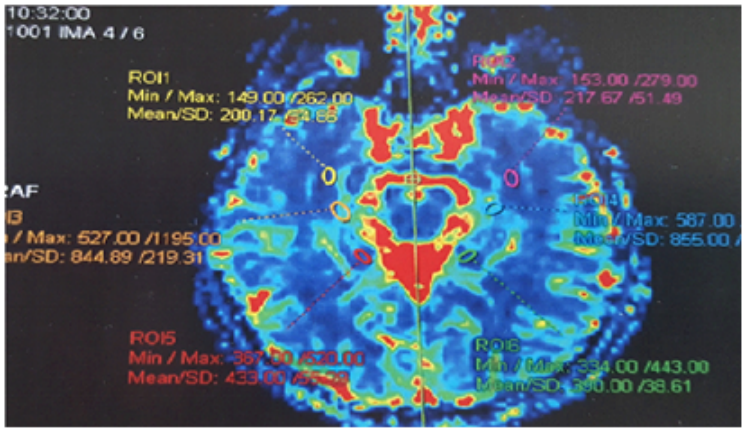

Fig. 1. Results for the MR Spectroscopy brain biochemical metabolites concentrations for the patients with epilepsy

- low NAA and NAAG (N-acetylaspartylglutamate) values;

- increased Glx (Glutamate + Glutamine);

-reduction of NAAt (N-acetylaspartate + N-acetylaspartylglutamate) in the frontal lobe, in IGE (idiopathic generalizesd epilepsy) (fig. 1).

We also observed, high values for the glutamate/glutamine, lactate/NAA, glutamate/Cr, Cho/Cr and low values for NAA/Cr, NAA/Cho, NAA/Cho + Cr ratios, showing the bilateral but dominant left hippocampal abnormalities especially in NAA/Cr; and low thalamic GABA/Cr ratio, in patients with epilepsy in comparison with healthy controls.

So that median right NAA/Cr = 0.78, left = 0.60 and normal values = NAA/Cr> or = 1.

Also the metabolite ratios Glx/NAAt and Glx/Ins showed elevation in IGE, in the frontal lobes and the thalami NAA/Cr were significantly decreased, as compared with healthy controls ($p < 0.001$)

These spectral abnormalities reflect the neuronal loss and damage [1, 2, 4, 6-9].

The parieto-occipital cerebral lesions were captured through the increase of the values of Lactate, Cho, NAA, Glx [19-21, 25, 26].

The results were also suggestive for frontal and parieto-occipital bilateral cerebral lesions, with the characteristics of gray matter heterotopy – specific for migration disorders of the cortex in the embryological period [6].

We also noticed strong negative correlations between GABA and NAA/Cr ratio, increased GABA correlated with decreasing NAA/Cr – Pearson correlations ($p < 0.001$).

Also, we found increased values for myo-inositol and glutathione, specific for edema and neuronal injury.

We detected reduction in the NAA signal, 19% increase in the Cr signal and 28% increase in the Cho signal (fig. 2).

The antiepileptic medication for the patients was chosen according to the guides and the type of epileptic seizures.

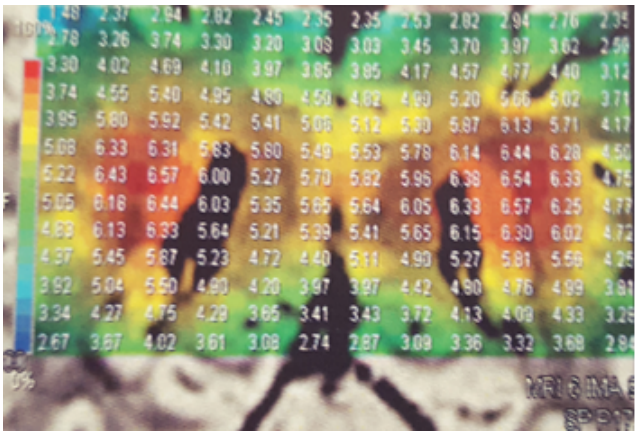

Fig. 2. MR Spectroscopy matrix quantifying the concentrations of brain biochemical metabolites captured

Anticonvulsants, also known as antiepileptic drugs or as antiseizure drugs are pharmacological agents used in the treatment of epileptic seizures. Anticonvulsants suppress the excessive rapid firing of neurons during epileptic seizures. Anticonvulsants also prevent the spread of the seizure within the brain.

