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Synergistic antinociceptive effect and gastric safety of the combination of docosahexaenoic acid and indomethacin in rats

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ABSTRACT

The use of analgesics is limited by the presence of significant adverse side effects. Thus, combinations of non-steroidal anti-inflammatory drugs (NSAIDs) with other antinociceptive agents are frequently used to decrease these adverse reactions. The aims of this work were to evaluate the antinociceptive interaction of the systemic administration of the combination of DHA and indomethacin through an isobolographic analysis of the theoretical and experimental antinociceptive effect and to demonstrate the gastric safety of the mixture compared with indomethacin alone. Female Wistar rats were orally administered indomethacin (1–10 mg/kg), DHA (100–300 mg/kg), or the DHA–indomethacin mixture at a fixed-ratio combination (1:1, 1:3, 3:1), and the antinociceptive effects of these treatments were evaluated through the formalin (1%) test. An isobolographic analysis was performed to characterize the antinociceptive interaction between DHA and indomethacin. The degree of gastric injury in all of the rats was determined 1 h after the formalin test. The theoretical ED_{30} values (*Zadd*) for the 1:1, 1:3, and 3:1 combinations were 73.48 ± 8.96, 37.75 ± 4.50, and 109.2 ± 13.43 mg/kg, p.o., respectively, and the experimental ED_{30} values (*Zexp*) were 43.63 ± 5.18, 13.13 ± 1.61, and 54.20 ± 6.53, respectively. The isobolographic analysis showed that the three fixed-ratio combinations studied exhibited a synergistic interaction. Furthermore, the gastric damage induced by indomethacin was abolished when this drug was combined with DHA. These data suggest that the systemic administration of the DHA–indomethacin combination induces a synergistic and gastric safety effect.

1. Introduction

Pain is a multidimensional sensory experience that is intrinsically unpleasant and associated with hurting and soreness (Woolf et al., 2004). Conventionally, non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin or diclofenac, and opioids, such as morphine, are commonly used for pain relief (Fornasari, 2012; Pasternak, 2012). However, the use of these analgesics is limited by the presence of significant adverse side effects; for example, non-selective NSAIDs cause gastric injury or thromboembolic problems (McDaid et al., 2010; Wallace et al., 2000), and opioids are frequently accompanied by side effects such as constipation, sedation, nausea, vomiting, and respiratory depression (McDaid et al., 2010). Several reports show that a combination of drugs that induce similar effects is used for treatment (Tallarida, 2000), thereby allowing the use of lower doses from each agent to improve their therapeutic effect without enhancing their adverse reactions. Recently, the combination of NSAIDs with opioids and other antinociceptive agents has been used in clinical practice; for example, the administration of codeine and diclofenac via a systemic route resulted in a synergistic interaction in rat (Jimenez-Andrade et al., 2003), and the same result was found with gabapentin and diclofenac at the peripheral level (Picazo et al., 2006). In addition, rilmenidine, a second-generation imidazoline-alpha-2-adrenoreceptor agonist, is able to increase the analgesic effects of ibuprofen in mice, as determined through the writhing test (Soukupova et al., 2009). Furthermore, the combination of *Heliopsis longipes* with diclofenac was found to induce a synergistic interaction in a murine model of thermal hyperalgesia (Acosta et al., 2009). Recently, the combination of NSAIDs with natural products is an alternative for the enhancement of the antinociceptive and anti-inflammatory effects without increasing gastric injury. Citral, a monoterpene that occurs naturally in herbs, plants, and citrus fruits, combined with naproxen induces an additive effect with less gastric injury than that induced by naproxen alone (Ortiz et al., 2010).

More recently, our group found that docosahexaenoic acid (DHA) protects against indomethacin-induced gastric injury in rat (Pineda-Pena et al., 2012). DHA is an omega-3 long-chain polyunsaturated fatty acid (PUFA) that is present in fish oil and exhibits several activities,

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such as anti-inflammatory, neuroprotective, and cardioprotective effects (Mayurasakorn et al., 2011). Furthermore, the antinociceptive effect of DHA has been reported in several experimental pain models of thermal and chemical nociception (tail flick test, acetic acid writhing test, and formalin test in mice) (Nakamoto et al., 2010). It is likely that n − 3 PUFAs reduce pain by inhibiting the production of proinflammatory eicosanoids and cytokines (Zaloga and Marik, 2001). Another mechanism involved in the antinociceptive effect of PUFAs involves the binding of fatty acids to several G-protein-coupled cell membrane receptors, such as GPR40 and GPR120 (Calder, 2013). Therefore, based on the antinociceptive and gastroprotective effects of DHA, the aims of this work were to evaluate the antinociceptive interaction of the systemic administration of the combination of DHA and indomethacin through an isobolographic analysis of the theoric and experimental antinociceptive effect and to demonstrate the gastric safety of the mixture compared with the administration of indomethacin alone.

