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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

present an increased risk of sudden unexpected death (SUDEP) and suicide (10-12).

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 suggests 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 dimmer (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 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

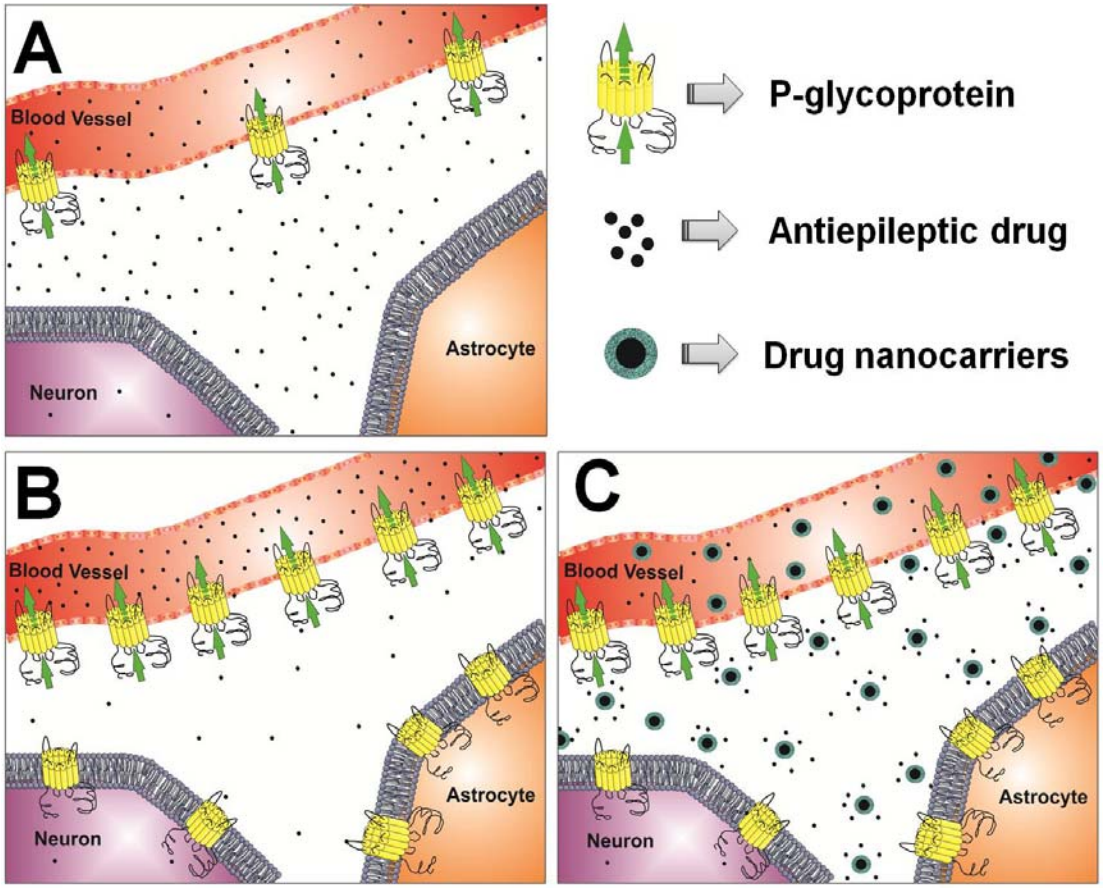

Figure 1. Schematic representations of P-glycoprotein (P-gp) expression and antiepileptic drugs (AEDs) in blood stream and cerebral parenchyma under different conditions. A) Under healthy conditions, in which P-gp expression is low and limited to the endothelial cells of the brain microvessels, the AEDs 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. NANOTHECHNOLGY 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

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 polylactic 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.

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 multiresistance 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(D,L-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 ions 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, proinflammatory 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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11. REFERENCES

1. Fisher, R. S., W. van Emde Boas, W. Blume , C. Elger , P. Genton , P. Lee, and J. Jr. Engel: Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 46, 470-472 (2005)

2. Kwan, P., A. Arzimanoglou , A. T. Berg, M. J. Brodie, W. Allen Hauser, G. Matthern, S. L. Moshé, E. Perucca, S. M Wiebe, and J. French: Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* 51, 1069-1077 (2010)

3. Kwan, P., and M. J. Brodie: Effectiveness of first antiepileptic drug. *Epilepsia* 42,1255-1260 (2001)

4. Kwan, P., and M. J. Brodie: Drug treatment of epilepsy: when does it fail and how to optimize its use?. *CNS Spectr* 9, 110-119 (2004)

5. García-Contreras, F., P. Constantino-Casas, A. Castro-Ríos, A. Nevárez-Sida, G. del C. Estrada Correa, F. Carlos Rivera, J. Guzmán-Caniupan, L. del P. Torres-Arreola, I. Contreras-Hernández, J. Mould-Quevedo, and J. Garduño-Espinosa: Direct medical costs for partial refractory epilepsy in Mexico. *Arch Med Res* 37, 376-383 (2006)

6. Bishop, M., and C. A. Allen: The impact of epilepsy on quality of life: a qualitative analysis. *Epilepsy Behav* 4, 226-233 (2003)

7. Jacoby, A., and G. A. Baker: Quality-of-life trajectories in epilepsy: a review of the literature. *Epilepsy Behav* 12, 557-571 (2008)

8. Rogawski, M. A.: The intrinsic severity hypothesis of pharmacoresistance to antiepileptic drugs. *Epilepsia* 54, 33-40 (2013)

9. Hermann, B. P., M. Seidenberg, C. Dow, J. Jones , P. Rutecki , A. Bhattacharya, and B. Bell: Cognitive prognosis in chronic temporal lobe epilepsy. *Ann Neurol* 60, 80-87 (2006)

10. Chapell, R., J. Reston, D. Snyder, J. Treadwell, S. Treager, and C. Turkelson: Management of treatment-resistant epilepsy. Evidence report/technology assessment No. 77 (Prepared by the ECRI Evidence-based Practice Center under contract 290-97-0020). Rockville, MD: Agency for Healthcare Research and Quality. AHRQ publication 03-0028, 1-8 (2003).

