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Antinociceptive synergism in preclinical studies: A review

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Abstract

In the treatment of acute and chronic pain the most frequently used drugs are nonsteroidal anti-inflammatory drugs (NSAIDs), e.g., paracetamol; opioids, e.g., tramadol, and a group of drugs called coanalgesics or adjuvants (e.g., antidepressants, anticonvulsants). When the administration of an isolated drug produces a minimal analgesic effect, multimodal analgesia is usually used, which consists of the co-administration of two drugs, which can produce an increase in the sum of the effects of each component, generating a synergistic effect or supradditivity or superadditivity. Synergism has been the objective of pharmacology, due to its biomedical orientation, due to its outstanding effect in the therapeutics of pain and mainly cancer. Among the advantages of synergism is that (i) reduction of drug doses and increase in therapeutic effect, (ii) reduction of side effects of each component, (iii) possibility of an increase in the speed of appearance of the effect and prolongation of its action. The exact mechanism of the synergistic interaction has not been exhaustively described, there are only theories of events that can occur at the pharmacokinetic level, due to changes in the concentration of the agents in the site of action or at the pharmacodynamic level, due to changes in the mechanism of action of drugs. This work, was reviewed reports of the preclinical analgesic synergism of NSAIDs with opioids and the mechanisms of action involved in the therapy of these useful analgesic drugs, which may be relevant for pain relief.

Keywords: Opioids; NSAIDs; Pain; Analgesia; Synergism

1 Introduction

The co-administration of 2 drugs can produce three alternatives of effect: (a) a simple sum of the individual effects (additivity); (b) a decrease in the sum of the effects (subadditivity or antagonism or underadditivity) and (c) an increase in the sum of effects (synergism or supraadditivity or superadditivity**).** The synergism has also been denominated as potentiation, augmentation, superadditivity, and supra-additivity [1-3].

There are extensive studies, both theoretical and experimental, of drug interactions that, through the analysis of dose-effect data, make it possible to evaluate the nature of the combinations. Several models have been developed for the detection of synergism. Among them, the graphic quantitative method of Loewe and Muischnek [4], updated by Tallarida [2] as isobolographic analysis. It consists of a Cartesian graph with the component doses linked by an additivity line

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(isobole) on each axis. The experimental doses below this line are synergistic, those above are antagonistic, and those on the line are additive, as shown in Figure 1.

Another method is the Chou-Talalay method, which based on the use of the Michaelis-Menten equation, the Hill equation, the Henderson-Hasselbalch equation, and the Scatchard equation, it was possible to establish a relationship that allows evaluating the magnitude of the interaction between the dose and the effect of the agents in combination. Thus, the combination index (IC = experimental ED_{50} / theoretical ED_{50}) can be calculated, so that if IC <1 corresponds to an index of synergism.

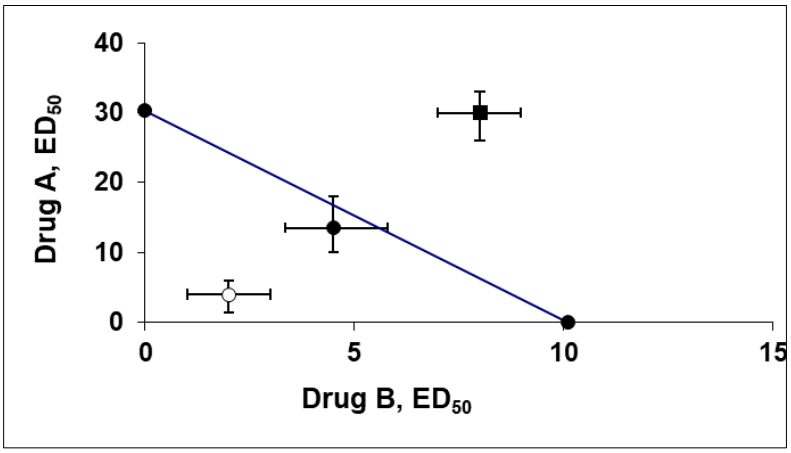

Figure 1 Isobolographic representation of drug interaction. (■) synergism, (●) additivity and (O) antagonism

Other methods are worth mentioning, such as the Response Surface model that creates a three-dimensional surface from the doses of the combined drugs on the X and Y-axis respectively and the Z-axis is the response to a given combination dose. The various experimental responses obtained plotted in relation to Z indicate the nature of the combination. However, this method has been developed with differences in its approach [5,6].

