

PRELIMINARY COMMUNICATION

EVALUATION OF INTERACTION BETWEEN VALPROATE AND BACLOFEN IN THE FORMALIN TEST IN MICE

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Evaluation of interaction between valproate and baclofen in the formalin test in mice.
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Valproate and baclofen dose-dependently inhibited both phases of the formalin test. Combination of valproate and baclofen exerted the additive antinociceptive effect on both phases of the formalin test.

Key words: *baclofen, valproate, antinociception, formalin test, interaction, mice*

Valproic acid a short, branched-chain fatty acid and its salts, sodium or magnesium valproate (VPA), are effective antiepileptics with a broad spectrum of activity. Although VPA has no effect upon acute thermal nociception, its concomitant administration with morphine showed a potentiation of morphine-induced analgesia in mice [1]. VPA and other antiepileptic drugs are used for the treatment of chronic intractable pain [13]. VPA causes an increased concentration of natural inhibitor, GABA (γ-aminobutyric acid) in the central nervous system (CNS) synapses [4]. Among antiepileptics, only gabapen-

tine (an analog of GABA) was studied in the formalin test [15] and in surgically induced neuropathic pain model [9], the experimental models of facilitated pain processing responsible for allodynia and hyperalgesia in neuropathy. To our knowledge, antinociceptive effect of VPA has never been studied in the formalin test.

Baclofen (BAC) is a derivative of GABA and the prototypic GABA_B agonist [3]. BAC has major clinical use as an antispastic agent, reducing pain associated with spasticity, and it is useful in the treatment of trigeminal neuralgia [8]. Contrary to

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VPA, BAC produces analgesia in a variety of available tests of acute nociception [17]. BAC also produces a dose-dependent antinociceptive effect in the formalin test in mice [15]. Subcutaneous (*sc*) injection of dilute formalin into the mice hind-paw produces biphasic nociceptive response: phase 1 reflects an acute pain response and phase 2 represents the injury-induced spinal sensitization, responsible for facilitated pain processing [6].

Recently, GABAergic mechanism was proposed to explain the antinociception in the formalin test [15]. VPA and BAC possess GABAergic activity and we hypothesized that the interaction between both drugs may have an additive nature. Therefore, the antinociceptive effects of both drugs, given alone or in combination, were studied in the formalin test. Additionally, the effects of both drugs on motor performance were studied in the chimney test.

All animal experiments were conducted on adult female Swiss mice (20–24 g). The mice were pretrained 24 h before behavioral tests and those unable to perform the chimney test were rejected from experimental groups. Each group consisted of 10 animals. Magnesium valproate (VPA, Dipromal Polfa, Rzeszów, Poland) and baclofen (BAC, Baklofen Polfa, Warszawa, Poland), were dissolved in 0.9% saline. Control animals received an equivalent volume of the vehicle. VPA and BAC were administered intraperitoneally (*ip*) 5 and 30 min before formalin and chimney test, respectively.

The formalin test was performed according to Rosland et al. [16]. Formalin (20 μ l of a 5% solution) was injected *sc* into the plantar surface of right hind paw using a 26-gauge needle. Immediately after formalin injection, animals were placed individually in a plexiglas chamber and observed for 30 min. The total time (s) spent licking the injected paw during periods of 0–5 min and 10–30 min after formalin injection were measured as an indicator of nociceptive behavior. For the dose-response analysis, data from phase 1 and phase 2 were considered separately, and mean values \pm SEM were compared. The effective dose producing a 50% reduction of nociceptive response in the control was defined as the inhibitory dose 50 (ID_{50}). The log dose response lines were fitted using least square linear regression and the ID_{50} and 95% confidence limits (CL) for each drug were calculated.

The chimney test of Boissier et al. [2] was used to assess the range of doses of the studied drugs producing motor impairment. The animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm long). Motor impairment was assessed as the inability of mice to climb backwards up the tube within 60 s. The values of ID_{50} with respective 95% CL of inhibiting activities in chimney tests were calculated on a computer by probit analysis [10].

