ORIGINAL ARTICLE

ISOBOLAR ANALYSIS OF THE BINARY FIXED-RATIO COMBINATION OF ACETYLSALICILIC ACID-ACETAMINOPHEN

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Abstract

The present study is part of a series of larger investigations concerning combinations of paracetamol with several other drug substances, as a rational modality of pain therapy. By using a nociception model with chemical stimulus (Zymosan A) and the administration of fixed-ratio dose sequences of paracetamol and acetylsalicylic acid we attempt the identification of the type of pharmacodynamic interaction between those two substances. The evaluation of the nociceptive response is quantal, while for the evaluation of the interaction we have used the isobolar analysis (composite additive line method). The data obtained suggested a synergistic interaction, demonstrated by the following parameters: Zadd = 253.16 ± 33.90 mg/kg body weight (b.w.), Zmix = 75.00 ± 19.02 mg/kg b.w, Zmix < Zadd, $\gamma = 0.296$ (Ft = 4.460, Fc = 14.414, Tt = 3.925, Tc = 4.275 $p < 0.01$).

Rezumat

Studiul de față face parte dintr-o serie de cercetări mai ample care au în vedere asocieri ale acetaminofenului (paracetamolului) cu o serie de substanțe medicamentoase ca o modalitate rațională de terapie a durerii. Prin utilizarea unui model de nocicepție cu stimul chimic (Zymosan A) și administrarea unor secvențe de doze în proporție fixă de paracetamol și acid acetil salicilic se testează în aceste cercetări identificarea tipului de interacțiune farmacodinamică între cele două substanțe. Evaluarea răspunsului nociceptiv este de tip cuantal iar pentru evaluarea interacțiunii s-a utilizat analiza izobolară (metoda dreptei aditive compuse). Datele obținute sugerează o interacțiune de tip sinergic pusă în evidență prin următorii parametri: Zadd = 253,16 ± 33,90 mg/kg corp, Zmix = 75,00 ± 19,02 mg/kg corp, Zmix < Zadd, $\gamma = 0,296$.

Keywords: paracetamol, Zymosan A, acetylsalicylic acid, abdominal constrictive response

Introduction

Nowadays, acetylsalicylic acid (ASA) and paracetamol (acetaminophen) are the two drugs most commonly used for the safe and efficient treatment of pain and fever, to which adds for ASA the platelet anti-aggregating action and the anti-inflammatory effect. Even if paracetamol demonstrates anti-pyretic and analgesic effects which are similar to nonsteroidal anti-inflammatory drugs (NSAIDs), unlike these, when it is administered in therapeutic doses it has a very small or non-existing anti-inflammatory and anti-platelet activity and it does not show a NSAID-type profile of the secondary effects. This is why paracetamol widely replaced ASA and other salicylates in the treatment of light to moderate pain, which are not associated with inflammatory processes, like headaches, dental pain and dysmenorrhea [1].

In order to explain the action of paracetamol on the activity of the cyclooxygenase (COX) there are two main theories: the first suggests that paracetamol inhibits a distinct isoform of the COX (COX-3) [2]; while the second theory suggests that paracetamol does not have any affinity for the active site of COX but it still blocks its activity by reducing the oxidized active form of the cyclooxygenase to an inactive form [3]. Significant efforts have been made for explaining the action mechanism of paracetamol with or without the activation of the supposed COX-3. Even in the conditions in which it really exists, its inhibition by paracetamol is not very specific and is quite reduced, thus not being able to completely solve the mystery through which paracetamol is an analgesic without affecting COX-

1 or COX-2. Most of the studies which promoted the existence of the COX-3 variant were made in an effort to explain the pharmacology of paracetamol [4]. In a revolutionary experiment, Raffa *et al.* [5] demonstrated the auto-synergic effect of paracetamol.

In spite of more than a century of use, the therapeutic use of ASA is still in a state of revolution. Irreversible acetylation, both of COX-1 and of COX-2, with the inhibition of the prostaglandin synthesis is well-known and explains some, but not all, of the actions of ASA [6].