Synergism has been mainly developed in the field of pharmacology, especially with a biomedical orientation, with a marked inclination to the therapeutics of pain and primarily cancer. However, the synergistic methodology has allowed advances in fields such as toxicology, epidemiology. Also, synergistic interactions have been studied in nutrition and food. There are several advantages of synergism and among them, it is worth mentioning that (i) it allows reducing the usual doses of drugs, with an increase in the therapeutic effect (ii) it entails the reduction of the side effects of each synergistic component and that they can even be suppressed (iii) there is the possibility of an increase in the speed of appearance of the effect and jointly the prolongation of its action. Nonetheless, the exact mechanism of the synergistic interaction has not been exhaustively described, there are only theories of events that can occur between the participating drugs of a pharmacokinetic nature, due to changes in the concentration of the agents at the site of action or pharmacodynamics due to changes in the mechanism of action of drugs.

The complex pain process compromises the activation of multiple neural pathways and other processes at both the SNP and CNS levels that are the target of antinociceptive drugs that modulate pain transmission. The combination of analgesic drugs, especially synergistic drugs, by increasing their effectiveness, is a highly fundamental therapeutic alternative. This work reviews the synergistic analgesic combinations of NSAIDs and opioids in their preclinical antinociceptive therapy.

2 NSAIDs synergism

NSAIDs are the most widely used drugs to relieve acute and chronic pain, so their participation is of prime importance in the synergistic association of drugs in the antinociceptive therapy.

The antinociception induced by the intraperitoneal co-administration of paracetamol with diclofenac, ibuprofen, ketoprofen, meloxicam, metamizole, naproxen, nimesulide, parecoxib, or piroxicam was analyzed by isobolographic analysis. All combinations turned out to be synergistic validated by the acetic acid writhing test. These results demonstrate interactions between NSAIDs that validate their clinical use in pain therapy [7].

The administration of dexketoprofen [(S)- (+) enantiomer of ketoprofen] and meloxicam-induced dose-dependent antinociception with different potencies of phase I and phase II in the orofacial formalin test and the interaction between NSAIDs synergistic in both phases determined by isobolographic analysis was synergic. Furthermore, this antinociceptive activity was not modified by the opioid antagonist's naltrexone, naltrindole, and norbinaltorphimine [8].

In this study and by isobolographic analysis in the orofacial test of formalin in mice, it was determined that the interaction between dexketoprofen and dexibuprofen is synergistic in the two phases of the trial. This synergism could be related to the different degrees of inhibition of COXs and other mechanisms that contribute to the efficacy of analgesics [9].

Among advances in chemoprevention evidence has been found to suggest a synergistic interaction of celecoxib, aspirin, and other NSAIDs in the treatment of estrogenic-sensitive breast cancer in different clinical settings, including metastatic therapy, adjuvant therapy, and ductal carcinoma in situ [10].

The study demonstrated that the analgesic interaction of ibuprofen with ketorolac, ketoprofen or paracetamol, ketorolac with ketoprofen or paracetamol, ketoprofen with meloxicam or paracetamol and meloxicam with paracetamol is synergistic. The antinociception was measured in a murine pain model, the tail flick test with an automatic algesimeter, and the pretreatment with L-NAME and NTX did not modify the previous isobolographic parameters of the mixture of NSAIDs [11].

The orofacial formalin test allows discriminating between the effect on antinociception and anti-inflammation. In this study, using this test and by isobolographic analysis, the co-administration of dexketoprofen with diclofenac or with piroxicam or with metamizole was found to be synergistic, with different potency, in phase I (of nociception) and phase II (of inflammation) of the test. The data corroborate the synergistic interaction capacity of NSAIDs and requires for its interpretation an additional complementary mechanism of action to the inhibition of COXs. These findings allow a novel approach in the multimodal management of orofacial pain strategy [12].

