Pharmacoresistant epilepsy and nanotechnology

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1. ABSTRACT

Epilepsy is one of the most common chronic neurological disorders. Furthermore, it is associated to diminished health-related quality of life and is thus considered a major public health problem. In spite of the large number of available and ongoing development of several new antiepileptic drugs (AEDs), a high percentage of patients with epilepsy (35-40%) are resistant to pharmacotherapy. A hypothesis to explain pharmacoresistance in epilepsy suggests that overexpression of multidrug resistance proteins, such as P-glycoprotein, on the endothelium of the blood brain barrier represents a challenge for effective AED delivery and concentration levels in the brain. Proven therapeutic strategies to control pharmacoresistant epilepsy include epilepsy surgery and neuromodulation. Unfortunately, not all patients are candidates for these therapies. Nanotechnology represents an attractive strategy to overcome the limited brain access of AEDs in patients with pharmacoresistant epilepsy. This manuscript presents a review of evidences supporting this idea.

2. INTRODUCTION

Epilepsy is a chronic disorder of the brain characterized by an enduring predisposition to generate epileptic seizures (1). Although there are several available antiepileptic drugs (AEDs), seizures do not remit in 35-40% of the patients despite pharmacological treatment (2-4). Pharmacoresistance in epilepsy has been defined by the International League Against Epilepsy as the “failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom” (2).

It is well known that the impact of epilepsy is multidimensional and extends far beyond the harm induced by seizures themselves. The unpredictability of seizures imposes severe lifestyle restrictions on patients with epilepsy, resulting in significant impairments in psychological, emotional, economical and/or social spheres (5, 6). These negative impacts of epilepsy are increased in case of pharmacoresistant epilepsy (7-9). Compared to the general population, patients with drug-resistant epilepsy...
Pharmacoresistant epilepsy represents an important economical burden since patients require continuous medical assistance, diagnostic tests, AEDs, protective equipment and eventual hospitalizations (5, 7, 13, 14). The cost of pharmacoresistant epilepsy in adults exceeded 2,817 USD per patient per year in the United States, whereas it was estimated at approximately 2,610 € in Germany (13, 15). In Mexico, the Mexican Institute of Social Security (IMSS) indicated that the annual cost per patient is $2,646 USD (5). Altogether, these data underscore the necessity of developing new therapeutic strategies to control pharmacoresistant epilepsy.

3. HYPOTHESES TO EXPLAIN PHARMACORESISTANCE IN EPILEPSY

There are several hypotheses that explain the mechanisms associated to pharmacoresistance in epilepsy. The neural network hypothesis suggests that recurring episodes of excessive neuronal activity induce structural changes such as neuronal degeneration, gliosis, axonal sprouting, necrosis and synaptic reorganization. These alterations could contribute to the formation of aberrant neural networks that may lead to pharmacoresistance (16). The methylation hypothesis indicates that seizures are associated to long-lasting epigenetic mechanisms such as acetylation, methylation, phosphorylation, ubiquitination of DNA and alterations in multidrug transporter molecules that contribute to the development of pharmacoresistance in epilepsy (17-19). The impaired mitochondrial function hypothesis suggest that failed responses to AEDs result from alterations in mitochondrial production of energy (20). The hypothesis of the intrinsic severity of epilepsy postulates that drug resistance is a consequence of an elevated excitatory neurotransmission that underlies higher seizure intensity and frequency (8, 21, 22). The target hypothesis establishes that patients with pharmacoresistant epilepsy present intrinsic or acquired changes in specific targets, rendering them less sensitive to AEDs effects (23, 24). Finally, the transporter hypothesis indicates that pharmacoresistant epilepsy results from restricted penetration of AEDs to the cerebral parenchyma and a consequent reduced concentration in target sites as a consequence of increased expression of drug transporters such as multidrug resistance-associated proteins (MRPs) and P-glycoprotein (P-gp) (2, 21). According to the last hypothesis, many studies have aimed to design new strategies to ensure effective drug delivery in pharmacoresistant epilepsy.

4. OVEREXPRESSION OF MULTIDRUG TRANSPORTERS AND PHARMACORESISTANT EPILEPSY

Normally, the role of multidrug transporters is to efficiently remove drugs or limit their access and accumulation in the brain (25) (Figure 1). The most studied transporters are P-gp, MRPs and Breast-Cancer Resistance Protein (BCRP), all members of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter protein superfamily (26). P-gp and MRPs are multidomain integral membrane proteins consisting of two transmembrane domains and two cytosolic nucleotide binding domains (27). BCRP has only one transmembrane domain and one nucleotide binding domain and is assumed to function as a dimer (28-30).