2. Material and methods

2.1. Animals

Female Wistar rats aged 7–9 weeks (weight range: 180–220 g) were obtained from Cinvestav-IPN, México. The sample size per group was five to twelve animals. Efforts were made to minimize animal suffering and to reduce the number of animals used. Each rat was used in only one experiment and sacrificed in a CO_2 chamber at the end of the experiment. All of the experiments followed the Guidelines on Ethical Standards for Investigation using Animals (Zimmermann, 1983), and the protocol was approved by the Institutional Animal Care and Use Committee.

2.2. Drugs

Indomethacin (I7378) and docosahexaenoic acid (DHA; D2534) were purchased from Sigma-Aldrich (Toluca, México). Formaldehyde was purchased from J.T. Baker.

2.3. Formalin-induced nociception test

To minimize stress, the rats were acclimated on the day of the experiment to individual open acrylic observation chambers until explorative behavior was observed (30 min). The pain and antinociception were assessed using the previously described formalin test (De Paz-Campos et al., 2012; Ortiz and Castañeda-Hernández, 2008). Briefly, fifty microliters of diluted formalin (1%) was injected subcutaneously (s.c.) into the plantar surface of the right hind paw, and the incidence of spontaneous flinching behavior was quantified during 1 min every 5 min for a period of 60 min after injection. The data collected between 0 and 10 min post-formalin injection represent the first phase, and the data collected between 15 and 60 min represent the second phase.

Sixty minutes before the formalin insult, the animals were orally administered a vehicle or increasing doses of indomethacin (0.3–10 mg/kg), or 14 h before the formalin insult, the rats received DHA (100–300 mg/kg) or the DHA–indomethacin combination in the respective dosing time (DHA 14 h and indomethacin 1 h before formalin injection) at a fixed ratio-combination of 1:1, 3:1, and 1:3 based on fractions (1/2, 1/4, 1/8, and 1/16) of their effective dose $(ED)_{30}$ values (1:1 combination, 9.18, 18.37, 36.74, and 73.48 mg/kg, p.o.; 3:1 combination, 13.65, 27.30, 54.60, and 109.20 mg/kg, p.o.; 1:3 combination, 4.71, 9.43. 18.87, and 37.75 mg/kg, p.o.). The person performing the experiments was unaware of the treatments that the rats received. The rats in all of the groups were observed for any changes in their behavior or motor function that could have been induced by the treatments. The ability of the animals to stand and walk at a normal posture was assessed but not quantified.

2.4. Gastric damage

One hour after completion of the formalin experiments, all of the rats (regardless of the treatment that they received) were killed in a CO_2 chamber. The stomach was removed and opened along the greater curvature. An observer, who was blind to the experimental treatment status of the animals, measured the area (mm^2) of each gastric lesion. The damaged area was determined by measuring the width and the length of each lesion. For each animal, the area of all of the lesions in the corpus of the stomach was calculated by adding the values and is reported as the gastric lesion area (mm^2) (Pineda-Pena et al., 2012; Wallace et al., 2000).

2.5. Data analysis

The results are presented as the means ± S.E.M. from five to twelve animals per group. The time courses of the antinociceptive responses resulting from the administration of the individual drugs and the drug combination were constructed by plotting the mean number of flinches as a function of time. The areas under the resulting curves (AUC) were calculated using the trapezoidal rule. The AUC was calculated for the two phases of the assay, and the percent of antinociception for each phase was calculated according to the following equation (Ortiz and Castañeda-Hernández, 2008):

$$\text{Percent of antinociception} = \left[\left(AUC_{vehicle} - AUC_{post\ compound}\right)/AUC_{vehicle}\right] \times 100.$$

Dose response curves were constructed using least-squares linear regression, and the antinociceptive ED_{30} ± S.E.M. values for DHA and indomethacin were calculated according to Tallarida (2000). The interaction between DHA and indomethacin was characterized through an isobolographic analysis assuming that the combinations comprised equieffective doses of the individual component drugs. The theoretical additive doses (*Zadd*) and their S.E.M. for each combination in the same component ratio (1:1, 1:3, or 3:1) were computed from the doses resulting in 30% of the effect (ED_{30}) of the single drugs according to the method described by Tallarida (1992) using the following equation:

$$Zadd = fA + (1-f)B,$$

where A is the ED_{30} of DHA, and B is the ED_{30} of indomethacin. For a fixed-ratio of 1:1, the value of f is 0.5, and $(1 - f)$ is also 0.5. The value $fA = a$ represents the fraction of the ED_{30} of DHA in the combination, and $(1 - f)B = b$ represents the fraction of the ED_{30} of indomethacin in the combination (Tallarida, 2000). *Zadd* represents the total additive dose of the drugs, and *Zexp* is the experimentally determined total dose of the mixture of the two component drugs, which were administered

Table 1
Dosing amount of each drug in the combination.

