Synergistic interaction between acetaminophen and carbamazepine in mice

Interacțiunea de tip sinergic dintre paracetamol (acetaminofen) și carbamazepină la şoarece

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Key words: carbamazepine, synergism, cytokine, acetaminophen
Cuvinte cheie: carbamazepină, sinergism, cytokine, acetaminophen

Abstract

This study aims to demonstrate the type of pharmacodynamic interaction and the demonstration of an antinociceptive action of fixed-ratio binary combinations between a non-opioid analgesic (acetaminophen) and an anticonvulsant (carbamazepine) as a rational way to treat pain. All experimental procedures used in this study were closely in line with international bioethical regulations on experiments conducted on laboratory animals. For quantifying the interaction, we used the method of the additive composite curve, which allows the quantitative assessment of the pharmacodynamic interactions of binary combinations in fixed proportion. The interaction between these substances is the synergistic (potentiation), as evidenced by the left shift of the regression curve of the combination compared to the composite additive curve. The statistical parameters of regression analysis reveal the synergism between the two substances (Zmix = 39.276 ± 7.54 mg / kg (Zmix < Zadd) the interaction index γ = 0.387 , p < 0.05, t_c = 3.753 , t_t = 3.511 , F_c = 10.825 , Ft = 4.260). The synergism between the two substances is explained by the specific action mechanisms of each substance

Rezumat

Studiul de faţă îşi propune investigarea tipului de interacţiune farmacodinamică şi demonstrarea acţiunii antinociceptive a unei combinaţii binare în proporţie fixă între un analgezic non-opoid (Acetaminofen) şi un anticonvulsivant (carbamazepină) ca o modalitate raţională de tratament al durerii. Toate procedeele experimentale utilizate în realizarea acestui studiu au fost în strânsă concordanţă cu reglementările bioetice internaţionale referitoare la experienţele realizate pe animale de laborator. Pentru cuantificarea interacţiunii s-a utilizat metoda dreptei aditive compuse, care permite evaluarea cantitativă a interacţiunilor de tip farmacodinamic din combinaţiile binare în proporţie fixă. Interacţiunea între aceste substanţe este de tip sinergic (potenţare), dovedită prin deplasarea la stânga a dreptei de regresie a asocierii faţă de dreapta aditivă compusă. Parametrii statistici ai analizei de regresie pun în evidenţă sinergismul dintre cele două substanţe (Zmix = 39.276 ± 7.54 mg/kg (Zmix < Zadd), indice de interacţiune γ = 0,387, p < 0.05, tc = 3.753, tt = 3.511, Fc = 10.825, Ft = 4.260). Sinergismul dintre cele două substanţe se explică prin mecanismele de acţiune specifice fiecărei substanţe.

Introduction

Modern analgesic therapy is currently dominated by two major classes of analgesic drugs namely opioid and non-opioid analgesics (analgesic, antipyretic and anti-inflammatory drugs NSAIDs).

In what concerns the analgesic NSAID substances, a host of new improved synthetic variants have been developed, as well as a set of optimized routes of administration, but the conceptual innovations have not been entirely satisfactory. This is probably due to the relatively slow advance in the understanding of the pathogenesis of inflammatory pain.

On the other hand, a principle formulated in the pharmacology of analgesic

combinations, as a reasonable way of improving the treatment of pain, is that associating drugs with different mechanisms of action, we can obtain a multimodal coverage of a broader spectrum of types of pain. Therefore we can create the possibility that the interaction to be greater than additive, i.e. synergistic (potentiation) [14].

In our studies were taken into consideration the role of pro-inflammatory cytokines in pathological pain states [22].

In this paper, we attempt to consider the prospects of associations (combinations) between drugs that influence pain in inflammatory processes

1. Materials and Methods

The research we used Swiss male mice (Source Cantacuzino Institute, Bucharest) weighing 20-25 g. Habitation conditions were established within the laboratory of experimental pharmacodynamics of the department of Pharmacodynamics and Clinical Pharmacy, University "Grigore T. Popa" Iaşi, in a room with controlled temperature and humidity (21°C ±2°C), and a cycle of light/dark, 12 / 12 hours (07.00AM / 07.00PM).

The animals were housed in plexiglas cages (8-10 individuals per cage) provided with water-drippers. They received standard adfood and water (Băneasa Biobase).

The tests were conducted from 10.00 AM. 3 hours before the test, access to food and water was discontinued.

All experimental procedures used in this study were in close agreement with the specific norms approved by the "Grigore T. Popa" University of Medicine following the bioethical and international regulations relating to experiments conducted on laboratory animals [23].

In this study, the following substances were used:

- acetaminophen (paracetamol) (Sigma),
- carbamazepine (Sigma),
- Zymosan A (Sigma),
- Carboxy-methyl-cellulose-Na (Sigma)
- saline (Zentiva).

Carbamazepine and acetaminophen were administered alone and in combination orally, with doses in a geometric sequence.

We used a model of nociception with chemical stimulus, specific for the inflammatory pain, in this study the abdominal constriction response test (the method of Siegmund et al. 1957, the technique of Koster et al. 1959) [20].