11. Ryvlin, P., M. Cucherat, and S. Rheims: Risk of sudden unexpected death in epilepsy in patients given adjunctive antiepileptic treatment for refractory seizures: a meta-analysis of placebo-controlled randomised trials. *Lancet Neurol* 10, 961-968 (2011)

12. Lhatoo, S. D., and J. W. Sander: Cause-specific mortality in epilepsy. *Epilepsia* 46, 36-39 (2005)

13. Hamer, H. M., A. Spottke, C. Aletsee, S. Knake, J. Reis, A. Strzelczyk, W. H. Oertel, F. Rosenow, and R. Dodel: Direct and indirect costs of refractory epilepsy in a tertiary epilepsy center in Germany. *Epilepsia* 47, 2165-2172 (2006)

14. Scambler, G., P. Afentouli, and C. Selai: Discerning biological, psychological and social mechanisms in the impact of epilepsy on the individual: a framework and exploration. In Eds Scambler, G & Scambler, S: New Directions in the Sociology of Chronic and Disabling Conditions. London; Palgrave Macmillan (2010)

15. Murray, M. I., M. T. Halpern, and I. E. Leppik: Cost of refractory epilepsy in adults in the USA. *Epilepsy Res* 23, 139-148 (1996)

16. Fang, M., Z. Q. Xi, Y. Wu, and X.F. Wang: A new hypothesis of drug refractory epilepsy: neural network hypothesis. *Med Hypotheses* 76, 871-876 (2011)

17. Bannister, A. J., and T. Kouzarides: Regulation of chromatin by histone modifications. *Cell Res* 21, 381-395 (2011)

18. Kobow, K., A. El-Osta, and I. Blümcke: The methylation hypothesis of pharmacoresistance in epilepsy. *Epilepsia* 54, 41-47 (2013)

19. Kobow. K., and I. Blümcke: The methylation hypothesis: do epigenetic chromatin modifications play a role in epileptogenesis?. *Epilepsia* 52, 15-19 (2011)

20. Yuen, A. W., and J. W. Sander: Impaired mitochondrial energy production: the basis of pharmacoresistance in epilepsy. *Med Hypotheses* 77, 536-540 (2011)

21. Rogawski, M. A., and M. R. Johnson: Intrinsic severity as a determinant of antiepileptic drug refractoriness. *Epilepsy Curr* 8, 127-130 (2008)

22. Arroyo, S., M. J. Brodie, G. Avanzini, C. Baumgartner, C. Chiron, O. Dulac, J. A. French, and J. M. Serratosa: Is refractory epilepsy preventable? *Epilepsia* 43, 437-444 (2002)

23. Remy, S., and H. Beck: Molecular and cellular mechanisms of pharmacoresistance in epilepsy. *Brain* 129, 18-35 (2006)

24. Schmidt, D., and W. Löscher: Drug resistance in epilepsy: putative neurobiologic and clinical mechanisms. *Epilepsia* 46, 858-77 (2005)

25. Higgins, C. F.: ABC transporters: from microorganisms to man. *Annu Rev Cell Biol* 8, 67-113 (1992)

26. Girardin, F.: Membrane transporter proteins: a challenge for CNS drug development. *Dialogues Clin Neurosci* 8, 311-321 (2006)

Ford, R., A. Kamis, I. Kerr, and R. Callaghan: The ABC Transporters: Structural insights into drug transport. In Ecker G, Chiba P, editors. Transporters as Drug Carriers. Weinheim: Wiley-VCH (2009)

Glavinas, H., P. Krajcsi, J. Cserepes, and B. Sarkadi: The role of ABC transporters in drug resistance, metabolism and toxicity. *Curr Drug Deliv* 1, 27-42 (2004)

Ejendal, K. F., and C. A. Hrycyna: Multidrug resistance and cancer: the role of the human ABC transporter ABCG2. *Curr Protein Pept Sci* 3, 503-511 (2002)

Löscher, W., and H. Potschka: Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. *NeuroRx* 2, 86-98 (2005)

27. Juliano, R. L., and V. Ling: A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 455, 152-62 (1976)

28. Ling, V., and L. Thompson: Reduced permeability in CHO cells as a mechanism of resistance to colchicine. *J Cell Physiol* 83: 103-116 (1974)

29. Aftab, D.T., J. M. Yang, and W. H. Hait: Functional role of phosphorylation of the multidrug transporter (P-glycoprotein) by protein kinase C in multidrug-resistant MCF-7 cells. *Oncol Res* 6, 59-70 (1994)

30. Su, L., P. Jenardhanan, D. D. Mruk , P. P. Mathur, Y. H. Cheng, K. W. Mok, M. Bonanomi , B. Silvestrini, and C. Y. Cheng: Role of P-glycoprotein at the blood-testis barrier on adjudin distribution in the testis: a revisit of recent data. *Adv Exp Med Biol* 763, 318-333 (2012).