DHA	Indomethacin	Total
Combination 1:1 DHA:indomethacin (mg/kg, p.o.)		
72.46	1.01	73.48
36.23	0.50	36.74
18.11	0.25	18.37
9.05	0.12	9.18
Combination 3:1 DHA:indomethacin (mg/kg, p.o.)		
108.70	0.50	109.20
54.35	0.25	54.60
27.17	0.12	27.30
13.58	0.06	13.65
Combination 1:3 DHA:indomethacin (mg/kg, p.o.)		
36.23	1.51	37.75
18.11	0.75	18.87
9.05	0.37	9.43
4.52	0.18	4.71

at a 1:1, 1:3, or 3:1 fixed-ratio combination. The *Zexp* values (and their 95% confidence limits) were determined from the respective drug-dose effect curves of the drug combinations according to a standard linear regression analysis of the log dose–response curve (Tallarida, 2000), and the 95% confidence limits were subsequently transformed into S.E.M.

To construct the experimental antinociceptive effect–dose curve, each group of rats received one of the drugs at the dose used in Table 1.

2.6. Statistical analysis

All of the data are expressed as the means ± S.E.M. The dose–response data were analyzed by one-way analysis of variance (ANOVA) using the Newman–Keuls test for the post hoc comparisons. The statistical comparisons between the theoretical additive ED_{30} value and the experimentally derived ED_{30} value were performed using Student's *t* test according to procedures previously described by Tallarida et al. (1989), who proposed the use of this statistical test for the analysis of the data included in an isobologram. *Zexp* values that were lower than the *Zadd* value, with differences with $p < 0.05$ in both the X and Y directions, were interpreted as significant *super-additive* interactions. Values of *Zexp* that were higher than *Zadd* values, with differences of $p < 0.05$ in both the X and Y directions, were interpreted as significant *sub-additive* interactions. The absence of a significant difference between the *Zexp* and *Zadd* values was interpreted as no interaction, and an additive relationship (*additivity*) was thus established in the combination (Tallarida, 2000).

Graphical representations of the observed interactions in the shape of isoboles (iso-effect curves or isobologram), which is a simple way to visualize interactions, facilitated the interpretation of the interactions between DHA and indomethacin. The isobologram was constructed by

