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Epilepsy 2019: Epigallocatechin-3-gallate loaded PEGylated-PLGA nanoparticles: A new anti-seizure strategy for temporal lobe epilepsy - Amanda Cano- University of Barcelona

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Temporal lobe epilepsy is the most common type of pharmacoresistant epilepsy in adults. Epigallocatechin-3-gallate has aroused much interest because of its multiple therapeutic effects, but its instability compromises the potential effectiveness. PEGylated-PLGA nanoparticles of Epigallocatechin-3-gallate were designed to protect the drug and to increase the brain delivery. Nanoparticles were prepared by the double emulsion method and cytotoxicity, behavioural, Fluoro-Jade C, Iba1 and GFAP immunohistochemistry studies were carried out to determine their effectiveness. Nanoparticles showed an average size of 169 nm, monodisperse population, negative surface charge, encapsulation efficiency of 95% and sustained release profile. Cytotoxicity assays exhibited that these Nano carriers were non-toxic. Neurotoxicity and immunohistochemistry studies confirmed a decrease in neuronal death and neuroinflammation. In conclusion, Epigallocatechin-3-gallate PEGylated-PLGA nanoparticles could be a suitable strategy for the treatment of temporal lobe epilepsy.

Background:

Epilepsy is a disorder of the central nervous system derived from an imbalance in the electrical activity of neurons, which gives rise to convulsive events. The severity can range from mild attention deficit to intense convulsions, associated with a loss of consciousness. It is estimated that a third of patients do not respond to current treatments, increasing the prevalence of this disease 1% worldwide.

One of the most common forms of epilepsy in adulthood is the temporal lobe epilepsy (TLE). Besides, this type of epileptic disorder presents great differences among patients, having some of them a severe form resistant to antiepileptic drugs (AED). Recently, many studies are focused on finding new treatments for those forms of epilepsy, which do not respond to the available drugs. Nowadays, one of the molecules that are mostly being evaluated for the treatment of many different diseases is the Epigallocatechin-3-gallate (EGCG). EGCG antioxidant activity is the main mechanism responsible for its beneficial health effects.

Oxidative stress is related to neurochemical changes that occur during seizures, due to the large detrimental alterations produced in the neuronal function during spontaneous recurrent seizures and status epilepticus (SE).

A recent study of Kang et al. showed that EGCG decreased cell death processes and the neurotoxic effects produced by oxidative stress in the hippocampal area. Demonstrated that green tea extract treatment in rats ameliorated the state of oxidative stress during SE induced by pilocarpine. Showed that previous treatments with this drug ameliorated the oxidative damage and cognitive deficits derived from pentylenetetrazole-induced seizures in rats. Therefore, all these evidences suggest that EGCG could be an effective treatment for TLE. Although this drug has a potential antiepileptic activity, the main problem of this type of molecules is its instability. This fact significantly reduces the total amount of EGCG in a few hours, decreasing the bioavailability and effectiveness of the drug. For this reason there is an arising need to create a formulation that guarantees the stability and integrity of the EGCG.

In recent years, colloidal systems have been increasingly studied due to its potential for highly targeted drug delivery. These systems act as a vector directing the drug to the site of action, improving its delivery and providing protection from external aggressions. Smith et al. developed a nanolipidic system which improved both the penetration of EGCG through the blood brain barrier (BBB) as well as its oral bioavailability 24. The main problem of these nanosystems is their short physical stability. In another study chitosan nanoparticles (NPs) of EGCG were designed, enhancing its intestinal absorption. However, the positive surface charge may increase the adherence to mucous membranes.

Therefore, the aim of this work is the development of EGCG PEGylated-PLGA NPs to protect the drug from the degradation and to evaluate the effectiveness of this new formulation for the treatment of TLE.

Methods:

EGCG was purchased from Capotchem (Hangzhou, P.R.China) and diblock copolymer PLGA-PEG 5% Resomer® was obtained from Evonik Corporation (Birmingham, USA). Tween® 80, β-glucuronidase (G7396), sulfatase (S-9754) and KA (K-0250, monohydrate) were purchased from Sigma Aldrich (Madrid, Spain). The other reagents were of analytical grade.

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Preparation of EGCG

NPs EGCG NPs were prepared by a modification of the W/O/W double emulsion-solvent evaporation technique described by Freytag et al. 36 . Tween® 80 and ethyl acetate were the selected surfactant and oil phase, respectively. For details, see Supplementary Material.