31. Melaine, N., M. O. Lienard, I. Dorval, C. Le Goascogne, H. Lejeune, and B. Jegou: Multidrug resistance genes and p-glycoprotein in the testis of the rat, mouse, Guinea pig, and human. *Biol Reprod* 67: 1699-1707 (2002)

32. Edwards, J.E., J. Alcorn, J. Savolainen, B. D. Anderson, and P. J. McNamara: Role of P-glycoprotein in distribution of nelfinavir across the blood-mammary tissue barrier and blood-brain barrier. *Antimicrob Agents Chemother* 49, 1626-1628 (2005)

33. Saito, T., Z. J. Zhang, H. Tsuzuki, T. Ohtsubo, T. Yamada, T. Yamamoto, and H. Saito: Expression of P-glycoprotein in inner ear capillary endothelial cells of the guinea pig with special reference to blood-inner ear barrier. *Brain Res* 767, 388-392 (1997)

34. Thiebaut, F., T. Tsuruo, H. Hamada, M. M. Gottesman, I. Pastan, and M. C. Willingham: Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* 84, 7735-7738 (1987)

35. Beaulieu, E., M. Demeule, L. Ghitescu, and R. Béliveau: P-glycoprotein is strongly expressed in the luminal membranes of the endothelium of blood vessels in the brain. *Biochem J* 326, 539-544 (1997)

36. Jette, L., B. Tétu, and R. Béliveau: High levels of P-glycoprotein detected in isolated brain capillaries. *Biochim Biophys Acta* 1150, 147-154 (1993)

37. Schinkel, A. H.: P-Glycoprotein, a gatekeeper in the blood-brain barrier. *Adv Drug Deliv Rev* 36, 179-194 (1999)

38. Schinkel, A. H., J. J. Smit, O. van Tellingen, J. H. Beijnen, E. Wagenaar, L. van Deemter, C. A. Mol, M. A. van der Valk, E. C. Robanus-Maandag, H. P. J. te Riele, A. J. M. Berns, and P. Bors: Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* 77, 491-502 (1994)

39. Abbott, N. J., E. U. Khan, C. M. Rollinson, A. Reichel, D. Janigro, S. M.Dombrowski, M.S. Dobbie, and D. J. Begley: Drug resistance in epilepsy: the role of the blood-brain barrier. *Novartis Found Symp* 243, 38-47, 180-185 (2002)

40. French. J.A.: Refractory epilepsy: clinical overview. *Epilepsia* 48, 3-7 (2007)

41. Potschka, H., M. Fedrowitz, and W. Löscher: P-Glycoprotein-mediated efflux of phenobarbital, lamotrigine, and felbamate at the blood-brain barrier: evidence from microdialysis experiments in rats. *Neurosci Lett* 327, 173-176 (2002)

42. Tishler, D. M., K. I. Weinberg, D. R. Hinton, N. Barbaro, G. M. Annett, and C. Raffel: MDR1 gene expression in brain of patients with medically intractable epilepsy. *Epilepsia* 36, 1-6 (1995)

43. Rizzi, M., S. Caccia, G. Guiso, C. Richichi, J. A. Gorter, E. Aronica, M. Aliprandi, R. Bagnati, R. Fanelli, M. D'Incalci, R. Samanin, and A. Vezzani: Limbic seizures induce P-glycoprotein in rodent brain: functional implications for pharmacoresistance. *J Neurosci* 22, 5833-5839 (2002)

44. Ak, H., B. Ay, T. Tanriverdi, G. Z. Sanus, M. Is, M. Sar, B. Oz, C. Ozkara, E. Ozyurt, and M Uzan: Expression and cellular distribution of multidrug resistance-related proteins in patients with focal cortical dysplasia. *Seizure* 16, 493-503 (2007)

45. Aronica, E., J. A. Gorter, M. Ramkema, S. Redeker, F. Ozbas-Gerceker, E. A. van Vliet, G. L. Scheffer, R. J. Scheper, P. van der Valk, J. C. Baayen, and D. Troost: Expression and cellular distribution of multidrug resistance-related proteins in the hippocampus of patients with mesial temporal lobe epilepsy. *Epilepsia* 45, 441-451 (2004)

46. Dombrowski, S. M., S. Y. Desai, M. Marroni, L. Cucullo, K. Goodrich, W. Bingaman, M. R. Mayberg, L. Bengez, and D. Janigro: Overexpression of multiple drug resistance genes in endothelial cells from patients with refractory epilepsy. *Epilepsia* 42, 1501-1506 (2001)

47. Liu, X., Z. Yang, J. Yang, and H. Yang: Increased P-glycoprotein expression and decreased phenobarbital distribution in the brain of pentylenetetrazole-kindled rats. *Neuropharmacology* 53, 657-663 (2007)

48. Potschka, H., H.A. Volk, and W. Löscher: Pharmacoresistance and expression of multidrug transporter P-glycoprotein in kindled rats. *Neuroreport* 15, 1657-1661 (2004)