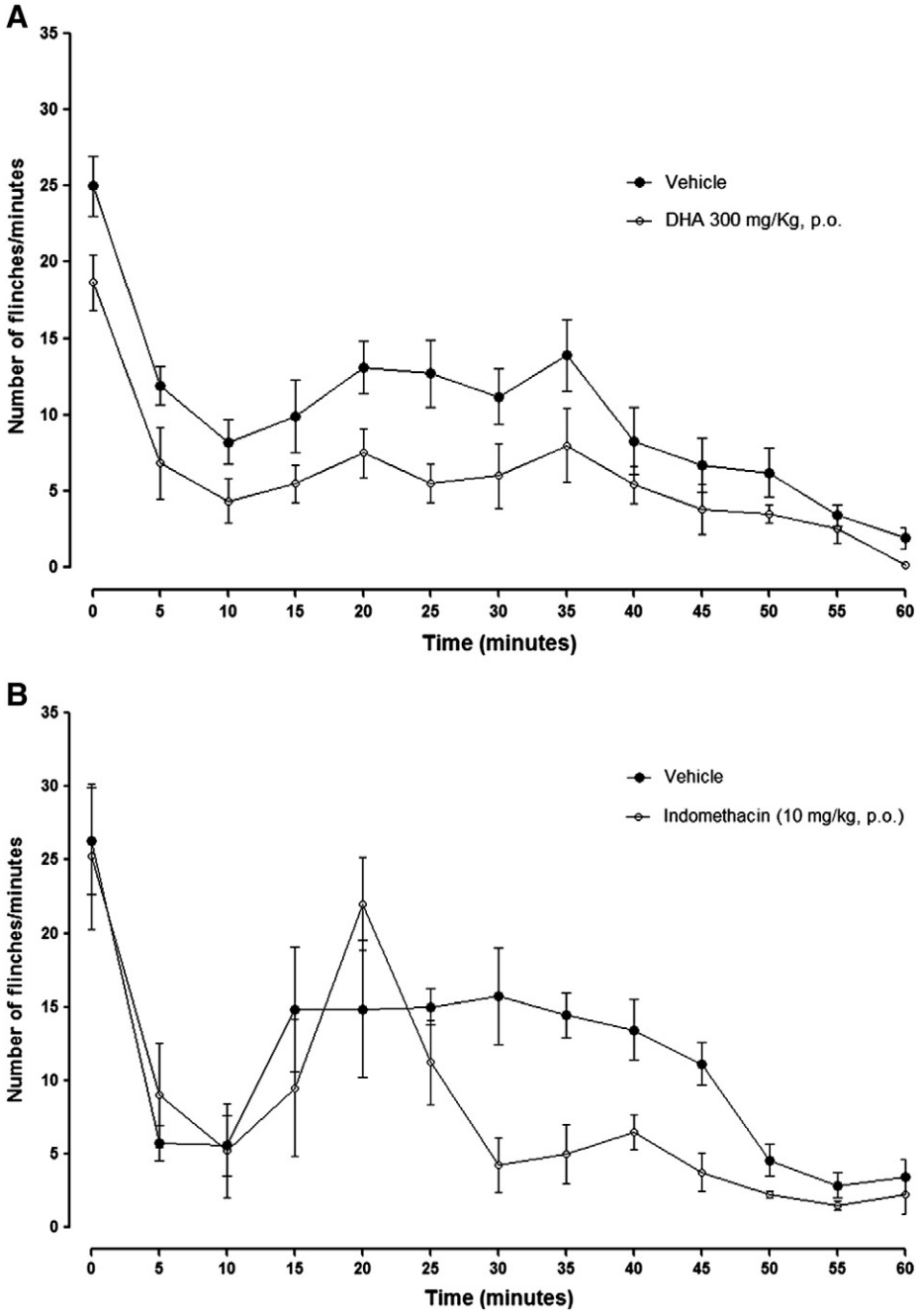

Fig. 1. Time course of the number of flinches per minutes in rats treated with vehicle, (A) DHA (300 mg/kg, p.o.) or (B) indomethacin (10 mg/kg, p.o.) in the 1% formalin test in the paw of the rat. Data are presented as mean ± S.E.M. (n = 5–12).

connecting the ED_{30} of DHA on the abscissa with the ED_{30} of indomethacin on the ordinate to obtain the additivity line (Tallarida, 2000). The amounts of each component in the combination [experimental (*Zexp*) and theoretical additive (*Zadd*) doses] were also plotted in the same graph. The theoretical additive point lies on a line connecting the ED_{30} values of the individual drugs. The experimental values that lie below and to the left of this additive line are considered to be synergistic or super-additive, and the values that lie above and to the right of the line demonstrate an attenuated or sub-additive interaction.

To obtain a value describing the magnitude of the interaction, a fractional analysis was performed for each combination using the ED_{30} values of DHA, indomethacin, and their combination according to the following equation:

$$a/\mathrm{A} + b/\mathrm{B},$$

where A and B are the ED_{30} of each drug (DHA and indomethacin) alone, and *a* and *b* are the ED_{30} values of each drug in the combination. These fractional values measure the divergence between the experimental dose (*Zexp*) of the combination and the theoretical (*Zadd*) additive dose (Tallarida, 2000). A significant difference ($p < 0.05$) from 1 for the relation $a/\mathrm{A} + b/\mathrm{B}$ is interpreted as a *super-additive* interaction if $a/\mathrm{A} + b/\mathrm{B}$ was less than 1.0 and as *sub-additive* interaction if $a/\mathrm{A} + b/\mathrm{B}$ was greater than 1.0, whereas the absence of a significant difference ($p > 0.05$) was interpreted as an additive effect (Tallarida, 2000).