All these NSAIDS synergism findings are summarized in Table 1.

3 Opioids synergism

Opioids are widely used drugs in therapy, especially for chronic pain, and their synergistic association constitutes a pole of vital interest for analgesic therapy.

A study evaluated the analgesic effect of intrathecal co-administration of analgesic doses of combinations of MOR / DOR or MOR / KOR and suggests that the co-administration of opioids that act on different receptors may constitute a useful synergism for the treatment of pain [13].

Antinociceptive interactions of combinations of morphine with DOR agonists were examined using the tail flick test and a synergistic antinociceptive effect was obtained. Data support the concept of a functional interaction between MOR and DOR opioid receptors [14-16].

Table 1 Summary of preclinical analgesic synergism of NSAIDs

Assay	drugs	Reference
Acetic acid writhing	paracetamol with diclofenac, ibuprofen, ketoprofen, meloxicam, metamizole, naproxen, nimesulide, parecoxib or piroxicam	7
Orofacial formalin	dexketoprofen with meloxicam	8
Orofacial formalin	dexketoprofen with dexibuprofen	9
Tail flick	ibuprofen with ketorolac, ketoprofen or paracetamol, ketorolac with ketoprofen or paracetamol, ketoprofen with meloxicam or paracetamol meloxicam with paracetamol	11
Orofacial formalin	dexketoprofen with diclofenac, piroxicam or metamizole	12

Supraspinal opioid antinociception is mediated by sites that include ventrolateral periaqueductal gray (PAG), the rostral ventromedial medulla (RVM), the locus coeruleus, and the amygdala. Synergistic antinociceptive interactions were detected after administration of opioid agonists at the mentioned sites [17].

The analgesic synergism of L-methadone with morphine, codeine, and 6-acetyl morphine was reported. Morphine synergizes only with L-methadone. However, L-methadone like morphine showed only additive effects when combined with oxymorphone, oxycodone, fentanyl, alfentanil, or meperidine. These synergies suggest that these drugs have different mechanisms of action [18].

A study evaluates the site-site interaction of the antinociception produced by tramadol by two different routes (subcutaneous and intraperitoneal) using the formalin test. In phase II, of the trial, isobolographic analysis demonstrated a "self-synergism" between the two routes of administration, which is antagonized by naloxone. Tramadol is shown to be an atypical opioid with a complex mechanism of action that includes the synergistic interaction between the parent drug and an active metabolite [19].

Microinjection of fixed-dose combinations of MOR and DOR agonists in an inflammatory pain model (Freund's complete adjuvant) produces a synergistic antinociceptive interaction. [20]. In other work, the interaction produced by the MOR agonist fentanyl and the delta agonist CNS243A was examined in a thermal pain test. The antinociceptive drug interaction analysis turned out to be supraadditive or synergistic in nature, confirming the MOR / DOR synergy [21]. In the following report, the analgesic interaction of DOR agonists with MOR in tail movement pain, using isobolographic analysis, resulted synergistic, both at the synaptic and behavioural levels, dependent on the pathway of phospholipase A2 (PLA2) and cAMP/protein kinase A (PKA) [22].

The antinociceptive combination of intracerebroventricular morphine and fentanyl evaluated in the tail flick trial proved to be synergistic. The increase in efficacy of the combination is directly related to the increase in β-arresting 2 and with the internalization of the MOR opioid receptor in the periaqueductal gray matter and the locus coeruleus [23].