49. Jing, X., X. Liu, T. Wen, S. Xie, D. Yao, X. Liu, G. Wangm, and L. Xie: Combined effects of epileptic seizure and phenobarbital induced overexpression of P-glycoprotein in brain of chemically kindled rats. *Br J Pharmacol* 159, 1511-1522 (2010)

50. Lazarowski, A., A. J. Ramos, H. Garcia-Rivello, A. Brusco, and E. Girardi: Neuronal and glial expression of the multidrug resistance gene product in an experimental epilepsy model. *Cell Mol Neurobiol* 24, 77-85 (2004)

51. Seegers, U., H. Potschka, and W. Löscher: Transient increase of P-glycoprotein expression in endothelium and parenchyma of limbic brain regions in the kainate model of temporal lobe epilepsy. *Epilepsy Res* 51, 257-268 (2002)

52. Volk, H. A., and W. Löscher: Multidrug resistance in epilepsy: rats with drug-resistant seizures exhibit enhanced brain expression of P-glycoprotein compared with rats with drug-responsive seizures. *Brain* 128, 1358-1368 (2005)

53. Hocht, C., A. Lazarowski, N. N. Gonzalez, J. Auzmendi, J. A. Opezzo, G.F. Bramuglia, C.A. Taira, and E. Girardi: Nimodipine restores the altered hippocampal phenytoin pharmacokinetics in a refractory epileptic model. *Neurosci Lett* 413, 168-172 (2007)

54. Brandt, C., K. Bethmann, A. M. Gastens, and W. Loscher: The multidrug transporter hypothesis of drug resistance in epilepsy: Proof-of-principle in a rat model of temporal lobe epilepsy. *Neurobiol Dis* 24, 202-211 (2006)

55. Sisodiya, S. M., W. R. Lin, B. N. Harding, M. W. Squier, and M. Thom: Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. *Brain* 125, 22-31 (2002)

56. Sisodiya, S. M., W. R. Lin, M. V. Squier, and M. Thom: Multidrug-resistance protein 1 in focal cortical dysplasia. *Lancet* 357, 42-43 (2001)

57. Sisodiya, S. M., L. Martinian, G. L. Scheffer, P. van der Valk, R. J. Scheper, B. N. Harding, and M. Thom: Vascular colocalization of P-glycoprotein, multidrug-resistance associated protein 1, breast cancer resistance protein and major vault protein in human epileptogenic pathologies. *Neuropathol Appl Neurobiol* 32, 51-63 (2006)

58. Czornyj, L., F. Lubieniecki, S. Camarero, H. Pomata, M. Bartoluchi, A. L. Taratuto, and A. Lazarowski: Unusual transmantle focal-cortical dysplasia (TD) and Refractory Epilepsy (RE) with brain expression of Breast Cancer Resistant Resistant Protein (BCRP). *Rev Mex de Neurociencias* 5, 229 (2004)

59. Czornyj, L., F. Lubienieky, H. Pomata, and A. Lazarowski: Breast cancer resistant protein (BCRP) in cortical dysplasia with refractory epilepsy and failure to produce pharmacological coma. *Epilepsia* 46, 326 (2005)

60. Vogelgesang, S., C. Kunert-Keil, I. Cascorbi, I. Mosyagin, E. Schröder, U. Runge, G. Jedlitschky, H. K. Kroemer, J. Oertel, M. R. Gaab, J. Pahnke, L. C. Walker, and R. W. Warzok: Expression of multidrug transporters in dysembryoplastic neuroepithelial tumors causing intractable epilepsy. *Clin Neuropathol* 23, 223-231 (2004)

61. Cerveny, L., P. Pavek, J. Malakova, F. Staud, and Z. Fendrich: Lack of interactions between breast cancer resistance protein (BCRP/ABCG2) and selected antiepileptic agents. *Epilepsia* 47, 461-468 (2006)

62. Lazarowski, A. J., F. J. Lubieniecki, S. A. Camarero, H. H. Pomata, M. A. Bartuluchi, G. Sevlever, and A. L. Taratuto: New proteins configure a brain drug resistance map in tuberous sclerosis. *Pediatr Neurol* 34, 20-24 (2006)

63. van Vliet, E. A., E. Aronica, S. Redeker, and J. A. Gorter: Expression and cellular distribution of major vault protein: a putative marker for pharmacoresistance in a rat model for temporal lobe epilepsy. *Epilepsia* 45, 1506-1516 (2004)

67. Wieser, H. G.: Epilepsy surgery: past, present and future. *Seizure* 7,173–184 (1998)

68. Engel, J. Jr.: Surgery for seizures. *N Engl J Med* 334, 647-652 (1996)

69. Hauser, W. A.: The natural history of drug resistant epilepsy: epidemiologic considerations. *Epilepsy Res* 5, 25-28 (1992)

70. Handforth, A., C. M. DeGiorgio, S. C. Schachter, B. M. Uthman, D. K. Naritoku, E. S. Tecoma, T. R. Henry, S. D. Collins, B. V. Vaughn, R. C. Gilmartin, D. R. Labar, G. L. Morris, M. C. Salinsky, I. Osorio, R. K. Ristanovic, D. M. Labiner, J. C. Jones, J. V. Murphy, G. C. Ney, and J. W. Wheless: Vagus nerve stimulation therapy for partial-onset seizures: a randomized active-control trial. *Neurology* 51, 48-55 (1998)