The method consists of the intraperitoneal administration of a suspension of zymosan A at a dose of 40mg/kg to the mouse. The number of abdominal constriction responses is recorded, for a duration of 12 minutes after the administration of irritants [21].

Data interpretation was quantal, characterized by the presence or absence of responses, calculating the maximum possible effect:

% (antinociception) inhibition = (no. non-responder / total No. of animals) x 100 [19].

For quantifying the interaction, we used the method of the additive composite curve, which allows the quantitative assessment of pharmacodynamic interactions of binary combinations in fixed proportion [17, 18].

The method is based on the analysis of the regression curve, the regression curve of the addition compared to the additive composite line for a 50% level of activity.

The interaction index indicates the strength of the synergism. The statistical analysis for all tests statistical significance was considered statistically significant for P values < 0.05 (ANOVA test, t - *test*).

2. Results and Discussions

We administered orally successive doses of acetaminophen (paracetamol) in geometric progression. The dose range was of 50.00-600.00 mg kgbw^{-1}.

Using such an administration pattern, it was possible to obtain a maximum effect of 100% for a dose of 600 mgkgbw^{-1}.

The data allowed determining the ED_{50} of acetaminophen (paracetamol). (Table 1)

We administered orally successive doses of carbamazepine in geometric progression, between 7.5-60.00 mgkgbw^{-1}.

We were able to obtain a maximum effect of 80% for the dose of 60 mg/ kgbw^{-1}.

The data allowed the determination of ED_{50} value of carbamazepine. (Table 1).

Table 1

ED50 for the drugs administered alone

Value	Acetaminophen	Carbamazepine (CBZ)
ED50 (SEM)[1] mgkgbw^{-1} p.o	162.48 (26.24) Y = -2.367 +3.33*X R = 0.948	41.14 (12.20) Y = 0.59 +2.72*X R = 0.976

[1]SEM, Standard error of the mean

The data in Table 1 allow for plotting the regression line for the composite additive curve, necessary for the determination of the Zadd value (Fig. 1).

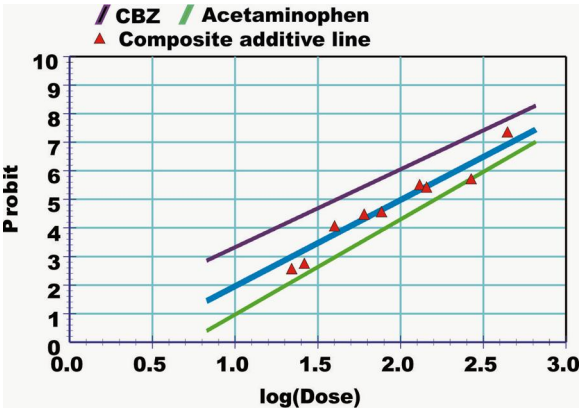

Fig. 1 Analysis of the composite additive line of the combination CBZ-Acetaminophen (paracetamol)

From the analysis of the regression line (Fig 1) and table 1 we can observe that we have been able to demonstrate an antinociceptive effect of carbamazepine (ED_{50} = 41.14 ±12.20 mgkgbw^{-1}) and of acetaminophen (paracetamol) (162.48 ± 26.24 mg/kg) using the nociception model taken in study. After finding those values, we have calculated a value of Zadd = 101.59 ± 14.54 mgkgbw^{-1} and the ratios of the two substances. After administration of the combination in fixed proportion, successive series of doses in geometric progression, we found the following:

Zmix = 39.276 ± 7.54 mg / kg (Zmix < Zadd), an interaction index γ = 0.387, p <0.05 (Table 2). The interaction between these substances is of the synergistic (potentiation) type. This is proven by the left shift of the regression line compared to the composed additive line for the combination (Fig 2).

The statistical parameters of the regression analysis reveal synergism between the two substances (tc = 3.753, tt = 3.511, Fc = 10.825, Ft = 4.260).

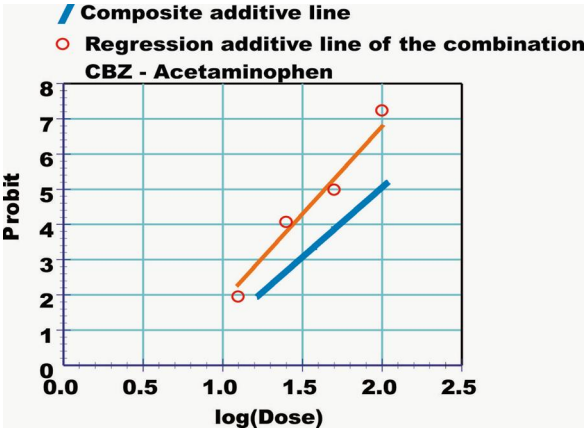

Fig. 2 Regression line for the combination acetaminophen (paracetamol)-CBZ compared to the additive composite line

Table 2

ED50 for the combinations of the drugs taken in the study

Substance	Total dose mgkgbw^{-1}, p.o.	Maximal possible effect (MPE) %	ED50 (SEM)	
			Zadd (SEM)	Zmix (SEM)
Acetaminophene / CBZ (0.798/0.202)	12.20	16.66	101.59 (14.54) Y = 3.02 + 0.57*X R = 0.959	39.276 (7.54)[1] Y = -3.17 + 4.97*X R = 0.953 Interaction index=0.387
	25.40	33.33		
	50.80	66.66		
	101.59	100.00		

[1]Synergistic combination

The abdominal constriction response test allows the assessment of central and peripheral analgesia. The test can use a number of irritating agents such as acetylcholine bromide, acetic acid, Zymosan A. Using the irritating agent Zymosan A the model receives a specificity for the pathogenesis of inflammatory pain [12, 21].