Table 2 Summary of preclinical analgesic synergism of opioids

ASSAY	DRUGS	REFERENCE
Tail flick	morphine with DPDPE, or DELT	14,15
Tail flick	DAMG0 with deltorphin	16
Several tests	L-methadone with morphine, morphine-6β-glucuronide, codeine, and 6-acetylmorphine. Morphine with l-methadone.	18
Freund's, thermal, tail flick	MOR with DOR	20, 21, 22,27
Tail flick	Morphine with fentanyl	23
Acetic acid writhing, tail flick, orofacial facial formalin	codeine with morphine	24
Acetic acid writhing, hot plate	fentanyl with methadone, morphine, or tramadol	25
Hargreaves, von Frey, Complete Freund's adjuvant	Loperamide with oxymorphindole	26

In mice using isobolographic analysis, the type of interaction of codeine and morphine, after intraperitoneal or intrathecal administration, in three nociceptive behavioural models (acetic acid writhing, tail flick and orofacial formalin tests) was synergistic. This synergy may relate to the different pathways of pain transmission and to the different intracellular signal transduction. The findings suggest a possibility of potential clinical advantages in combining opioids in pain management [24].

In a tonic pain model (writhing test) and in a phasic model (hot plate assay), the antinociceptive interaction between fentanyl, methadone, morphine, and tramadol was evaluated. The result of the different combinations was a synergistic i with the exception of methadone/tramadol and fentanyl/tramadol, which were additive. With the use of opioid receptor antagonists the synergism of the morphine combinations was found that it is due to the activation of MOR with

the partial contribution of DOR and KOR; however, the combinations of fentanyl and methadone are due in part to the activation of MOR and DOR. And the lack of involvement of the KOR. The effects of tramadol combinations are due partially to the activation of the opioid subtypes MOR, DOR, and KOR. The results suggest that the efficacy and magnitude of opioid interactions depend of the pain stimulus [25].

The authors demonstrated that loperamide, a peripheral MOR agonist, and oxymorphindole, a central DOR agonist, exhibit synergistic antinociceptive and analgesic action, as measured by isobolography, in the Hargreaves tests for thermal nociception, the von Frey test for mechanical nociception, and Freund's model for inflammatory pain [26].

All these opioids synergism results are summarized in Table 2.

4 NSAIDs plus opioids synergism

The isolated administration of NSAIDs or opioids have marked therapeutic effects, both in terms of antinociception and anti-inflammation. Based on these characteristics, different studies have been designed to know the effects of the association of these agents in synergistic multimodal therapy.

The co-administration of morphine with diclofenac, or ketoprofen, or meloxicam, or metamizole, or paracetamol or with piroxicam induced synergistic antinociception measured by isobolographic analysis in the writhing test. The synergy obtained was partially antagonized by different selective opioid receptor antagonists and dependent on the intracellular signal transduction mechanisms of action of the co-administered agents [27]. Antinociception induced by intrathecal co-administration of morphine with naproxen, piroxicam, metamizole, diclofenac, or ketoprofen was studied by isobolographic analysis in the writhing test. All combinations were synergistic, demonstrating potent interactions between morphine and NSAIDs [28].

The intrathecal co-administration of morphine with nimesulide, meloxicam, and parecoxib, studied using a chemical model of visceral pain, the acetic acid writhing test, and through isobolographic analysis, were synergistic. The findings show that COX-2 inhibitor NSAIDs significantly increase morphine analgesia, suggesting a useful alternative in the treatment of pain accompanied by an opioid-sparing effect with the consequent reduction of adverse effects [29].

The antinociceptive and anti-inflammatory activity of dexketoprofen co-administered with morphine or paracetamol or ketoprofen was evaluated in preclinical pain tests and by isobolographic analysis demonstrated a synergistic interaction. The results allow us to deduce that the synergistic associations of analgesics allow improving the effective pharmacological treatment of pain, minimizing the specific adverse effects of the drugs [30].

The antinociceptive interaction of ketoprofen with a mixture of morphine and paracetamol was evaluated using a model of acute visceral tonic pain, the acetic acid writhing test in mice. The administration of the combination of NSAIDs with morphine turned out to be synergistic, which is not modified by atropine, increased by prazosin, and reduced by naltrexone and tropisetron. The findings of this work report that the descending noradrenergic inhibitory systems participate in the described synergy and serotonergic as well as the opioid route. In conclusion, the synergistic combination of morphine, paracetamol, and ketoprofen with high analgesic efficacy could be of interest for multimodal clinical analgesia [31].