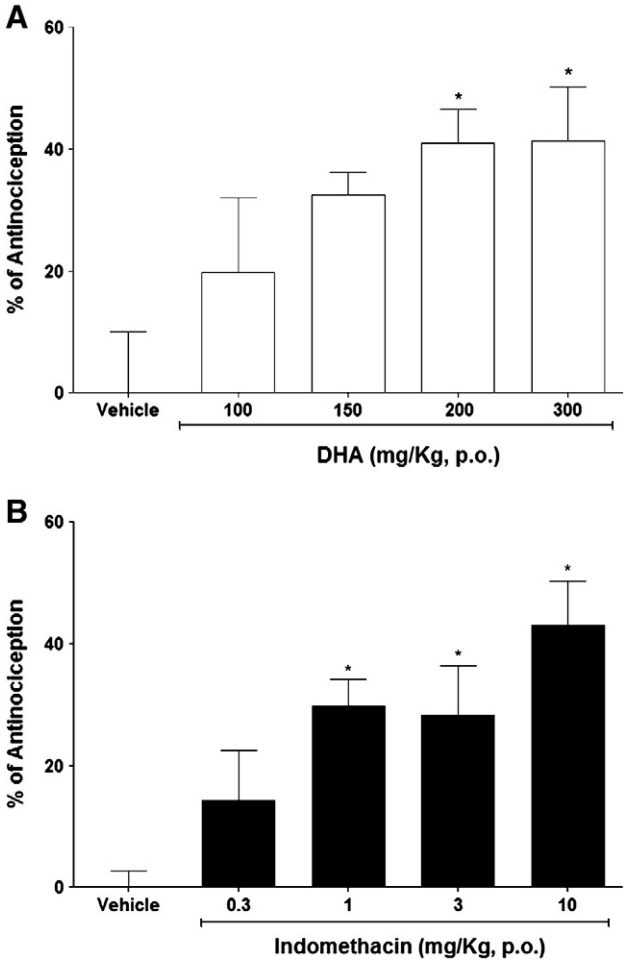

Fig. 2. Dose response curve of the antinociceptive effect of DHA and indomethacin on the second phase in the 1% formalin test in the paw of the rat. (A) Rats were treated with DHA at 100, 150, 200 and 300 mg/kg, p.o. (B) Rats were treated with indomethacin at 0.3, 1, 3 and 10 mg/kg, p.o. Data are presented as mean ± S.E.M. (n = 5–12) $^{*}p \leq 0.05$ vs. respective vehicle (olive oil or sodium bicarbonate).

3. Results

3.1. Systemic antinociceptive effect of DHA and indomethacin

The administration of formalin (1%) into the plantar surface of the right hind paw produced a typical pattern of flinching characterized by a biphasic time course. The initial phase started immediately after administration and then diminished gradually over the next 10 min. The second phase started 15 min after administration and lasted up to 1 h post administration (Fig. 1A and B). The time courses of DHA and

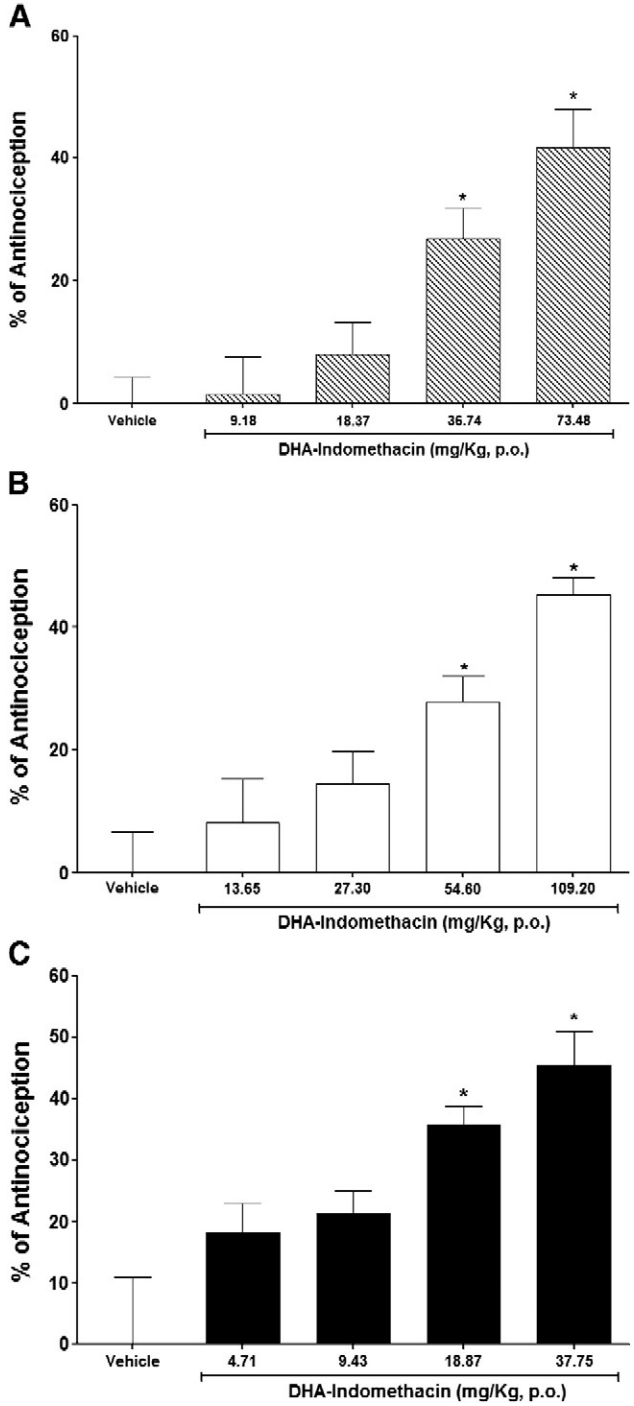

Fig. 3. Dose response curve of the antinociceptive effect of the combination DHA–indomethacin on the second phase in the 1% formalin test in the paw of the rat. (A) Combination 1:1, (B) combination 3:1, and (C) combination 1:3. Data are presented as mean ± S.E.M. (n = 5–12) $^{*}p \leq 0.05$ vs. vehicle.