Antiepileptic drugs may block sodium channels or enhance γ-aminobutyric acid (GABA) function. Next to the voltage-gated sodium channels and components of the GABA system, their targets include $GABA_A$ receptors, the GAT-1 GABA transporter, and GABA *transaminase*, voltage-gated *calcium channels*, SV2A, and α2δ. By blocking sodium or calcium channels, antiepileptic drugs reduce the release of excitatory glutamate, whose release is considered elevated in epilepsy, but also that of GABA. This is probably a side effect or even the actual mechanism of action for some antiepileptic drugs, since GABA can itself, directly or indirectly, act proconvulsively. Another potential target of antiepileptic drugs is the *peroxisome proliferator-activated receptor alpha*.

Main anticonvulsivants are from the following groups:

- Carboxamides -Carbamazepine -$C_{15}H_{12}N_2O$ and Oxcarbazepine - $C_{15}H_{12}N_2O_2$;

- Fatty acids – Valproate, Valproic acid, Sodium valproate, Divalproex sodium - $C_8H_{16}NO_2$;

- Fructose derivatives-Topiramate - $C_{12}H_{21}NO_8S$;

- Gaba analogs – Gabapentine - $C_9H_{17}NO_2$;

- Pyrolidines- Levetiracetam - $C_8H_{14}N_2O_2$;

- Succinimides-Ethosuximide - $C_7H_{11}NO_2$;

- Triazines- Lamotrigine -$C_9H_7Cl_2N_5$;

- Benzodiazepines - Diazepam-$C_{16}H_{13}ClN_2O$, Clonazepam - $C_{15}H_{10}ClN_3O_3$,

Nitrazepam - $C_{15}H_{11}N_3O_3$, Lorazepam -$C_{15}H_{10}Cl_2N_2O_2$;

- Barbiturates - Phenobarbital - $C_{12}H_{12}N_2O_3$.

Of these three drugs have a broader spectrum, namely:

- Levetiracetam - $C_8H_{14}N_2O_2$ -is effective against partial seizures (including secondary generalized tonic-clonic) and is somewhat effective against primary generalized tonic-clonic seizures;

- Valproate or valproic acid - $C_8H_{16}NO_2$ - is commonly used in children with generalized seizures who are prone to *absence* seizures and *tonic-clonic* seizures. It is also used for a variety of other seizures, in both children and adults, including tonic-clonic, myoclonic, *complex partial* seizures;

- Topiramate - $C_{12}H_{21}NO_8S$ is a broad-spectrum drug, effective against *partial seizures*, including secondary generalized tonic-clonic seizures and primary generalized

tonic-clonic seizures and somewhat effective against absence and tonic–atonic seizures. Is being good for multiple seizure types or patients refractory to other drugs.

In the therapeutic practice, other drugs are also used, namely:

- Ethosuximide – $C_7H_{11}NO_2$ is used to control absence seizures;
- Carbamazepine - $C_{15}H_{12}N_2O$ is effective in treating complex partial seizures and simple partial seizures;
- Oxcarbazepine - $C_{15}H_{12}N_2O_2$ is effective against partial seizures, including secondary generalized tonic-clonic seizures;
- Lamotrigine – $C_9H_7Cl_2N_5$ is used in conjunction with other anti-epileptic drugs to aid in controlling absence, tonic-clonic seizures;
- Lacosamide - $C_{13}H_{18}N_2O_3$ is used in controlling partial seizures especially, but also generalized tonic-clonic seizures;
-- Clonazepam - $C_{15}H_{10}ClN_3O_3$ may be used alone or with other antiepileptic drugs to treat absence seizures and myoclonic seizures in children;
- Gabapentine $C_9H_{17}NO_2$ is effective against partial seizures (including secondary generalized tonic-clonic seizures);
- Phenobarbital - $C_{12}H_{12}N_2O_3$ is generally used to control tonic-clonic and simple partial seizures.

