Role of P-glycoprotein inhibitors in children with intractable epilepsy

Thesis

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Introduction

Medical management with Anti-Epileptic Drugs (AEDs) remains the first-line of treatment in patients with epilepsy (Shinnar and Berg, 1996). Epilepsy appears intractable in more than 35% of treated patients (Kwan and Brodie, 2000).

Medically refractory epilepsy can be defined as inadequate seizure control despite appropriate medical therapy with at least two AEDs in maximally tolerated doses for 18 months–2 years, or adequate seizure control with unacceptable drug-related side effects. There are several factors to be considered in a definition of medical intractability, including the number of AED failures, minimum frequency at which seizures must occur to be considered intractable (daily, monthly, and so forth), duration of unresponsiveness to medication, epilepsy syndrome involved, cause of seizures in the absence of a clear-cut epilepsy syndrome, and patient age at the onset of seizures (Berg, 2006).
At the present time, two main mechanisms of drug resistance have been proposed, increased expression of multidrug transporters (also called drug resistance proteins) that remove antiepileptic drugs from the epileptogenic zone and for reduced sensitivity of the drug target in the epileptogenic zone (Schmidt and Loscher, 2005).

With respect to pharmacoresistant epilepsy, one favored putative hypothesis is the over expression of multidrug transporters, such as P-glycoprotein (Pgp), which is a drug efflux transporter that limits the access of antiepileptic drugs to their site of action in the brain lowering their in situ concentrations (Schmidt and Loscher, 2005).

P-glycoprotein (permeability glycoprotein, abbreviated as P-gp or Pgp) is a well-characterized ATP-Binding Cassette (ABC-transporter) of the Multi-Drug Resistance/Trasporter Antigen Peptide (MDR/TAP)subfamily (Dean and Michael, 2002).

P-glycoprotein can be inhibited by blocking drug binding site either competitively, non-competitive or allosterically, or by interfering ATP hydrolysis (Schmidt and Loscher, 2005), and by altering integrity of cell membrane lipids (Shapiro and Ling, 1997).

The overexpression of P-glycoprotein in the central nervous system may be one mechanism of pharmacoresistance in
patients with epilepsy. The calcium-channel blocker verapamil is a known inhibitor of P-glycoprotein and may function to block P-glycoprotein–modulated efflux of antiepileptic drugs in the brain, thereby raising the intracellular concentration of antiepileptic drugs and ultimately decreasing seizure burden in patients with refractory epilepsy (Summers et al., 2004).

Verapamil may offer pharmacoresistant patients hope of improved seizure control due to its potential P-glycoprotein inhibitory effects (Summers et al., 2004).
**Hypothesis:**

Intractable epilepsy might be the result of the overexpression of P-glycoprotein due to its nature as one of the multidrug resistance transporter family.

Verapamil as a P-gp inhibitor is expected to improve intractability.

**Aim of the work:**

The aim of this work is to measure the serum level of P-Glycoprotein in patients with intractable epilepsy & to assess the efficacy (The extent to which a specific intervention, procedure, regimen, or service produces a beneficial result under ideal conditions) of Verapamil (as a P-Glycoprotein inhibitor) in those patients.
Subjects and methods:

Subjects:

The study is an exploratory pilot study. It will be conducted on thirty (30) patients with intractable epilepsy with an age range of 5-15 years, presenting to the Pediatric Neurology Clinic, Children Hospital, Ain –Shams University.

Studied subjects will be divided into 2 groups:

**Group (1):** includes 30 patients with intractable epilepsy. This group will be divided into 1a and 1b.

1a will include 15 patients on Verapamil

1b will include 15 patients on placebo (looks, smells, and tastes exactly as Verapamil)

**Group (2):** includes 30 controlled epileptic patients (no seizures in the last six months), serving as controls.

- Informed consents (patients and controls) will be taken from the parents or guardians of each studied subject before the commencement of the study (*Appendix I*)
• Diagnosis of epilepsy and seizure type will be done according to the guidelines of the International League against Epilepsy (ILAE classification, 2006).

• **INCLUSION CRITERIA:**

  1. Age: 5-15 years

  2. Probable or definite localization-related, primary generalized or symptomatic generalized epilepsy that is medically-refractory, as defined by treatment failure of at least 2 anti-epilepsy drugs at standard doses, despite medication compliance as determined by the treating neurologist. Epileptic patients are considered controlled if they are seizure free for more than six months.