71. Pereira, E. A., A. L. Green, R. J. Stacey, and T. Z. Aziz: Refractory epilepsy and deep brain stimulation. *J Clin Neurosci* 19, 27-33 (2012)

72. DeGiorgio, C. M., and S. E. Krahl: Neurostimulation for drug-resistant epilepsy. Continuum (Minneap Minn) 19 (3 Epilepsy), 743-755 (2013)

73. Hammond, E. J., B. M. Uthman, S. A. Reid, and B. J. Wilder: Electrophysiological studies of cervical vagus nerve stimulation in humans: I. EEG effects. *Epilepsia* 33, 1013-1020 (1992)

74. Henry, T. R., R. A. Bakay, P. B. Pennell, C. M. Epstein, and J. R. Votaw: Brain blood-flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: II. prolonged effects at high and low levels of stimulation. *Epilepsia* 45, 1064-1070 (2004)

75. Ko, D., C. Heck, S. Grafton, M. L. Apuzzo, W. T. Couldwell, T. Chen, J. D. Day, V. Zelman, T. Smith, and C. M. DeGiorgio: Vagus nerve stimulation activates central nervous system structures in epileptic patients during PET H2(15)O blood flow imaging. *Neurosurgery* 39, 426-430, 30-31 (1996)

76. Ben-Menachem, E.: Neurostimulation-past, present, and beyond. *Epilepsy Curr* 12,188-191 (2012)

77. Velasco, A. L., J. M. Núñez, D. Vázquez, J. D. Carrillo-Ruiz, M. Cuéllar-Herrera, R. Conde, and F. Velasco: Neuromodulation Advances for Seizure Control. Topics in Neuromodulation Treatment, Dr. José Carrillo-Ruiz (Ed.), ISBN: 978-953-51-0395-0, InTech (2012)

78. Suh, W. H., K. S. Suslick, G. D. Stucky, and Y. H. Suh: Nanotechnology, nanotoxicology, and neuroscience. *Prog Neurobiol* 87, 133-170 (2009)

79. Weissleder, R., G. Elizondo, J. Wittenberg, A. S. Lee, L. Josephson, and T. J. Brady: Ultrasmall Superparamagnetic Iron-Oxide - an Intravenous Contrast Agent for Assessing Lymph-Nodes with Mr Imaging. *Radiology* 175, 494-498 (1990)

80. Varallyay, C. G., L. L. Muldoon, S. Gahramanov , Y. J. Wu, J. A. Goodman, X. Li , M. M. Pike, and E. A. Neuwelt: Dynamic MRI using iron oxide nanoparticles to assess early vascular effects of antiangiogenic versus corticosteroid treatment in a glioma model. *J Cerebr Blood F Met* 29, 853-860 (2009)

81. Neuwelt, E. A., P. Várallyay, A. G. Bagó, L. L. Muldoon, G. Nesbit, and R. Nixon: Imaging of iron oxide nanoparticles by MR and light microscopy in patients with malignant brain tumours. *Neuropathol Appl Neurobiol* 30, 456-471 (2004)

82. Rausch, M., A. Sauter, J. Fröhlich, U. Neubacher, E.W. Radü, and M. Rudin: Dynamic patterns of USPIO enhancement can be observed in macrophages after ischemic brain damage. *Magnet Reson Med* 46, 1018-1022 (2001)

83. Saleh, A., M. Schroeter, A. Ringelstein, H. P. Hartung, M. Siebler, U. Mödder, and S. Jander: Iron oxide particle-enhanced MRI suggests variability of brain inflammation at early stages after ischemic stroke. *Stroke* 38, 2733-2737 (2007)

84. Vellinga, M. M., R. D. Oude Engberink, A. Seewann, P. J. Pouwels, M. P. Wattjes, S. M. van der Pol, C. Pering, C. H. Polman, H. E. de Vries, J. J. Geurts, and F. Barkhof: Pluriformity of inflammation in multiple sclerosis shown by ultra-small iron oxide particle enhancement. *Brain* 131, 800-807 (2008)

85. Floris, S., E. L. A. Blezer, G. Schreibelt, E. Dopp, S. M. A. van der Pol, I. L. Schadee-Eestermans, K. Nicolay, C. D. Dijkstra, and H. E. de Vries: Blood-brain barrier permeability and monocyte infiltration in experimental allergic encephalomyelitis: A quantitative MRI study. *Brain* 127, 616-627 (2004)

86. Sykova, E., and P. Jendelova: In vivo tracking of stem cells in brain and spinal cord injury. *Prog Brain Res* 161, 367-83 (2007)

87. Hoehn, M., D. Wiedermann, C. Justicia, P. Ramos-Cabrer, K. Kruttwig, T. Farr, and U. Himmelreich: Cell tracking using magnetic resonance imaging. *J Physiol* 584, 25-30 (2007)

88. Akhtari, M., A. Bragin, M. Cohen, R. Moats, F. Brenker, M. D. Lynch, H. V. Vinters, and J. Jr. Engel: Functionalized magnetonanoparticles for MRI diagnosis and localization in epilepsy. *Epilepsia* 49, 1419-1430 (2008)

89. Soroceanu, L., Y. Gillespie, M. B. Khazaeli and H. Sontheimer: Use of chlorotoxin for targeting of primary brain tumors. *Cancer Res* 58, 4871-4879 (1998)