P-gp was initially discovered in cancer cells (31, 32). Under normal conditions, P-gp is expressed in various tissues associated with barrier and/or secretory functions (33), such as the blood-testis barrier (34), the blood-mammary barrier (35, 36) and the endothelial cells of the cochlea and vestibule (37, 38). P-gp is expressed in endothelial cells of the blood brain barrier (BBB), specifically at the luminal end of the endothelium side (39, 40), and it plays an important role in brain protection (41, 42).

Several studies in patients and experimental models support the role of P-gp overexpression in pharmacoresistant epilepsy (43-45). In 1995 Tishler et al. (46) were the first authors to report that the gene expression of multidrug resistance protein 1 (MDR1), which encodes for P-gp, was significantly increased in the endothelium of the BBB and astrocytes of epileptic brain tissue obtained from patients with refractory epilepsy. At present, it is known that P-gp is also overexpressed in neurons, astrocytes and endothelial cells of patients and in experimental models of pharmacoresistant epilepsy (47-55).

Experiments carried out in animal models of pharmacoresistant seizures support the idea that low intracerebral concentrations of AEDs are correlated with a high expression and function of P-gp (56) (Figure 1). Indeed, the administration of P-gp blockers such as tariquidar or nimodipine, facilitates AED delivery into the brain of animals with pharmacoresistant epilepsy (57, 58).

Overexpression of MRP has been detected in blood vessels, dysplastic neurons and glia of patients with pharmacoresistant epilepsy (59, 60). BCRP is normally located at the luminal surface of the microvessel endothelium of the BBB, but its overexpression has been associated to different types of pharmacoresistant epilepsy (61-64). At present, the role of BCRP in resistance to epilepsy is controversial due to the low affinity of AEDs for this transporter (65). The Mayor Vault Protein (MVP), a non-ABC multidrug resistant transporter, is also overexpressed in balloon cells of tissue from patients with pharmacoresistant tuberous sclerosis (66) and experimental animal models of temporal lobe epilepsy (67).

5. THERAPEUTIC STRATEGIES TO CONTROL PHARMACORESISTANT EPILEPSY

Different therapeutic strategies have been developed to control pharmacoresistant epilepsy. Epilepsy surgery has the potential to render some patients seizure free and/or significantly improve their quality of life (68). This strategy may involve removal of brain areas that cause seizures and/or interruption of seizure propagation pathways (69). Unfortunately, a high percentage of patients present an increased risk of sudden unexpected death (SUDEP) and suicide (10-12).
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Figure 1. Schematic representations of P-glycoprotein (P-gp) expression and antiepileptic drugs (AEDs) in blood stream and cerebral parenchyma under different conditions. A) Under healthy conditions, in which P-gp expression is low and limited to the endothelial cells of the brain microvessels, the AEDs could raise the brain parenchyma at therapeutic concentrations and induce anticonvulsant effects; B) In pharmacoresistant epilepsy, P-gp is abnormally expressed in neurons and astrocytes and overexpressed in the endothelial cells of the brain microvessels. This last condition prevents achieving effective concentrations of the AEDs in the brain parenchyma. C) Representation of the application of drug nanocarries of AEDs in the brain with pharmacoresistance epilepsy. The drug nanocarriers transport the AEDs across the BBB, a situation that allows achieving their effective concentrations in the brain.

with refractory epilepsy (30%) are not candidates for surgery (70). For these patients, neuromodulation represents a promising strategy for seizure control (71). Neuromodulation strategies include electrical stimulation of the vagal or trigeminal nerve, deep brain areas and transcranial magnetic stimulation (72, 73). Although neuromodulation is a powerful therapeutic alternative, it entails invasive procedures associated to certain risks such as bleeding or infections (82), and side effects such as hoarseness, changes in voice quality, or breathing impairment in the case of vagal nerve stimulation (74-77). In addition, the different neuromodulation strategies are expensive (78) and, therefore, inaccessible for many patients.

6. NANOTECHNOLOGY AND DRUG NANOCARRIERS

Nanotechnology is the engineering of functional systems with domain dimensions below 100 nm (79). Nanotechnology has been used as a diagnostic modality in brain disorders. For example, iron oxide (magnetic) nanoparticles (MnPs) are applied as contrast agents in magnetic resonance imaging (MRI) (80) of cerebral pathologies such as tumors (81, 82), stroke (83, 84), multiple sclerosis (85), acute disseminated encephalomyelitis (86) and trauma (87, 88). Attached to nonradioactive drugs, such as alpha methyl tryptophan, magnetic nanoparticles are employed for MR imaging of normal cerebral functions and changes induced by epileptic activity (89). Conjugation of nanoparticles with specific markers (90) or antibodies (91) may improve diagnostic specificity.