To determine the nature of the drug-drug interaction we applied an isobolographic analysis, conducted according to [19]. VPA and BAC were administered in combination as fixed ratios of the ID_{50} dose for each drug (1:1). The experimental ID_{50} value and 95% CL for drug combination were calculated. The isoboles were drawn by plotting the experimentally determined ID_{50} value of VPA on the x-axis and that of BAC on the y-axis, delivered alone and in combination. The theoretical additive ID_{50} dose was calculated according to [18]. For statistical comparison of the difference between the experimentally derived ID_{50} value and the theoretical additive value, Student's *t*-test was used.

VPA and BAC administered *ip* produced a dose-dependent inhibition in the formalin and chimney tests in mice. The effective doses of both drugs (ID_{50}) in the formalin test were significantly, 3–4 times, lower than in the chimney test (Tab. 1). Both drugs inhibited the phase 1 and 2 of the formalin test at similar doses. Interaction of VPA and BAC in the second phase in the formalin test was clearly additive (Fig. 1).

It has been demonstrated for the first time that VPA possesses inhibitory, dose-dependent effect in the formalin test in mice. Another antiepileptic drug, gabapentine, exerted a similar inhibitory effect in this test [15]. VPA increases GABA concentration in CNS synapses and also exerts direct ef-

Table 1. The inhibitory activity of valproic acid (VPA) and baclofen (BAC) in the formalin and chimney tests in mice

Treatment	The ID_{50} (mg/kg) values (with 95% confidence limits)		
	Formalin test		Chimney test
	phase 1	phase 2	
VPA	102.5 (72.7–145.5)	106.8 (74.3–153.5)	374.9 (339.4–414.0)
BAC	2.3 (1.5–3.5)	2.6 (1.7–4.0)	9.6 (6.4–14.6)

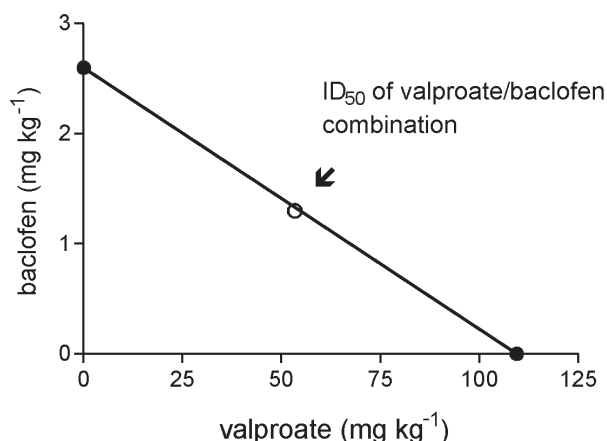


Fig. 1. Isobologram showing the additive interaction of valproate and baclofen in the second phase of the formalin test in mice. ID₅₀ values of each drug and their combination are plotted on the X- and Y-axis. The line connecting ID₅₀ values of each drug is the line of additivity. Values of SEM are not shown for clarity. ID₅₀ value of drugs combination lies exactly on the line of additivity

fect on cellular membranes, by blocking discharge in neurons, or through the excitatory amino acids (EAA) in CNS [7, 11]. The second phase of formalin test is a composite of the ongoing neuronal activity plus the generation of a facilitated state thought to result from the sensitization of the spinal cord (wind-up) [5]. Wind-up phenomenon is mediated, at least partly, by glutamate receptor of N-methyl-aspartate type [5]. VPA may inhibit the second phase of formalin test through the GABA or EAA mechanisms. If fundamentally different mechanism contribute to the observed actions of BAC and VPA, a synergic interaction is considered likely. Therefore, we could not exclude the synergy between VPA and BAC. Previous drug interaction analysis using the formalin test has demonstrated synergy [14] and additivity [12] between several drug combinations, so it is clear that the type of interaction can be determined with this test system. Our present results clearly show that the interaction of VPA and BAC with regard to antinociceptive activity in the second phase of the formalin test is of the additive nature. ID₅₀ value of combination of VPA and BAC lies exactly on the line of additivity. Probably, the same main mechanism (e.g. GABAergic) contributes to their antinociceptive effects in the formalin test. The same additive interactions of VPA and BAC were also observed in the first phase of the formalin test and in the chimney test (results not shown).

The combination of VPA and BAC may well have some clinical value. However, the observed additive effect of VPA and BAC on motor-impairment, may suggest that the side effects of both drugs (e.g. sedation) will not be reduced.

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