Until not long ago, the effect of ASA on poly-morphonuclear (PMN) recruitment in inflammation was unclear, but published data reported, that ASA triggers the synthesis of a new series of lipid mediators, the lipoxins. These substances co-activate the neutrophils and the cells of the reticulo-endothelial system and inhibit the activation of the cell-mediated mechanisms within the inflammation [7].

The purpose of this paper was to verify the type of pharmacodynamic interaction between ASA and paracetamol by the analysis of fixed-ratio combinations, a rigorous pharmacometric method of analysis.

Materials and Methods

Substances: paracetamol (Paracetamol, Sigma Aldrich, Germany); ASA (Sigma Aldrich, Germany); Zymosan A (Sigma Aldrich, Germany); CMC-Na (Sigma Aldrich, Germany), physiological saline (0.9 %), distilled water.

Animals: We used male Swiss mice, weighting 18 - 22 g, groups of 6, 10 respectively 15 animals which received orally dose sequences in geometric progression of the tests substances (50 - 400 mg/kg b.w. paracetamol, 100 - 600 mg/kg b.w. ASA), and their combination (Table I). 30 minutes after the administration the nociceptive testing was performed. All experimental procedures were made in agreement with international ethics regulations [8], and were approved by our University Ethics Committee.

The animals purchased from the "Cantacuzino" Institute (Bucharest) received food and water *ad libitum*. Three hours before the testing the access to food and water has been discontinued. The work groups were kept in adequate-sized Plexiglas cages with drippers in the laboratory of Experimental Pharmacodynamics, in conditions of controlled temperature (21.00 ± 2.00ºC) and a light/dark cycle of 12 hours, from 7.00 AM to 7.00 PM.

The study groups were set as follows: groups P1 - P4 10 animal/group, treated with paracetamol, groups A1 - A4 15 animals/group, treated with ASA, groups PA1 – PA4 6 animals/group treated with ASA and paracetamol in fixed ratios.

Nociception model: the test of the abdominal constrictive response (writhing test) induced with Zymosan A. The principle of the method relies on the fact that through the intraperitoneal injection of a suspension of Zymosan A, a characteristic response is induced, represented by the stretching followed by the torsion of the body, abdominal retraction and opistotonus. The test allows for the evaluation of the central and peripheral analgesia, and by using Zymosan, the test is more relevant for the pathogenesis of inflammatory pain [9, 10].

In mice, it has been demonstrated the release of pro-inflammatory cytokines like TNF-alfa, IL1-beta and IL8 [11].

The test consists of the intraperitoneal administration of a suspension of Zymosan A, 40 mg/kg b.w. and the number of abdominal constrictive responses was recorded for 12 minutes. The evaluation of the response was of the quantal type, characterized by the presence or the absence of the constrictive response. It was considered as inhibitory effect of the substances taken into study, or their combinations, the inhibition percentage obtained through the absence of the response of the total number of animals tested

$$\%(antinociception) = \frac{\text{number of non} - \text{responders}}{\text{total number of animals}} \times 100$$

Statistical analyses: for evaluating the type of interaction, the isobolar analysis was employed. The values obtained for Zadd, Zmix allowed the calculation of the γ parameter and establishing the type of interaction [12].

Results and Discussion

The results of these investigation allowed the identification of an ED50 value for ASA of (228.25 ± 60.65 mg/kg b.w.), and for paracetamol (161.07 ± 30.31 mg/kg b.w.), for the antinociceptive action. From the analysis of the data, we obtained a Zadd value of 253.16 ± 33.90 mg/kg b.w. Thus, of the ratios p1 = 0.682, p2 = 0.318 and the given dose sequence we obtained a Zmix value of 75.00 ± 19.02 mg/kg b.w. (Table I, Figure 1). We can see that Zmix < Zadd, γ = 0.296. By analysing the position of the regression line of the combination of the two substances compared to the composite additive line (Figure 1), we observed that this is shifted towards the left, which points to a synergistic interaction. The statistical parameters of the regression analysis demonstrate this (Ft = 4.460, Fc = 14.414, Tt = 3.925, Tc = 4.275, $p < 0.01$).

