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# Network pharmacology for antiepileptogenesis: Tolerability of multitargeted drug combinations in nonepileptic vs. post-status epilepticus mice

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ABSTRACT

Prevention of symptomatic epilepsy ("antiepileptogenesis") in patients at risk is a major unmet clinical need. Several drugs underwent clinical trials for epilepsy prevention, but none of the drugs tested was effective. Similarly, most previous preclinical attempts to develop antiepileptogenic strategies failed. In the majority of studies, drugs were given as monotherapy. However, epilepsy is a complex network phenomenon, so that it is unlikely that a single drug can halt epileptogenesis. We recently proposed multitargeted approaches ("network pharmacology") to interfere with epileptogenesis. One strategy, which, if effective, would allow a relatively rapid translation into the clinic, is developing novel combinations of clinically used drugs with diverse mechanisms that are potentially relevant for antiepileptogenesis. In order to test this strategy preclinically, we developed an algorithm for testing such drug combinations, which was inspired by the established drug development phases in humans. As a first step of this algorithm, tolerability of four rationally chosen, repeatedly administered drug combinations was evaluated by a large test battery in mice: A, levetiracetam and phenobarbital; B, valproate, losartan, and memantine; C, levetiracetam and topiramate; and D, levetiracetam, parecoxib, and anakinra. As in clinical trials, tolerability was separately evaluated before starting efficacy experiments to identify any adverse effects of the combinations that may critically limit the successful translation of preclinical findings to the clinic. Except combination B, all drug cocktails were relatively well tolerated. Based on previous studies, we expected that tolerability would be lower in the latent and chronic phases following status epilepticus in mice, but, except combinations C and D, no significant differences were determined between nonepileptic and post-status epilepticus animals. As a next step, the rationally chosen drug combinations will be evaluated for antiepileptogenic activity in mouse and rat models of symptomatic epilepsy.


## 1. Introduction

Epilepsy is one of the most common neurological diseases and often (~40%) a consequence of brain insults, such as traumatic brain injury or stroke (Löscher et al., 2013). It is widely believed that there is a seizure-free, pre-epileptic state, termed the "latent period", between brain injury and the onset of epilepsy, during which a cascade of complex brain alterations gradually mediates the process of "epileptogenesis" (Pitkänen and Engel, 2014). This latent period may offer a therapeutic window to interfere with epileptogenesis, resulting in modification or even prevention of epilepsy (Löscher and Brandt, 2010; Pitkänen and Lukasiuk, 2011). However, most previous attempts to develop antiepileptogenic strategies failed (Löscher and Brandt, 2010; Pitkänen and Lukasiuk, 2011; Löscher et al., 2013).

We recently proposed multitargeted approaches ("network pharmacology") to interfere with epileptogenesis (Löscher et al., 2013; White and Löscher, 2014). One strategy, which, if effective, would allow a relatively rapid translation into the clinic, is developing novel combinations of clinically used drugs with diverse mechanisms that are potentially relevant for antiepileptogenesis.

*Abbreviations:* AED, antiepileptic drug; AT1, angiotensin II type 1; COX, cyclooxygenase; i.h., intrahippocampal; IL, interleukin; NMDA, N-methyl-D-aspartate; PG, prostaglandin; SE, status epilepticus; SRS, spontaneous recurrent seizures; TGF-β, transforming growth factor beta; TLE, temporal lobe epilepsy.

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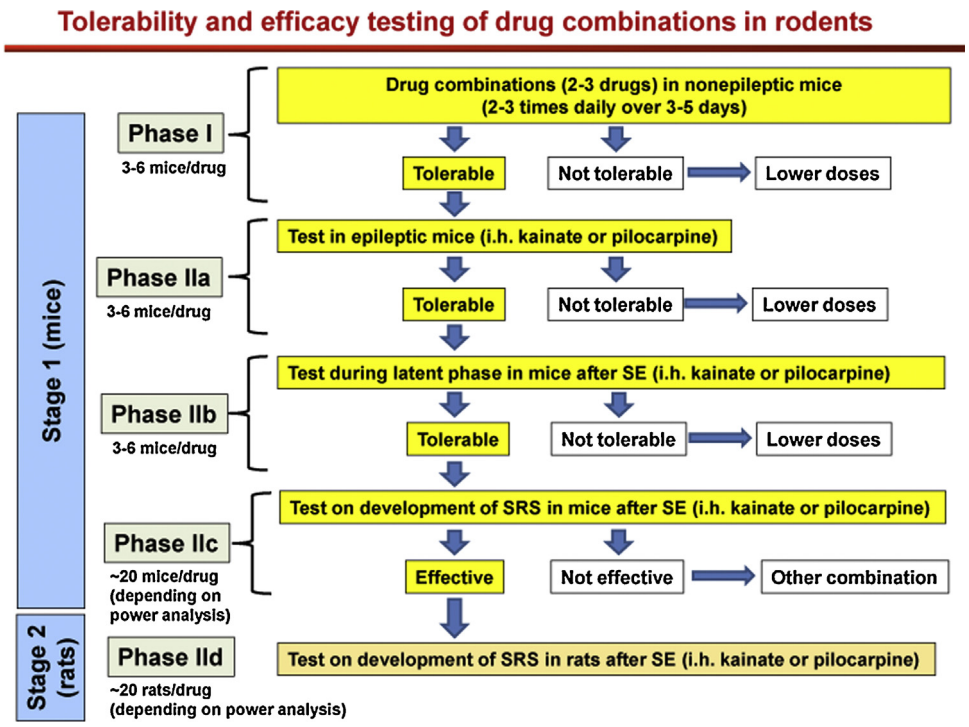