Major anticonvulsivants in function of the seizures types are represented in table 1.

Results of the metabolic abnormalities investigations

In the group of patients with epilepsy, who took Valproate as anticonvulsivant medication, highly modified and abnormal lipid profiles, weight gain, BMI increase and abnormal metabolic values were found, in comparison with the group of patients treated with other anticonvulsivants.

For the comparison of the values between the different groups, we applied the Friedman nonparametric test for pair values. For the patients from group I – with Valproate as treatment, the BMI values increased significantly since the baseline ($p < 0.001$, $\alpha = 0.001$). For insulin values, the differences were also statistically significant ($\alpha = 0.001$).

The increase of the BMI values from baseline is also statistically significant, with a threshold of significance $\alpha = 0.001$, meaning that the patients from group I were prone and exposed to adverse effects, expressed through weight gain.

So we identified some relevant metabolic abnormalities – hypercholesterolemia, high triglycerides, high blood insulin levels, high blood sugar levels, BMI-Body Mass Index increase, weight gain, especially in the group of patients, who were treated with Valproate.

We also made some correlations concerning the treatment benefits. So that after proper anticonvulsivant treatment, the patients showed also the normalization of the biochemical metabolites' levels identified through the MR Spectroscopy. The obtained results proved that the patients, who took proper antiepileptic medication, registered the improvement of the Spectroscopy biochemical metabolites, as a positive response to the chosen pharmacotherapy (fig. 3).

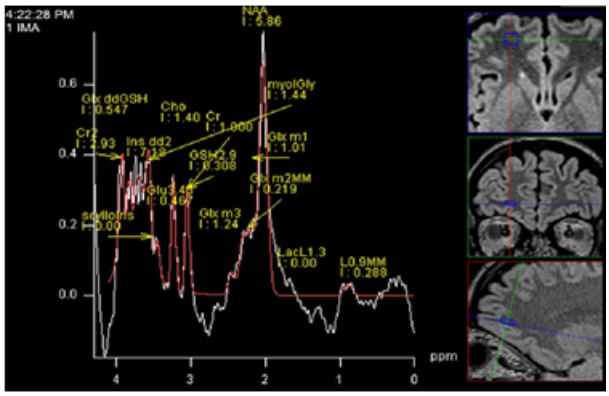

Fig. 3. Concentrations, peaks and correlations of MR Spectroscopy biochemical brain metabolites for the patients with epilepsy and anticonvulsivant treatment

The approach must be ethical, personalized, avoiding as much as possible the emergence of adverse events [27-32]. The attitude must take, with great attention, into account, all the present signs, symptoms and associated pathologies, the medical and family history and the heredo-collateral antecedents [33-41].

The aspects concerning the potential adverse events, quality of life and vulnerabilities, must be carefully approached. The risk and resilience factors must be known and evaluated and the the therapeutic approach must be ethical with the lowest number of adverse events encountered [42-46]. We must be careful and also prevent

Anticonvulsivant Medication	Seizure types		
	Tonic-clonic seizures	*Absence seizures*	*Partial seizures*
Valproate $C_8H_{16}O_2$	+++	+++	+++
Levetiracetam $C_8H_{14}N_2O_2$	+++		+++
Lamotrigine $C_9H_7Cl_2N_5$	+++	+++	
Lacosamide $C_{13}H_{18}N_2O_3$	+++		+++
Ethosuximide $C_7H_{11}NO_2$		+++	
Carbamazepine $C_{15}H_{12}N_2O$			+++
Topiramate $C_{12}H_{21}NO_8S$	+++	+++	+++
Phenobarbital $C_{12}H_{12}N_2O_3$	+++		+++

Table 1
MAJOR ANTICONVULSIVANTS IN FUNCTION OF THE SEIZURES TYPES

the emergence of metabolic abnormalities [47-54].