Authors have shown that intraperitoneal administration of zymosan in mice induces an inflammatory response, characterized by the abdominal constriction response, plasma extravasations, leukocyte infiltration and biosynthesis of eicosanoids [Doherty cit. Pettipher [12].

Recently it was revealed that inflammatory agents do not directly stimulate the release of primary hyper nociceptive mediators, but that their release is preceded by a cascade of cytokines [5, 15]

It was revealed that the mouse abdominal constriction response to zymosan and acetic acid is mediated by TNF-α, IL1-β and IL8, which act synergistically and simultaneously [15].

Acetaminophen (paracetamol) has analgesic and antipyretic effects similar to NSAIDs.

However, in contrast, acetaminophen (paracetamol) taken in therapeutic doses has very low or non-existent antiplatelet or anti-inflammatory activity and does not show any side effect like of the NSAIDs, such as gastrointestinal injury or bronchoconstriction induced by acetyl salicylic acid.

Therefore, acetaminophen widely replaced aspirin and other salicylates in the treatment of mild to moderate pain that is not associated with inflammatory processes, such as headaches, toothaches and dysmenorrheal [2].

Although it has been synthesized many years ago and used extensively in the treatment of pain, its mechanism of action is still unknown. The most probable major mechanism however remains the inhibition of cyclooxygenase. Some studies have tested the possible interference between acetaminophen and NOS (nitric oxide synthase) both constitutive and inducible [2], while other studies support the hypothesis of an activating component of the opioid descending spinal pathways [13].

Other studies present actions on the spinal TRPA1, which is considered one of the most important triggers on pain at the spinal level.

Acetaminophen metabolites activate native TRPA1 and reduce voltage-gated calcium and sodium currents in primary sensory neurons, thus inducing an analgesic effect [1].

One of the analgesic mechanisms of acetaminophen is inhibiting the uptake of anandamide and other endocannabinoids from the extracellular space.

The results imply that modulation of the endocannabinoid system in addition to other mechanisms mediate the synergistic antinociceptive effects of acetaminophen combinations [10].

Carbamazepine is a drug with anticonvulsant action, useful in various types of epilepsy, but which is lately used in clinic in certain types of pain, like the trigeminal neuralgia [16].

The action of the drug is explained by their blocking of the Na^+ channels. We know several subtypes denoted by Na_v 1.1 to 1.9. Na_v1.7, 1.8 and 1.9 are expressed only in peripheral sensory neurons and are considered to be involved in both the initiation of the acute pain and in maintaining the post-lesion inflammatory hyperalgesia. Na_v 1.4 channels, 1.6, 1.7 are sensitive to tetrodotoxin (TTX), while Na_v 1.5, 1.8, 1.9, are TTX-resistant [7-9].

The Na_v1.7 subtype is expressed significantly in the dorsal root ganglion neurons (DRG) in the myenteric and sympathetic neurons, while Na_v1.8 is expressed preferentially in DRG and Gasser ganglia. Its implication in inflammatory pain

and cold-induced pain stimulus is well documented [4].

It is known that the $Na_v1.7$ subtype is sensitive to carbamazepine and its mutations cause a number of hereditary pain syndromes [11].

$Na_v1.9$ is expressed in sensory neurons and in the enteric nervous system, especially in the small neurons with nociceptive properties. In light of this knowledge $Na_v1.7$, 1.8, and 1.9 could be important targets for analgesic drugs [7].

Conclusions

The synergism obtained in research presented in this paper can be explained by the specific action mechanisms of the substances taken in study.

Usually, synergistic effects are obtained when two drugs with at least one different action mechanism are combined.

In the case of carbamazepine and acetaminophen (paracetamol), there are several mechanisms at play, which might concur for obtaining such a clear effect:

- carbamazepine, blocks the ionic channels involved in the generation and transport of nociceptive sensations at the level of the spinal nerve and the medulla,
- acetaminophen, might inhibit the local generation of inflammatory mediators by the presumed COX inhibition;
- inhibit the generation of sodium / calcium potentials in primary sensory nociceptive neurons by activating TRPV ;
- modulate the endocannabinoid spinal pathways, reducing pain transmission at the spinal level.

Anyway, acetaminophen (paracetamol) seems to remain a potent analgesic and might enjoy a new period of glory as member in various drug combination with stronger analgesic properties.

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