For therapeutic purposes, magnetic nanoparticles can be directed to a specific organ or tissue using an external magnetic field to induce local effects (92). For example, hyperthermia induced by MnPs exposed to a magnetic field has been applied...
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for termotherapy of malignant brain tumors in animal models (93) and in human patients (94, 95).

Nanotechnology represents an attractive therapeutic strategy to design drug nanocarriers that allow transportation of previously adsorbed, entrapped, encapsulated or covalently linked substances to nanomaterials (96, 97). The main advantage of the drug nanocarriers is their ability to mask molecules and then transport them into the cerebral parenchyma across the BBB (98). They may also prolong the release of drugs increasing their efficiency (99, 100). According to these characteristics, drug nanocarriers are considered ideal to augment cerebral penetration of substances (101) for the treatment of pharmacoresistant brain tumors (102) or neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (103).

Some examples of drug nanocarriers are polymer nanoparticles, liposomes, dendrimers, micelles, carbon nanotubes, solid lipid nanoparticles and nanostructured lipid carriers (104-112). Drug nanocarriers should fulfill some characteristics to be considered as therapeutic options. Size must be small to avoid reticuloendothelial system uptake (113). Other requirements include high drug-loading capacity, elevated stability in biological fluids and reduced toxicity as well as ability to provide protection from peripheral degradation to transported drugs (114).

7. NANOTECHNOLOGY, SEIZURES AND EPILEPSY

Several studies have studied the effects of anticonvulsant drugs transported by drug nanocarriers in animal models of seizures or epilepsy. Using the maximal electroshock model, Friese et al. (115) described that the duration of the anticonvulsant effect of MRZ 2/57, a non-competitive NMDA receptor antagonist, was longer when this drug was encapsulated in poly (butyl cyanoacrylate) nanoparticles coated with polysorbate-80. Eskandari et al. (116) described that the anticonvulsant effect of valproic acid is augmented when it is applied by intranasal route using nanostructured lipid carriers. Using the maximal electroshock model, Nair et al. (117) reported that the anticonvulsant effect induced by oral administration of carbamazepine is increased when the drug is loaded in solid lipid nanoparticles of chitosan. Yusuf et al. (118) described that the anticonvulsant effect of β-carotene in acute experimental models of seizures is improved when it is encapsulated in nanoparticles of poly(d,l-lactide-co-glycolide), an effect that is more effective when the nanoparticles are coated with polysorbate-80. Carbamazepine loaded in a nanoemulgel system and administered by intranasal route induces higher anticonvulsant effects in chemically- and electroshock-induced convulsions in mice (119). The subcutaneous administration of ethosuximide loaded in thermo-gelling nanogels of chitosan suppresses spontaneous spike-wave discharges in an experimental model of absence like seizures (120).

Fewer studies have evaluated the effects of drug nanocarriers in experimental models of epileptogenesis in rats. The intranasal administration of thyrotropin releasing hormone encapsulated in polyactic acid nanoparticles delays kindling-induced epileptogenesis (121, 122). A similar effect was induced by adenosine-releasing brain implants containing microspheres in nanofilm-coated silk fibroin scaffolds (123).

It is important to notice that treatment of pharmacoresistant disorders of the central nervous system is one of the greatest challenges in drug delivery. However, at present there are no studies aiming to evaluate the effects of AEDs transported by drug nanocarriers in experimental models or patients with pharmacoresistant epilepsy.

8. HOW TO APPLY NANOTECHNOLOGY TO CONTROL PHARMACORESISTANT EPILEPSY

The use of drug nanocarriers is potentially a very useful tool that may be applied to overcome the problem of drug delivery in pharmacoresistant epilepsy. Developing this technology requires the collaborative work of specialists in the field of nanotechnology and pharmacology in a multidisciplinary approach.