90. McAteer, M. A., N. R. Sibson, C. von Zur Muhlen, J. E. Schneider, A. S. Lowe, N. Warrick, K. M. Channon , D. C. Anthony, and R. P. Choudhury: In vivo magnetic resonance imaging of acute brain inflammation using microparticles of iron oxide. *Nat Med* 13, 1253-1258 (2007)

91. Kong, S. D., J. Lee, S. Ramachandran, B. P. Eliceiri, V. I. Shubayev, R. Lal, and S. Jin: Magnetic targeting of nanoparticles across the intact blood-brain barrier. *J Control Release* 164, 49-57 (2012)

92. Jordan, A., R. Scholz, K. Maier-Hauff, F. K. H. van Landeghem, N. Waldoefner, U. Teichgraeber, J. Pinkernelle, H. Bruhn, F. Neumann, B. Thiesen, A. von Deimling, and R. Felix: The effect of thermotherapy using magnetic nanoparticles on rat malignant glioma. *J Neurooncol* 78, 7-14 (2006)

93. Maier-Hauff, K., R. Rothe, R. Scholz, U. Gneveckow, P. Wust, B. Thiesen, A. Feussner, A. von Deimling, N. Waldoefner, R. Felix, and A. Jordan: Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: results of a feasibility study on patients with glioblastoma multiforme. *J Neurooncol* 81, 53-60 (2007)

94. Maier-Hauff, K., F. Ulrich, D. Nestler, H. Niehoff, P. Wust, B. Thiesen, H. Orawa, V. Budach, and A. Jordan: Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme. *J Neurooncol* 103, 317-324 (2011)

95. Kreuter, J.: Drug delivery to the central nervous system by polymeric nanoparticles: What do we know? *Adv Drug Deliv Rev* http://dx.doi.org/10.1.016/j.addr.2013.0.8.0.08 (2013)

96. Soppimath, K. S., T. M. Aminabhavi, A. R. Kulkarni, and W. E. Rudzinski: Biodegradable polymeric nanoparticles as drug delivery devices. *J Control Release* 70, 1-20 (2001)

97. Lockman, P. R., R. J. Mumper, M. A. Khan, and D. D. Allen: Nanoparticle technology for drug delivery across the blood-brain barrier. *Drug Dev Ind Pharm* 28, 1-13 (2002)

98. Vij, N., T. Min, R. Marasigan, C. N. Belcher, S. Mazur, H. Ding, K. T. Yong, and I. Roy: Development of

PEGylated PLGA nanoparticle for controlled and sustained drug delivery in cystic fibrosis. *J Nanobiotechnology* 8, 22. doi: 10.1.186/1477-3155-8-22 (2010)

99. Mu, L., and S. S Feng: A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS. *J Control Release* 86, 33-48 (2003)

100. Kreuter, J., P. Ramge, V. Petrov, S. Hamm, S. E. Gelperina, B. Engelhardt, R. Alyautdin, H. von Briesen, and D.J. Begley: Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. Pharm Res 20, 409-416 (2003)

101. Koziara, J. M., P. R. Lockman, D. D. Allen, and R. J. Mumper: Paclitaxel nanoparticles for the potential treatment of brain tumors. *J Control Release* 99, 259-269 (2004)

102. Modi, G., V. Pillay, Y. E. Choonara, V. M. Ndesendo, L.C. du Toit, and D. Naidoo: Nanotechnological applications for the treatment of neurodegenerative disorders. *Prog Neurobiol* 88, 272-285 (2009)

103. Muller, R. H., R. D. Petersen, A. Hommoss, and J. Pardeike: Nanostructured lipid carriers (NLC) in cosmetic dermal products. *Adv Drug Deliv Rev* 59, 522-530 (2007)

104. Saad, M., O. B. Garbuzenko, E. Ber, P. Chandna, J. J. Khandare, V. P. Pozharov, and T. Minko: Receptor targeted polymers, dendrimers, liposomes: which nanocarrier is the most efficient for tumor-specific treatment and imaging? *J Control Release* 130, 107-114 (2008)

105. Klingeler, R., S. Hampel, and B. Büchner: Carbon nanotube based biomedical agents for heating, temperature sensoring and drug delivery. *Int J Hyperthermia* 24, 496-505 (2008)

106. Bennewitz, M. F., and W. M. Saltzman: Nanotechnology for delivery of drugs to the brain for epilepsy. *Neurotherapeutics* 6, 323-336 (2009)

107. Allen, T. M., and P. R. Cullis: Liposomal drug delivery systems: from concept to clinical applications. *Adv Drug Deliv Rev* 65, 36-48 (2013)

108. Malik, A., S. Chaudhary, G. Garg, and A. Tomar: Dendrimers: A Tool for Drug Delivery. *Adv Biol Res* 6, 165-169 (2012)

109. Husseini, G. A., and W. G. Pitt: Micelles and nanoparticles for ultrasonic drug and gene delivery. *Adv Drug Deliv Rev* 60, 1137-1152 (2008)

110. Lacerda, L., A. Bianco, M. Prato, and K. Kostarelos: Carbon nanotubes as nanomedicines: From toxicology to pharmacology. Adv Drug Deliv Rev 58, 1460-1470 (2006)

111. Patel, T., J. Zhou, J. M. Piepmeier, and W. M. Saltzman: Polymeric nanoparticles for drug delivery to the central nervous system. *Adv Drug Deliv Rev* 64, 701-705 (2012)

Cho, K.J., X. Wang, S.M. Nie, Z. Chen, and D. M. Shin: Therapeutic nanoparticles for drug delivery in cancer. *Clin Cancer Res* 14, 1310-1316 (2008)

112. De Jong, W. H., and P. J. Borm: Drug delivery and nanoparticles: applications and hazards. *Int J Nanomedicine* 3, 133-149 (2008)

113. Friese, A., E. Seiller, G. Quack, B. Lorenz, and J. Kreuter: Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system. *Eur J Pharm Biopharm* 49, 103-109 (2000).