**Fig. 1.** Algorithm for testing drug combinations in a 2-stage approach in mice and rats. Abbreviations: i.h., intrahippocampal; SE, status epilepticus; SRS, spontaneous recurrent seizures.

In order to test this strategy preclinically, we developed an algorithm for testing such drug combinations, which is illustrated in Fig. 1. As a first step of this algorithm, tolerability of the chosen combinations was evaluated in mice. Epileptogenic brain injury may induce an increased drug adverse effect potential (Löscher and Schmidt, 1994), so that we tested all drug combinations in naive animals and animals during the latent and chronic phases following status epilepticus (SE; Fig. 1).

The idea for evaluating the tolerability of drug combinations before testing them for antiepileptogenic activity was inspired by the established clinical drug development phases in which in Phase I a new drug or treatment is administered in a small group of people, usually healthy volunteers, for the first time to evaluate its tolerability and safety, determine a safe dosage range, identify side effects, and evaluate its pharmacokinetics. In Phase II, the drug or treatment is given to a larger group of people who are ill to further evaluate its safety and to see if it is effective. We knew from previous experience with single and combined drug administration that kindled or epileptic animals often exhibit an increased response to drug adverse effects, which may result in interruption of an experimental drug trial or even mortalities (Löscher, 2011). Furthermore, certain adverse effects, i.e., those that impair motor functions, may affect the outcome of drug trials in rodents, but adverse effects are often not precisely evaluated or reported in published studies on antiepileptogenesis, which may form a serious bias when judging the reliability of reported data (Galanopoulou et al., 2012). Because preclinical trials on disease-modifying or antiepileptogenic drug effects are extremely complex and cost- and time-expensive (Löscher and Brandt, 2010), we decided to organize our experiments on the antiepileptogenic potential of rationally chosen drug combinations like the different phases of clinical trials and started with Phase I (pharmacokinetics, tolerability, safety) and the safety or tolerability part of Phase II, termed Phases IIa and IIb in Fig. 1. Phase IIa in epileptic mice was performed before Phase IIb, in which drugs were administered during the latent phase after SE, in order to prescreen tolerability of drug combinations during the chronic phase of epilepsy and thus avoid that intolerable or toxic combinations are administered in the much more laborious experiments during the latent period.

In order to obtain multitargeted drug combinations with a high chance of antiepileptogenic potency (cf., Löscher and Brandt, 2010; Pitkänen and Lukasiuk, 2011; Löscher et al., 2013), three categories of clinically available compounds were used:

- Antiinflammatory drugs: the cyclooxygenase (COX) 2 inhibitor parecoxib and interleukin (IL)-1β antagonists such as anakinra, for which disease-modifying effects in post-status epilepticus (post-SE) rodent models of temporal lobe epilepsy (TLE) have recently been reported (Polascheck et al., 2010; Noé et al., 2013); furthermore, the angiotensin II type 1 (AT1) receptor antagonist, losartan, which has recently been shown to block brain inflammatory transforming growth factor beta (TGF-β) signaling in astrocytes and to prevent epilepsy in the albumin or blood–brain barrier breakdown models of epileptogenesis (Bar-Klein et al., 2014), was included in the experiments;
- Neuroprotective drugs: the N-methyl-D-aspartate (NMDA) receptor antagonists ketamine and memantine, which were reported to reduce or prevent neurodegeneration when administered after SE in rodent models (Löscher and Brandt, 2010);
- Antiepileptic drugs (AEDs) for which disease-modifying effects have been described previously in epilepsy models, although these drugs were individually unable to prevent epilepsy (Löscher and Brandt, 2010): valproate, topiramate, phenobarbital, and levetiracetam. Except topiramate, all these AEDs have been also evaluated previously for antiepileptogenic activity in human trials for prevention of posttraumatic epilepsy (Temkin, 2009; Pearl et al., 2013).

Usually, in clinical trials the pharmacokinetics are also determined in Phase I, but this was not necessary for the drugs tested here, because all of them are clinically approved, so that pharmacokinetic data for both rodents and humans were available from the literature (see Section 3).