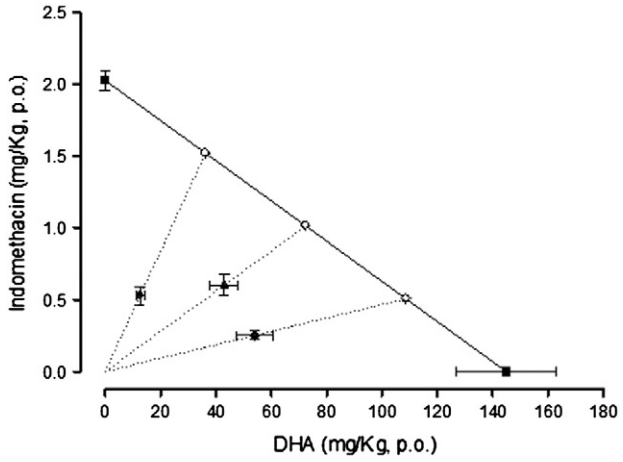

Fig. 4. Isobolograms of the combination DHA–indomethacin on the second phase in the formalin test. The individual ED_{30} values in each combination (■), the theoretical calculated ED_{30} value for an additive effect (Zadd) in a fixed ratio 1:1, 1:3 or 3:1 (○) and its corresponding experimental ED_{30} values in a fixed ratio 1:1 (*Zexp*, ▲), 1:3 (*Zexp*, ●) or 3:1 (*Zexp*, ♦) are represented in the graph. Horizontal and vertical bars indicate S.E.M. The values of *Zexp* were close to *Zadd*, indicating an additive relationship for all the combinations studied.

indomethacin based on the flinching behavior are illustrated in Fig. 1A and B, and these graphs indicate that only DHA alters the nociceptive behavior in the first phase (Table 2), both drugs reduced the flinching behaviors during the second phase. The administration of DHA or indomethacin produced only a dose-dependent antinociceptive effect during the second phase (Fig. 2A and B); how isobolographic analysis is used when both agents are active, only the data from the second phase were subjected to further analysis.

3.2. Antinociceptive interaction of DHA and indomethacin after systemic administration

The administration of DHA and indomethacin at 1:1, 1:3, and 3:1 fixed ratios produced a dose-dependent antinociceptive effect (Fig. 3A, B, and C). The theoretical ED_{30} values (*Zadd*) for the 1:1, 1:3, and 3:1 combinations were 73.48 ± 8.96, 37.75 ± 4.50, and 109.2 ± 13.43 mg/kg, p.o., respectively, and the experimental ED_{30} (*Zexp*) values were 43.63 ± 5.18, 13.13 ± 1.61, and 54.20 ± 6.53, respectively (Fig. 4 and Table 3). The *Zexp* values obtained for the simultaneous administration of DHA and indomethacin were significantly lower ($p < 0.05$) than the respective *Zadd* values. In addition, the fractional analysis of these combinations demonstrated that the relation $a/A + b/B$ was significantly less than 1.0 ($p < 0.05$), indicating a super-additive or synergistic interaction for all of these combinations (Table 3).

Table 2
Percentage of antinociceptive effect in the first phase of the formalin test in the rat.

Drug	Dose (mg/kg, p.o.)	% of antinociceptive effect
Indomethacin	0	0
	0.3	−8.59 ± 8.7
	1	−6.45 ± 20.1
	3	8.60 ± 1.3
	10	5.91 ± 17.7
DHA	0	0
	100	33.05 ± 9.3*
	150	42.12 ± 5.2*
	200	42.82 ± 7.6*
	300	36.08 ± 9.6*

Dose response curve of the antinociceptive effect of DHA and indomethacin on the first phase in the 1% formalin test in the paw of the rat. Data are presented as mean ± S.E.M. (n = 5–12). * $p \leq 0.05$ vs. respective vehicle (olive oil or sodium bicarbonate).

Table 3
Theoretical and experimental ED_{30} values ± S.E.M. for combinations of DHA with indomethacin and magnitude of the interaction values.

DHA:indomethacin combination	Theoretical ED_{30} (*Zadd*)	Experimental ED_{30} (*Zexp*)	Interaction index
1:1	73.48 ± 8.96	43.63 ± 5.18	0.5938*
1:3	37.75 ± 4.50	13.13 ± 1.61	0.3478*
3:1	109.20 ± 13.43	54.20 ± 6.53	0.4963*

* *Super-additive* interaction $a/A + b/B$ was statistically <1.0 ($p < 0.05$).

3.3. Gastric safety

The administration of indomethacin induced gastric hemorrhagic lesions in a dose-dependent manner, DHA administration resulted in zero gastric lesions (Table 4). Gastric injury did not appear in any of the three dose–response curves obtained for the fixed ratios of the DHA–indomethacin combinations studied (Fig. 5; Table 4).

4. Discussion

The current study demonstrates that the systemic administration of indomethacin, DHA, and the combination of DHA and indomethacin produces dose-dependent antinociception, as determined through the formalin test, without gastric injury.