114. Eskandari, S., J. Varshosaz, M. Minaiyan, and M. Tabbakhian: Brain delivery of valproic acid via intranasal administration of nanostructured lipid carriers: in vivo pharmacodynamic studies using rat electroshock model. *Int J Nanomedicine* 6, 363-371 (2011)

115. Nair, R., A. C. Kumar, V. K. Priya, C. M. Yadav, and P. Y. Raju: Formulation and evaluation of chitosan solid lipid nanoparticles of carbamazepine. *Lipids Health Dis* 11, 72 (2012)

116. Yusuf, M., R. A. Khan, M. Khan, and B. Ahmed: Plausible antioxidant biomechanics and anticonvulsant pharmacological activity of brain-targeted beta-carotene nanoparticles. *Int J Nanomedicine* 7, 4311-4321 (2012)

117. Samia, O., R. Hanan, and T. Kamal el: Carbamazepine mucoadhesive nanoemulgel (MNEG) as brain targeting delivery system via the olfactory mucosa. *Drug Deliv* 19, 58-67 (2012)

118. Hsiao, M. H., M. Larsson, A. Larsson, H. Evenbratt, Y.Y. Chen, Y.Y. Chen, and D. M. Liu: Design and characterization of a novel amphiphilic chitosan nanocapsule-based thermo-gelling biogel with sustained in vivo release of the hydrophilic anti-epilepsy drug ethosuximide. *J Control Release* 161, 942-948 (2012)

119. Kubek, M. J., A. J. Domb, and M. C. Veronesi : Attenuation of kindled seizures by intranasal delivery of neuropeptide-loaded nanoparticles. *Neurotherapeutics* 6, 359-371 (2009)

120. Veronesi, M. C., Y. Aldouby, A. J. Domb, and M. J. Kubek: Thyrotropin-releasing hormone d,l polylactide nanoparticles (TRH-NPs) protect against glutamate toxicity in vitro and kindling development in vivo. *Brain Res* 1303, 151-160 (2009)

121. Wilz, A., E. M. Pritchard, T. Li, J. Q. Lan, D. L. Kaplan, and D. Boison: Silk polymer-based adenosine

release: therapeutic potential for epilepsy. *Biomaterials* 29, 3609-3616 (2008)

122. Mason, C. R., and R. M. Cooper: A permanent change in convulsive threshold in normal and brain-damaged rats with repeated small doses of pentylenetetrazol. *Epilepsia* 13, 663-674 (1972)

123. Goddard, G. V., D. C. McIntyre, and C. K. Leech: A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* 25, 295-330 (1969)

124. Loscher, W., C. Rundfeldt, and D. Honack: Pharmacological characterization of phenytoin-resistant amygdala-kindled rats, a new model of drug-resistant partial epilepsy. *Epilepsy Res* 15, 207-219 (1993)

125. Zavala-Tecuapetla, C., and L. Rocha: Experimental Models to Study Pharmacoresistance in Epilepsy. In: L. Rocha and Esper A. Cavalheiro, Eds. Pharmacoresistance in epilepsy: from genes and molecules to promising therapies. Springer. New York, Heidelberg, Dordrecht, London. pp. 185-98 (2013)

126. Lazarowski, A., L. Czornyj, F. Lubieniecki, S. Vazquez, C. D'Giano, G. Sevlever, A. Lia Taratuto, A. Brusco, and E. Girardi: Multidrug-resistance (MDR) proteins develops refractory epilepsy phenotype: clinical and experimental evidences. *Curr Drug Ther* 1, 291-309 (2006)

127. Park, K.: Nanotechnology: What it can do for drug delivery. *J Control Release* 120, 1–3 (2007)

128. United States Pharmacopeia 33. (2010)

129. US Food and Drug Administration. Guidance for Industry: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients. (2005)

130. Bajpai, A. K., and R. Gupta: Magnetically mediated release of ciprofloxacin from polyvinyl alcohol based superparamagnetic nanocomposites. *J Mater Sci Mater Med* 22, 357-369 (2011)

131. Gaihre, B., M. S. Khil, D. R. Lee, and H. Y. Kim: Gelatin-coated magnetic iron oxide nanoparticles as carrier system: Drug loading and in vitro drug release study. *Int J Pharm* 365, 180-189 (2009)

132. Tong, Q., H. Li, W. Li, H. Chen, X. Shu, X. Lu, and G. Wang: In vitro and In vivo Anti-Tumor Effects of Gemcitabine Loaded with a New Drug Delivery System. *J Nanosci Nanotechnol* 11, 3651-3658 (2011)

133. Miller T., S. Breyer, G. van Colen, W. Mier, U. Haberkorn, S. Geissler, S. Voss, M. Weigandt, and A. Goepferich: Premature drug release of polymeric micelles and its effects on tumor targeting. *Int J Pharm* 445, 117-124 (2013)