Results:

Optimization study

The results obtained in the optimization study. Regarding to Zav, the most influential variables were the percentage of Tween® 80 and the amount of polymer, being inversely proportional to the concentration of surfactant, and directly proportional to the concentration of polymer. The analysis of PI showed that the most influential parameters were the EGCG and Tween® 80 concentrations. ZP was greatly influenced by the concentration of surfactant, increasing in absolute value as this variable increase. Higher concentrations of EGCG did not significantly affect EE, suggesting that the developed NPs could be loaded with larger amounts of drug than those studied in this factorial design.

To promote drug release from the polymeric matrix, the highest drug loading formulation and the lowest amount of surfactant able to maintain good characteristics were selected.

Physicochemical and morphological characteristics

The trend of the results was analysed and a final formulation with 1.5 mg/ml of EGCG, 14 mg/ml of PLGA-PEG and Tween® 80 1.5% was selected. These characteristics led to a monodisperse population (PI < 0.1) of particles with a Zav of 168.5 ± 9.9 nm and a EE higher than 95%. This particle size (lower than 200 nm) and the surfactant selected (Tween® 80) improve the penetration of NPs through the BBB 48–50 . Although the PEG layer reduces the surface negative charge of PLGA-NPs 51 , the optimized formulation showed a ZP value (-23.3 ± 5.3 mV) large enough to increase the repulsion forces between the NPs, thereby increasing the stability of the sample. As is shown in Figure 1D, PEG chains were oriented towards the aqueous phase creating a hydrophilic layer surrounding the particles. Furthermore, TEM images revealed that the particles had a smooth surface and spherical shape without signals of aggregation phenomena.

Discussion:

The major finding of the current study is the significant decrease in the number and intensity of the seizure pattern together with a diminution of neuropathological alterations in a mouse model of TLE by the EGCG PEGylated-PLGA NPs pre-treatment. The results evidenced that EGCG possesses an effective anticonvulsant activity which is ameliorated by the developed nanostructured system.

In conclusion, the current study demonstrates that EGCG PEGylated-PLGA NPs significantly improve the anticonvulsive and neuroprotective effect of the free drug, mainly reducing the neuroinflammatory process and seizure threshold. In addition, EGCG NPs are safe for the brain cells and capable of increasing drug integrity and bioavailability. For these reasons we suggest that EGCG-loaded PEGylated PLGA NPs could be a promising, effective and safe strategy for the treatment and prevention of TLE.

References:

1. Cunliffe, V. T.(2016) Building a zebrafish toolkit for investigating the pathobiology of epilepsy and identifying new treatments for epileptic seizures. Journal of Neuroscience Methods 260: 91–95.

2. AlQassmi, A., Burneo, J. G., McLachlan, R. S. and Mirsattari, S. M.(2016) Benign mesial temporal lobe epilepsy: A clinical cohort and literature review. Epilepsy and Behavior 65: 60–64.

3. Burtscher, J. and Schwarzer, C(2017) The Opioid System in Temporal Lobe Epilepsy : Functional Role and Therapeutic Potential. Frontiers in Molecular Neuroscience 10: 1–13.

4. Mirza, N., Sills, G. J., Pirmohamed, M. and Marson, A. G.(2017) Identifying new antiepileptic drugs through genomics-based drug repurposing. Human Molecular Genetics 26, 527–537.

5. Chowdhury, A., Sarkar, J., Chakraborti, T., Pramanik, P. K. and Chakraborti, S.(2016) Protective role of epigallocatechin-3-gallate in health and disease: A perspective. Biomedicine and Pharmacotherapy 78: 50–59.

6. Kim, H. S., Quon, M. J. and Kim, J. a.(2014) New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. Redox Biology 2: 187–195.

7. de la Torre, R., Sola, S., Hernández, G., Farré, M., Pujol, J., Rodríguez, J. et al.(2016) Safety and efficacy of cognitive training plus epigallocatechin-3-gallate in young adults with Down's syndrome (TESDAD): A double-blind, randomised, placebo-controlled, phase 2 trial. The Lancet Neurology 15: 801–810.

8. Singh, B. N., Shankar, S. and Srivastava, R. K.(2011) Green tea catechin, epigallocatechin-3-gallate (EGCG): Mechanisms, perspectives and clinical applications. Biochemical Pharmacology 82, 1807–1821.