  3. Ability of the patient or guardian to understand the concept of a placebo-controlled clinical trial.

• **EXCLUSION CRITERIA:**

  1. Any patient with pre-existing cardiac condition.

  2. Low blood pressure as regard to the patient age, below 25th percentiles.

  3. Known hypersensitivity to Verapamil or any component of the formulation.

  4. Patient with co-existing renal or hepatic illness.
**End Point:**

One year from date of starting Verapamil, unless serious problems related to the drug adverse effects occurred, or if patient didn't respond (seizure severity evaluation, based on Chalfont severity scale) in period of 12 weeks at highest tolerated dose.

**Premature termination of the drug:**

As mentioned above, treatment (with Verapamil) will stop if he took the drug for >12 weeks on highest tolerated dose and didn't respond, or anytime he experienced intolerable side effects.
**Study Design:**

Treatment, Randomized, Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor), Placebo Control, Parallel Assignment, Efficacy Study

**Justification of the study:**

There might be a direct benefit of administering Verapamil to patients with intractable epilepsy. Studying its efficacy and measuring P-Glycoprotein level in these patients might contribute to the understanding of the effect that P-Glycoprotein might exhibit on the action of Anti-epileptic drugs in patients with intractable epilepsy, and the usefulness of verapamil (as a P-Glycoprotein inhibitor) on these patients.
Methods:

Patients will be subjected to the following:

(I) Full history taking in a private room in the pediatric neurology clinic by a specialized doctor to conduct a questionnaire, respecting the privacy of such information with special emphasis on:

A) Personal history

B) Analysis of seizures:

1. History of the epilepsy disorder (Epilepsy type, seizure type, frequency of attacks, age of onset, date of first seizure, duration of disease and medication(s) history).

2. The severity of seizures at time of recruitment will be rated according to Chalfont seizure severity scale (Duncan and Sander, 1991)

Chalfont severity scale:

- Loss of awareness: no=0, yes=1
- Warning (if loss of awareness): no=1, yes=0
- Drop/Spill a held object: no=0, yes=1
- Fall to the ground: no=0, yes=4
- Injury: no=0, yes=20
- Incontinence: no=0, yes=8
- Automatism: no=0
  mild=4
  severe=12
- Convulsion: no=0, yes=12
- Duration of seizure:  
  <10 sec = 0,  
  <1 min = 1  
  1-10 min = 4  
  >10 min = 16  
- Time to return to normal from onset:  
  < 1 min = 0  
  1-10 min = 5  
  10-30 min = 20  
  30-60 min = 30  
  1-3 hr = 50  
  >3 hr = 100  

If seizure event with total score = 0, add 1  
If only in sleep, divide score by 2  

In each section, score what usually occurs with fractionation as follows:  
- No score if it does not occur, quarter of score if occurs in up to 25% of attacks.  
- Half score if occurs in 25-50% of attacks, three quarters if in 50-75%  
- Full score if occurs in >75% of attacks  

Injury includes tongue biting, laceration and bruising  
Spill includes a held object even if the vessel is not dropped  
Convulsions is taken to mean clonic jerking of the limbs.  

C) **Antiepileptic drugs** including drugs taken by the patient,  
their number, duration of therapy, dose in mg/kg/day and preparations.
D) History of perinatal and/or neonatal insult including:

i. Antenatal history: teratogens, infections, drugs and maternal diseases during pregnancy.

ii. Natal history: gestational age, place of delivery, mode of delivery, weight at birth, first cry.

iii. Postnatal history: age of 1st convulsion, jaundice, cyanosis, Apgar score.

E) Developmental history for evidence of psychomotor retardation or regression including:

Gross motor, fine motor, communication, language and cognitive skills.

G) Family history of similar conditions or other neurological illnesses and history of consanguinity.

(II) Detailed general and neurological examination

1. General examination

With special emphasis on:

a. Anthropometric parameters (weight, height or length, span and occipitofrontal circumference).
b. Facial and skeletal abnormalities suggestive of any chromosomal or genetic diseases.
c. Skin examination (rash, hypopigmented and/or hyperpigmented lesions).