These results demonstrate that the antinociceptive efficacy of DHA, indomethacin, and the DHA–indomethacin combination treatment is consistent with previous reports that showed the antinociceptive effect of oral indomethacin (Gil-Flores et al., 2010; Ortiz et al., 2012) and DHA (Nakamoto et al., 2010; Nakamoto et al., 2012; Tokuyama and Nakamoto, 2011). Because indomethacin is an inhibitor of prostaglandin synthesis (Bingham et al., 2006), it reduces sensitization in the primary afferent neurons and at the spinal cord. Prostaglandins are present in the "inflammatory soup" to sensitize peripheral nociceptors

Table 4
Gastric lesions (mm^2) in the rat.

Drug	Dose (mg/kg, p.o.)		Gastric lesions (mm^2)
Indomethacin	0		0
	0.3		0
	1		20.83 ± 6.8*
	3		35.16 ± 9.2*
	10		53.83 ± 12.4*
DHA	0		0
	100		0
	150		0
	200		0
	300		0
Combination DHA–indomethacin	1:1	0	0
		9.18	0
		18.37	0
		36.74	0
		73.48	0
	1:3	0	0
		4.71	0
		9.43	0
		18.87	0
		37.75	0
	3:1	0	0
		13.65	0
		27.30	0
		54.60	0
		109.20	0

Rats were treated with indomethacin, DHA, or DHA–indomethacin combination in 1:1, 1:3 and 3:1 fixed ratio combination. Data are presented as mean ± S.E.M. n = 5–12.
* $p \leq 0.05$ vs. vehicle.

and enhance the excitability of the nerve fiber in order to increase inflammation and nociception (Basbaum et al., 2009).

Moreover, to the best of our knowledge, this study provides the first demonstration that the systemic administration of the DHA–indomethacin combination has a synergistic effect in the formalin test. Previously, the indomethacin–codeine combination was found to induce an additive but not a synergistic antinociceptive effect (Arredondo-Garza et al., 2007). The systemic administrations of combinations of NSAIDs, such as lumiracoxib with buprenorphine, an opiate, have been shown to result in antinociception in the formalin test (Capuano et al., 2009). In addition, diclofenac, another NSAID, produces a synergistic interaction when it is combined with codeine (Jimenez-Andrade et al., 2003).

The mechanism underlying the DHA–indomethacin interaction remains unknown. However, it was recently elucidated that one of the actions contributing to the antinociceptive mechanism of DHA is not performed directly on the opioid receptor but rather indirectly through the release of an endogenous opioid peptide β-endorphin (Nakamoto et al., 2011; Nishinaka et al., 2013).

Furthermore, indomethacin, an indole acetic acid-derivative NSAID (Polat et al., 2010), shows a clear action as a cyclooxygenase (COX) inhibitor (Summ and Evers, 2013). Recently, protectin DX (PDX), a docosahexaenoic acid di-hydroxylated product, was found to inhibit COXs in vitro (Liu et al., 2013). Thus, if both agents inhibit the same COX pathway, the effects would result in an additive interaction, but the results show that the DHA–indomethacin mixture exhibits a synergistic effect. To determine whether the interaction depends on the amount of each agent, we increased both the proportion of DHA with respect to indomethacin and the proportion of indomethacin with respect to DHA. Both interactions resulted in a synergistic antinociceptive effect because the experimental ED_{30} values were lower than the respective theoretical ED_{30} values.

The antinociceptive effects of the interaction between other NSAIDs and DHA have not been previously evaluated. In addition, clinical placebo-controlled double-bind studies reported that fish oil (rich in eicosapentaenoic acid (EPA) and DHA) used for the treatment of rheumatoid arthritis (RA) reduces the duration of morning stiffness and the number of tender joint pains and decreases the use of NSAIDs (Galarraga et al., 2008; Geusens et al., 1994; Lau et al., 1993). However, these studies did not include an isobolographic analysis to demonstrate whether the interaction is additive or synergistic.

Another mechanism involved in the antinociceptive effect of PUFAs involves the binding of fatty acids to several G-protein-coupled cell membrane receptors, such as GPR40 and GPR120 (Calder, 2013). GPR40 is preferentially expressed on β pancreatic cells, where it mediates insulin secretion (Feng et al., 2012; Zhao et al., 2013). Additionally, the GPR40 receptor is expressed in the olfactory, bulb, striatum, hippocampus, midbrain, hypothalamus, medulla oblongata, cerebellum, and cerebral cortex in the brain as well as in the spinal cord. Thus, it has been hypothesized that DHA can activate a signal to trigger GPR40 in

