PROGETTO

Supervisore::	Michele Simonato
Titolo/*Title*:	A combinatorial gene therapy for focal epilepsy based on coordinated expression of multiple GABA-A receptor subunits
Curriculum:	Neuroscienze e Neurologia Sperimentale/*Neuroscience and Experimental Neurology*
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://research.hsr.it/en/divisions/neuroscience/michele-simonato.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

In spite of the many available antiepileptic drugs (AEDs) and of other therapeutic approaches (surgery, brain stimulation, ketogenic diet, etc.), about one-third of epilepsy patients do not get control of their seizures. The search for conceptually new therapeutic approaches is therefore a priority, and gene therapy is a viable option. In fact, drug-resistant focal epilepsies represent an accessible target for gene therapy, because it may be sufficient to inject the vector directly in the brain parenchyma and express the therapeutic gene(s) in the relatively restricted brain area that generates focal seizures (1-3).

Attempts in this direction have been made with genes that can modify cell (or circuit) function and control hyperexcitability, such as some channels, neurotransmitters, or receptors (4,5). These strategies have shown some but not totally satisfactory success in pre-clinical models.

Rationale and hypothesis

We plan to use a conceptually new combinatorial gene therapy based on lentiviral vectors (LV), in the frame of an innovative chemogenetic approach.

Current chemogenetic approaches use Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), i.e. modified receptors that no longer respond to the endogenous ligand but to an otherwise inert drug (6). A problem with this approach is that the mutant receptor is a potentially immunogenic exogenous protein. In addition, the use of a new drug (the DREADD ligand) requires in depth assessment of its safety. The alternative approach that we propose is to overexpress endogenous receptors that mediate the response to clinically used AEDs, as these receptors will not cause immune reactions.

Objectives and specific aims

We will focus on GABA-A receptors highly sensitive to benzodiazepines and barbiturates, that contain the alpha1, beta3 and gamma2 subunits. We have already designed and tested in vitro and in vivo Lentiviral vectors expressing a combination of these subunits under control of a promoter (CaMKII) that is specifically active in excitatory neurons (7).

Therefore, the specific aims of the project will be

- to complete testing of these vectors in vivo in healthy animals;

- to test these vectors in a relevant model of temporal lobe epilepsy;

In vivo testing will include Immunohistochemistry to label GABA-A subunits and verify their selective expression in excitatory neurons, western blotting to detect expression levels of endogenous and over-expressed subunits, co-immunoprecipitation experiments to verify in vivo assembly of subunits .

Effects in rodent epilepsy models will be measured by monitoring spontaneous seizures by video-EEG telemetry. We will also explore anxiety (using the open field) and memory (Barnes maze tests).

Expected outcomes

These vectors are expected to increase endogenous GABA-mediated inhibition on excitatory neurons, and to increase their responsiveness to benzodiazepines and/or barbiturates in the injected epileptogenic animals, implicating a reduction in doses needed to obtain the desired effect and, thus, reduced drug concentrations in the brain, i.e., reduced side effects .

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

- Technical skills: the PhD student will learn molecular biology techniques; primary cell culture; engineering and production of LV vectors; micro-surgery; immunohistochemistry; confocal microscopy.
- Analysis & Problem-Solving skills: learn to identify problems and their possible causes; think independently; design experiments; identify goals to be accomplished in a realistic timeline.
- Interpersonal & Leadership Skills: learn to conduct group discussions; present data at scientific meetings; communicate ideas effectively; teach skills or concepts to undergraduate students.

Bibliografia (max. 15)

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12. Bankstahl M, Bankstahl JP, Löscher W. Inter-individual variation in the anticonvulsant effect of phenobarbital in the pilocarpine rat model of temporal lobe epilepsy. Exp Neurol. 2012 Mar;234(1):70-84.