Table I

Determination of Zadd and Zmix values

Combination	Total dose (mg/kg/p.o.)	Maximal possible effect (MPE) (%)	Responder/non-responder	ED50 (SEM)	
				Zadd (SEM)	Zmix (SEM)
Paracetamol/ASA (0.318/0.682)	30.19	16.67	1/6	253.14 (33.90)	75.00[1] (19.02)
	60.38	33.33	2/6		
	126.58	66.67	4/6		
	253.16	83.33	4/6		

[1]Synergistic combination (78.83% antinociception)

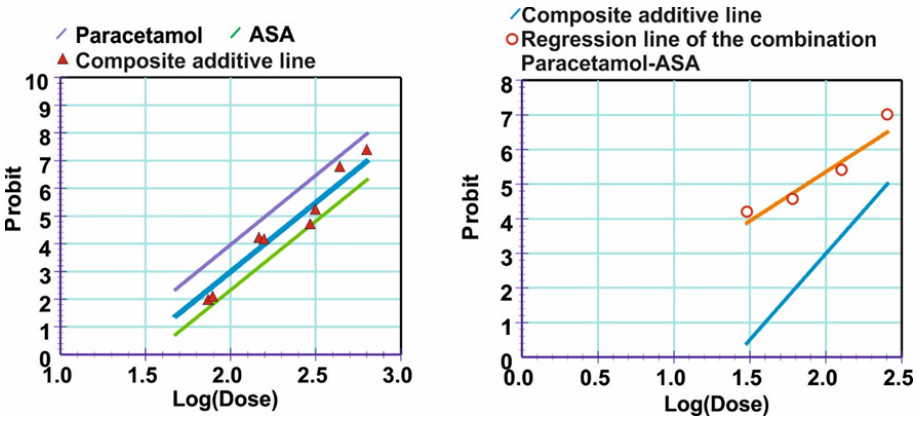

Figure 1.

Analysis of the regression lines of the combination paracetamol - ASA

The results obtained can be explained in the following manner: COX inhibition increases the amount of substrate (arachidonic acid) which is available to the action of other enzymes among which the most commonly found is lipoxygenase. Its action induces the synthesis of HETE (hydroxi-eicosa-tetraenoic) derivatives, with various moieties called commonly eicosanoids. In most of the clinical approaches, ASA is considered to act within the lipid metabolism as a COX inhibitor. However, the acetylated form of COX-2 is still active, due to an unusual L-shaped coupling of the arachidonic acid, in the substrate channel of COX-2, which is higher than in COX-1 [13, 14]. The active site of the COX-2 seems to be more flexible than in COX-1, the presence of an additional coupling pocket in COX-2 being exploited for the production of the COX-2 specific inhibitors [15]. It seems that the new anti-inflammatory pathways using omega3 acids and other substrates for non-COX enzymes in combined administration with ASA, acts at the level of the microinflammation and improve the anti-inflammatory effect of ASA using non-COX pathways or mechanisms that involve acetylated COX-2, which at the level of endothelial cells is the main source of 18-R-HEPE and 15-R-HEPE (hydroxy-eicosa-penta-enoic). Both 18-R and 15-R-HEPE proved to be inhibitors of the trans-endothelial migration of PMN, thus reducing the inflammatory responses and the level of the vascular micro-environment. These new action pathways raised the question if arachidonic acid is the only substrate with physiological relevance for COX-2 in the human tissue or the polyunsaturated fatty acids can be significant substrates for a COX-2 which has been acetylated following the action of ASA and might be responsible for its multitude of beneficial effects, mostly anti-inflammatory and vaso-protective [16, 17].

Conclusions

The classic literature presents the interaction between ASA and paracetamol as being additive. Likewise, in the product nomenclators, throughout the world there are countless preparations which associate various ratios of ASA/paracetamol.

In conclusion ASA is a COX non-selective inhibitor with a special characteristic. Unlike other NSAIDs that use various competitive and steric mechanisms for total or partial, temporary or definitive inactivation of COX, ASA chemically changes COX by the non-selective acetylation of the isozymes. Additionally, it produces a series of lipid metabolites through the acetylated COX-2, metabolites that are not yet well characterized and that may have analgesic effects beside their other effects [18]. Paracetamol acts at medullar and supra-medullar level through previously described mechanisms. The ASA/paracetamol combination is still of great future in the inflammatory pain.

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