2. **Neurological examination:**

   With special emphasis on:

   - **a- Mental status, cognition and alertness.**
   - **b- Motor examination:**
     i. Muscle tone, muscle state and muscle power.
     ii. Reflexes (superficial, deep and pathological).
     iii. Gait and posture.
   - **c- Sensory examination:**
     i. Superficial sensations.
     ii. Deep sensations.
     iii. Cortical sensations.
   - **d- Coordination.**
   - **e- Cranial nerves examination.**

*History taking and clinical examination will be carried out in a private room in the pediatric neurology clinic by specialized personnel to conduct a questionnaire and perform the examination, respecting the privacy of such procedures.*
III-Investigations:

(1) Clinical chemistry for epileptic patients:
   a. Complete blood count (CBC)
   b. Liver function tests (ALT, AST)

Five ml of blood will be collected once in the outpatient clinic through venipuncture after sterilization from every patient for hematological assessment and repeated again 3, 6, 9 and 12 months later. This procedure will be done by the most expert personnel of the investigators. The amount of blood that will be withdrawn according to age is not harmful without any consequences except for the needle prick at the site of venipuncture.

(2) Digital inter-ictal EEG (electroencephalogram): will be carried out in the pediatric neurology unit. The EEG tracings will be analyzed carefully as regards background activity, presence of generalized slowing or spike and wave, presence of focal slowing or spike and wave or focal with secondary generalization. Interictal abnormalities, consisting of spike/sharp, slow complexes and/or bilaterally independent inter-ictal epileptiform abnormalities will be considered positive findings of epileptic activity. This procedure will be done under sedation by intravenous Diazepam (0.1 -0.5 mg/kg/dose) with preparation of its antidote “intravenous Flumazenil”.
(3) Neuroimaging:
Computed Tomography (CT) and/or Magnetic Resonance Imaging (MRI) of the brain will be done if patients have any suspicious findings in history or clinical examination. These procedures will be done in the radiodiagnosis department, Ain Shams University hospitals by an expert personnel. Patients less than seven years of age will receive sedation by intravenous diazepam (0.1 -0.5 mg/kg/dose) with preparation of its antidote intravenous Flumazenil. Patients above the age of 7 years will undergo the procedure without sedation.

(5) Serum levels of P-Glycoprotein for both groups 1 & 2 at the start of the study and before verapamil administration. By Human permeability glycoprotein (P-gp) ELISA Kit obtained from Cusabio Biotech co., Ltd.
This immunoassay kit allows for the in vitro quantitative determination of human P-gp concentrations in serum, plasma and other biological fluids. The microtiter plate provided in this kit has been pre-coated with an antibody specific to P-gp. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for P-gp and Avidin conjugated to
Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3'5, 5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that Contains P-gp, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of P-gp in the samples is then determined by comparing the O.D. of the samples to the standard curve.

**IV- Special measures for Group 1 Patients :**

1. Cardiac examination including measuring blood pressure.
2. ECG before drug administration.
3. Start drug administration, Verapamil for group 1a (1 mg/kg/day to be increased slowly to 1.5 mg/kg/day Bid) and placebo for group 1b, which will be the exact form as the Verapamil tablets.

A previous study where Verapamil was used as P-glycoprotein inhibitor on two children with intractable epilepsy was carried by *Lanetti et al in 2009*, where they concluded that after concomitant verapamil therapy with AEDs dramatic seizure control was achieved. Another study was done by *Summers et al in 2004* on one patient
(24 years old) with intractable epilepsy, where Verapamil reduced seizure severity and frequency. Currently a similar study is being carried out in Columbia University on adult patient, using different P-Glycoprotein inhibitor than Verapamil.
http://clinicaltrials.gov/ct2/show/NCT00524134

4. Follow up of seizure frequency, severity and any drug adverse effects, with prompt cessation of the Verapamil at the presence of any adverse effect (as bradycardia, hypotension, postural hypotension, constipation, elevated liver enzymes, paresthesia, dizziness, headache, fatigue etc) or at the patient/guardian request.

5. Any change in the AEDs (type or dosage) during the study will result in exclusion of the patient.

6. Re-measure the P-gp level at the end of the study.

V-Follow-up of patients:

The duration of follow-up will be one year from the beginning of the patients’ recruitment. It will be carried out in the pediatric specialized neurology clinic. Follow up of patients will be done every month and it will include the assessment of the following:

a-Seizure frequency.
b-Seizure severity score.
c- Seizure control and response to treatment.

d- Serum levels of AEDs will be done every 3 months.

VI- Statistical Analysis of the results will be done

VII- Medical ethics issues will be highly respected during the work as regard to:

• Confidentiality

• Consents from both groups including all the details about drugs adverse effects and risks from needed procedures (blood sampling, sedation during EEG or Imaging)

• Ethical Committee approval
References