134. Li, L., Z. Bai, and P. A. Levkin: Boronate-dextran: an acid-responsive biodegradable polymer for drug delivery. *Biomaterials* 34, 8504-8510 (2013)

135. Foldvari, M., and M. Bagonluri: Carbon nanotubes as functional excipients for nanomedicines: I. pharmaceutical properties. *Nanomedicine* 4, 173-182 (2008)

136. Foldvari, M., and M. Bagonluri: Carbon nanotubes as functional excipients for nanomedicines: II. Drug delivery and biocompatibility issues. *Nanomedicine* 4, 183-200 (2008)

137. Koo, O. M., and S. A. Varia: Case studies with new excipients: development, implementation and regulatory approval. *Ther Deliv* 2, 949-956 (2011)

138. Gupta, A. K., and M. Gupta: Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials* 26, 3995-4021 (2005)

139. Kievit, F. M., and M. Zhang: Surface engineering of iron oxide nanoparticles for targeted cancer therapy. *Acc Chem Res* 44, 853-862 (2011)

140. Hilger, I., K. Fruhauf, W. Andra, R. Hiergeist, R. Hergt, and W. A. Kaiser: Heating potential of iron oxides for therapeutic purposes in interventional radiology. *Acad Radiol* 9, 198-202 (2002)

141. Faraji, M., Y. Yamini, and M. Rezaee: Magnetic Nanoparticles: Synthesis, Stabilization, Functionalization, Characterization, and Applications. *J Iran Chem Soc* 7,1-37 (2010)

142. Thorek, D. L., A. K. Chen, J. Czupryna, and A. Tsourkas: Superparamagnetic iron oxide nanoparticle probes for molecular imaging. *Ann Biomed Eng* 34, 23-38 (2006)

143. Huang, W. C., S. H. Hu, K. H. Liu, S. Y. Chen, and D. M. Liu: A flexible drug delivery chip for the magnetically-controlled release of anti-epileptic drugs. *J Control Release* 139, 221-228 (2009)

144. Jain, T. K., M. K. Reddy, M. A. Morales, D. L. Leslie-Pelecky, and V. Labhasetwar: Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats. *Mol Pharm* 5, 316-327 (2008)

145. Muller, K., J. N. Skepper, M. Posfai, R. Trivedi, S. Howarth, C. Corot, E. Lancelot, P. W. Thompson, A. P. Brown, and J.H. Gillard: Effect of ultrasmall superparamagnetic iron oxide nanoparticles (Ferumoxtran-10) on human monocyte-macrophages in vitro. *Biomaterials* 28, 1629-1642 (2007)

146. Hanini, A., A. Schmitt, K. Kacem, F. Chau, S. Ammar, and J. Gavard: Evaluation of iron oxide nanoparticle biocompatibility. *Int J Nanomedicine* 6, 787-794 (2011)

147. Kim, J. S., T. J. Yoon, K. N. Yu, B. G. Kim, S. J. Park, H. W. Kim, K. H. Lee, S. B. Park, J. K. Lee, and M. H. Cho: Toxicity and tissue distribution of magnetic nanoparticles in mice. *Toxicol Sci* 89, 338-347 (2006)

148. Medina, C., M. J. Santos-Martinez, A. Radomski, O. I. Corrigan and M.W. Radomski: Nanoparticles: pharmacological and toxicological significance. *Br J Pharmacol* 150, 552-558 (2007)

149. Nel, A., T. Xia, L. Madler, and N. Li: Toxic potential of materials at the nanolevel. *Science* 311, 622-627 (2006)

150. Wilczewska, A. Z., K. Niemirowicz, K. H. Markiewicz, and H. Car: Nanoparticles as drug delivery systems. *Pharmacol Rep* 64, 1020-1037 (2012)

151. Srinivas A, P. J. Rao, G. Selvam, A. Goparaju, P. B. Murthy, and P.N Reddy: Oxidative stress and inflammatory responses of rat following acute inhalation exposure to iron oxide nanoparticles. *Hum Exp Toxicol* 31, 1113-1131 (2012)

152. Xiong, F., Z. Mi, and N. Gu: Cationic liposomes as gene delivery system: transfection efficiency and new application. *Pharmazie* 66, 158-164 (2011)

153. Lenaz, G.: The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. *IUBMB Life* 52,159-164 (2001)

154. Howden, P. J., and S. P. Faux: Fibre-induced lipid peroxidation leads to DNA adduct formation in Salmonella typhimurium TA104 and rat lung fibroblasts. *Carcinogenesis* 17, 413-419 (1996)

155. Knaapen, A. M., P. J. Borm, C. Albrecht, and R. P. Schins: Inhaled particles and lung cancer. Part A: Mechanisms. Int J *Cancer* 109, 799-809 (2004)

156. Napierska, D., V. Rabolli, L. C. Thomassen, D. Dinsdale, C. Princen, L. Gonzalez, K. L. Poels, M. Kirsch-Volders, D. Lison, J. A. Martens, and P.H. Hoet: Oxidative stress induced by pure and iron-doped amorphous silica nanoparticles in subtoxic conditions. *Chem Res Toxicol* 25, 828-837 (2012)

157. Shi, H., L. G. Hudson, and K.J. Liu: Oxidative stress and apoptosis in metal ion-induced carcinogenesis. *Free Radic Biol Med* 37, 582-593 (2004)

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