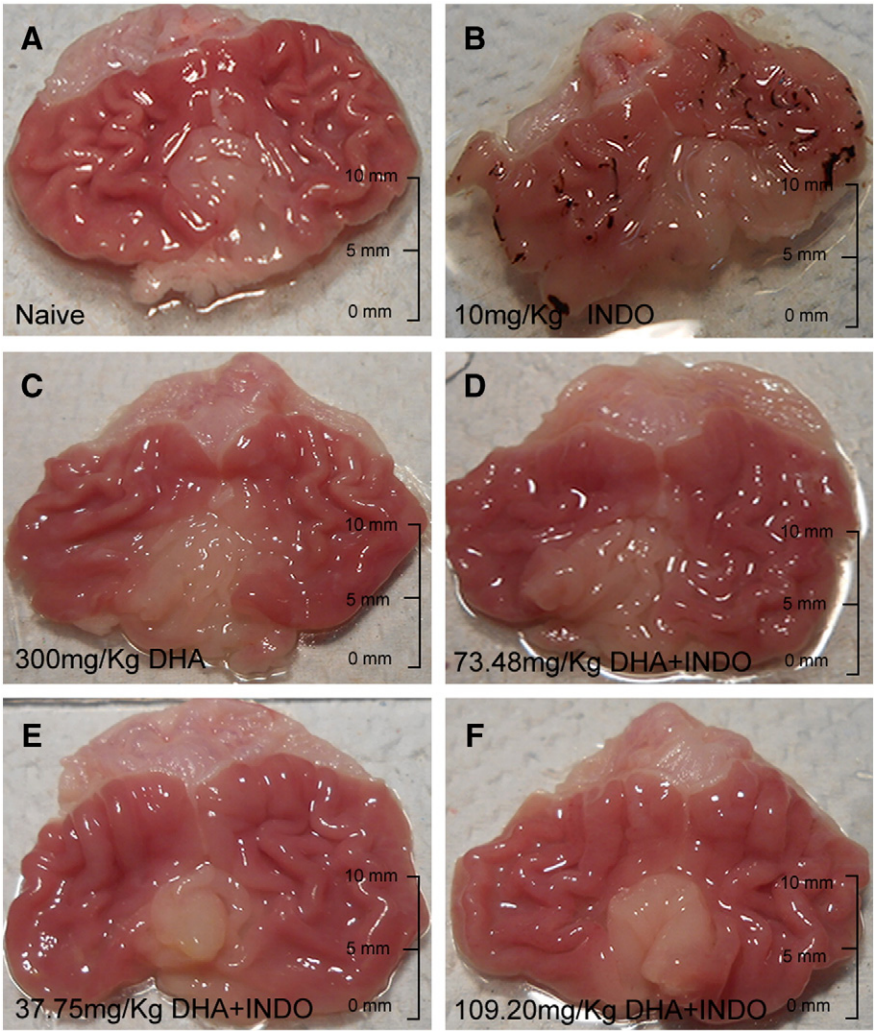

Fig. 5. Representative images of gastric lesions in the corpus of the stomach following different treatments. Naive, (Panel A), indomethacin (Panel B), DHA (Panel C), DHA + indomethacin in a 1:1 (Panel D), 3:1 (Panel E) and 1:3 (Panel F) fixed ratio.

the presynaptic neuron to liberate endorphins (Nishinaka et al., 2013). Furthermore, the inhibition of prostaglandin synthesis by indomethacin may interact with the synthesis of resolvins from series D induced by DHA administration to generate the observed synergistic interaction. Resolvins derived from the E and D series have shown an antinociceptive effect in several pain models, such as chronic pancreatitis-induced pain (Quan-Xin et al., 2012), inflammatory pain (Park et al., 2011; Xu et al., 2010), and arthritis-associated inflammatory pain (Xu and Ji, 2011).

NSAIDs are a group of drugs that are used worldwide for the treatment of pain, inflammation, and fever. However, their use is limited because their chronic consumption is strongly associated with gastric and cardiac adverse effects (Bhala et al., 2013; Rahme et al., 2004). The NSAID-induced gastric damage has been decreased through suppression of gastric acid secretion with proton bomb inhibitor drugs; however, it was recently found that proton bomb inhibitors such as omeprazole and lansoprazole exacerbate NSAID-induced small intestinal damage (Wallace et al., 2011). Thus, there is a medical need to identify new alternatives for the treatment of pain and inflammation that do not induce significant negative side effects.

Of all NSAIDs, indomethacin is the most toxic in the gastric mucosa (Wallace and Vong, 2008). In this study, we demonstrated that the DHA–indomethacin combination is safe at the three dosage combinations studied. Our group previously reported the gastroprotective effect of DHA in indomethacin-induced gastric injury (Pineda-Pena et al., 2012). The doses of indomethacin used in the DHA–indomethacin combination treatments were lower than those used in the indomethacin-induced gastric injury model. Nonetheless, in our study, indomethacin exhibits important gastric lesions at doses of at least 1 mg/kg, p.o.

5. Conclusions

The present study demonstrated that the systemic administration of the DHA–indomethacin combination induces a synergistic antinociceptive and gastric safety effect. Further studies need to be performed to evaluate the clinical efficacy and safety of the combination and to establish the mechanism involved in the interaction of these drugs.

Acknowledgments

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