This study is important, especially in the context, that there is a lack of studies on children and adolescents with epilepsy, concerning the neuroimagistic MR Spectroscopy correlations.

Some biochemical metabolic modifications of the brain metabolites, which can be captured through MR Spectroscopy, can induce epileptic seizures and a greater number of seizures damage the brain further [12].

Some of the strengths of our study are: the prospective observation of the children with convulsive seizures, in correlation with the neuroimagistic MR Spectroscopy investigations and the anticonvulsivant medication administered.

Our main focus was to investigate the neurobiological, EEG, neuroimagistic and clinical aspects, for the patients with epilepsy, in order to have a better management of intervention and care.

We considered the ethical foundation for our research, complied with the principles related to the child's rights, to the respect of the human dignity, the freedom of choice, the right to be informed and tried to solve any possible ethical issues, occurring from the nature of research.

Further studies are needed that evaluate the relationship between the MR Spectroscopy biochemical brain metabolites modifications and the type of anticonvulsivant medication, which would be needed to treat every type of convulsive seizure personalized and properly.

Further research would be needed in order to investigate the relationships of the individual EEG abnormalities to neuroimaging, neurocognitive, biochemical and molecular-genetic, pharmacogenetic data obtained from the same subjects with epilepsy.

Relatively little research has been conducted on resting EEG activity in correlation with MR Spectroscopy, in patients with epilepsy. Concerning the MR Spectroscopy, our obtained results are in line with some other existing studies. So, concerning the group with temporal lobe epilepsy, NAA changes were specific to the epileptogenic zone [1-5, 9-12].

While it is not surprising, that metabolic dysfunction follows from uncontrolled seizure activity, it has also been known that oxidative stress can cause also abnormalities in GABA and glutamate. So, Saransaari and Oja, 1997 [55], studied the mouse hippocampus with variable levels of peroxide stress and showed increase in basal GABA release, ranging from 30% to 550%. These observations are of great interest for epilepsy, where GABA is thought to be a key component, underlying the abnormal hyperexcitability.

So the GABA function might be in this case proconvulsant, like Woo et al., in 2002 [56] and Palma et al., in 2006 [57], suggested. GABA can also become excitatory and can further propagate seizure-linked injury.

Other important MR Spectroscopy metabolites identified for epilepsy included glutamate, myo-inositol, lactate and glutamine. Glutamate and GABA, in particular, are important targets, given their role in neurotransmission [58-60].

Another study of Filibian, in 2012 [9] found progressive increases in myo-inositol and glutathione with decreases in NAA in epilepsy, characterizing edema and neural injury.

This interesting study raises the question of what these changes might mean for epileptogenesis. So some studies and also, animal model studies, have identified changes in many of these compounds that are linked with epileptogenesis and seizures-related injury [9, 12].

Also idiopathic generalized epilepsy was associated, in our study, in line with other existing studies, with bilateral frontal lobe metabolite changes. Also elevation in Glx was observed, which may imply increased neuronal excitability, whereas reduction of NAA suggesting neuronal dysfunction.

Our obtained results are in line with the study of Simister et al [14] on 21 patients and 17 controls with idiopathic generalized epilepsy (IGE). So that, especially in the frontal lobes, glutamate and glutamine were increased, NAA and inositol were decreased in patients compared with controls.

Further research is needed in the field of psychiatry/child psychiatry and neurology especially, in order to develop some integrative correlations of the neurobiological, EEG abnormalities, neuroimagistic modifications and their clinical application.

Conclusions

So that, the fingerprinting of the EEG, neurobiological and spectroscopic MR Spectroscopy markers, represents strongly predictive factors of the clinical evolution in child epilepsy.

The evaluation of neuroimagistic markers in patients with epilepsy, proved high clinical utility in prevention, early detection and targeted intervention. Our research, pleads for the utility of this modern approach, which represents the only valid path for the future treatment management of high quality also in epilepsy. If technical advances would permit simultaneous acquisition of MR Spectroscopy, from several compounds in multiple brain regions, detecting key metabolites, pharmacologic challenges might be cleared, in order to address these complex issues.

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