One important consideration at the experimental level is that nanomaterials must be tried out in experimental models that reproduce the characteristics of pharmacoresistant epilepsy, such as the overexpression of transporters in the BBB. Epilepsy induced by kindling or spontaneous epileptic seizures as long-term consequence of status epilepticus may be appropriate models to identify animals with pharmacoresistant epilepsy. Electrical or chemical kindling requires the repeated application of initially subthreshold electrical or chemical stimuli, which induce progressive changes culminating in generalized epileptic seizures (124, 125). Once the process of epileptogenesis is completed, pharmacoresistant animals are identified by the absence of changes in afterdischarge thresholds despite administration of effective doses of AEDs (126). Another procedure entails induction of spontaneous seizures as a long-term consequence of status epilepticus induction by repeated electrical stimulation of sensitive brain regions or systemic administration of drugs such as lithium-pilocarpine or kainic acid. Pharmacoresistant animals present spontaneous seizures in spite of adequate treatment with AEDs. Animals with pharmacoresistant epilepsy exhibit characteristics that resemble findings in patients with pharmacoresistant epilepsy such as overexpression of transporters in the BBB. However, development of these models requires prolonged experimental periods and the percentage of pharmacoresistant subjects obtained is low (20% or less) (127). A short-term alternative for obtaining animals with overexpression of brain transporters is the repeated administration of 3-mercaptopropionic acid or pentylentetrazol (47, 128). However, these procedures do not induce spontaneous seizures and thus should not be considered epilepsy models.
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Other important requirements to be considered in the development of nano-sized transporters for AEDs as therapeutic options in pharmacoresistant epilepsy include: 1) nanosystems must be designed in such a way that AEDs can be masked and circumvent the effects of multi-resistance proteins on the BBB facilitating penetration into brain parenchyma (Figure 1); 2) nanosystems must result in effective prolonged and sustained delivery of the transported drugs enabling reduced administration frequencies; 3) synthesis should be easy and economical; 4) secondary effects of application should be minimal at the central and peripheral levels. About the last issue, all the components of the drug nanocarriers have to be safe and must be approved by agencies for the evaluation of medicinal products such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA) (129). Besides the active pharmaceutical ingredient (drug), the nanocarriers may include other inactive substances that are intentionally integrated for therapeutic-enhancing purposes called excipients (130). All the excipients authorized by the FDA are listed in the Inactive Ingredients Guide (131), including substances with different chemical nature such as sugars (sucrose, trehalose, dextran, gelatine), polymers (polyethylene glycol (PEG), polylactide (PLA), poly(DL-lactide-co-glycolide) (PGLA)) and macromolecules as albumin. Several of these substances are used as excipients with nanomaterials (118, 132-136) and some nanomaterials are used as excipients per se (137, 138). However, the design and application of new drug nanocarriers focused to induce sustained therapeutic effects may include the application of novel excipients not already approved by the FDA. Unfortunately, at present there are regulatory uncertainties of using new excipients in drug products, including nanodrugs. Therefore, the development process of novel nanocarriers and their excipients focused to control pharmacoresistant epilepsy requires a strong collaboration between pharmacologists and pharmaceutical industry, including excipient innovators (131, 139).

Among the different types of nanosystem evaluated at present, MnPs have proven to be one of the best strategies for different biomedical purposes, such as MRI contrast agents, cellular therapy, tissue repair, ablation by hyperthermia, drug delivery and carrier systems (140-142). In addition to their easy and economical synthesis process, MnPs offer special advantages such as their small size (<100 nm) allowing penetration through cell membranes (114, 143). Their biodegradability is carried out by lysosomal rupture of the iron oxide core resulting in iron that are incorporated back into the hemoglobin pool (144). Given the magnetic characteristic of MnPs, intracerebral release from special devices can be controlled by magnetic fields (145).

Some reports indicate that MnPs present high biocompatibility and its administration does not induce toxic effects (146-148). According to this information, MnPs could represent an excellent strategy to deliver AEDs into the brain parenchyma of patients with pharmacoresistant epilepsy. Indeed, a previous study indicates that the intraperitoneal administration of MnPs for 4 weeks does not produce “apparent” toxicity, histopathological changes or adverse effects on body development and behavior (149). However, it is relevant to consider that MnPs are also reactive in the biological environment and may induce chemical interactions and toxicological effects (150, 151). Concerning this issue, it is described that the MnPs could produce reactive oxygen species in excess, a situation that may result in oxidative stress (152, 153), neuronal damage, pro-inflammatory effects and modification of BBB permeability (154). The oxidative stress could induce cell death as result of mitochondrial membrane damage or electron chain dysfunction (150, 155), mutagenesis (156), the activation of oncogenes such as Ras (157) or production of DNA damaging end-products (158) and subsequent carcinogenesis (159). Therefore, it is indispensable to determine under different physiological conditions if undesirable effects are produced after the chronic administration of MnPs.

9. CONCLUSIONS

It is clear that AEDs-loaded in nanosystems could be a promising therapy for the treatment of pharmacoresistant epilepsy. The preparation procedure of some of these nanosystems, already used routinely for therapy of different brain disorders, is very easy. This advantage along with the diversity of available modern technologies, the availability of proper experimental models of AED resistant epilepsy plus a coordinated endeavor involving experts in basic science, bioengineering and clinicians will surely promote the development of new -more effective- nanotherapies for the treatment of pharmacoresistant epilepsy over the next years.

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