In the present study, by means of isobolographic analysis and using the acetic acid contortion test, the antinociceptive interaction of morphine with diclofenac, ketoprofen, meloxicam, metamizole, naproxen, nimesulide, parecoxib or piroxicam, co-administered by spinal route, was evaluated. The various combinations were found to be synergistic and not modified by naltrindole. The work showed that the synergistic effect is independent of COX-1 or COX-2 inhibitory selectivity. Furthermore, it is suggested that combinations of morphine and NSAIDs activate spinal processing of nociceptive information by mechanisms other than prostaglandin synthesis and modulation of opioid receptors. Therefore, these combinations could be a viable alternative to multimodal analgesia for the clinical management of pain [32].

Other study demonstrated synergistic antinociception of intrathecally administration of morphine with diclofenac, ketoprofen, meloxicam, metamizole, naproxen, nimesulide, parecoxib, or piroxicam. The effect was obtained with very low doses of each drug and was independent of the selectivity of inhibition of COX-1 or COX-2 of each NSAID and was not significantly modified by the administration of naltrexone. These findings suggest that the combination of morphine and NSAIDs has a direct action on spinal nociceptive processing, due to mechanisms unrelated to the activation of opioid receptors and indicate the participation of other complex pain regulatory systems. The usefulness of combinations in multimodal analgesia is proposed [33].

The antinociceptive activity of dexketoprofen was studied in mice using the formalin assay for orofacial pain, coadministered with tramadol, the interaction measured by isobolograms was synergistic only in phase I but antagonistic in phase II. Naltrexone did not change the previous condition, but naltrindole and norbinaltorphimine turn both phases to synergic. These results suggest that the opioid receptors modulate the interaction dexketoprofen with tramadol [34].

In neurons of the raphe magnus nucleus, and through isobolographic analysis, a synergistic interaction between DOR receptors with MOR was determined, dependent on the PLA2 and cAMP / PKA pathways [35].

The following study was undertaken to determine the nature of the interaction, characterized by isobolograms, between codeine and COXs inhibitors, in the visceral pain induced by acetic acid writhing test in mice. The isobolographic analysis displayed that the interactions between codeine and etoricoxib or celecoxib were sub-additive or additive, respectively and combinations of codeine with ibuprofen or paracetamol, which were synergic. The results obtained indicate that opioids increase the efficacy of COX-1 or COX-3 inhibitors and not that of COX-2 inhibitors [36].

In this study the interaction analgesic between dexketoprofen with tramadol in a chronic musculoskeletal pain model in mice, a fairly replicates of chronic osteoarticular pain in humans was evaluated. The isobolographic analysis revealed that the combination was synergistic. The findings suggest that the dexketoprofen with tramadol combination could be useful in the management of acute and chronic inflammatory musculoskeletal pains in humans [37].

All these synergisms between NSAIDs with opioids are summarized in Table 3.

Table 3 Summary of preclinical analgesic synergism of NSAIDs with opioids

Assay	**drugs**	**Reference**
Acetic acid writhing	morphine with diclofenac, or ketoprofen, or meloxicam, or metamizole, or paracetamol or naproxen, or piroxicam, or parecoxib or nimesulide	28, 29,31,33, 35
Acetic acid writhing, tail flick, formalin hind paw	dexketoprofen with morphine or paracetamol	30,32
Orofacial formalin	dexketoprofen with tramadol, in phase I	34
Acetic acid writhing	codeine with ibuprofen or paracetamol	36
von Frey, Complete Freund's adjuvant	dexketoprofen with tramadol	37
Acetic acid writhing	paracetamol with tramadol	38
Acetic acid writhing	tapentadol with ketorolac or diclofenac	39, 39
von Frey	ketorolac with tramadol	41
von Frey, Complete Freund's adjuvant	tramadol with paracetamol	42
Formalin hind paw	Codeine with diclofenac	43

The aim of the next work was to determine the nature of the interaction induced by intraperitoneal or intrathecal coadministration of paracetamol and tramadol and evaluated by means of isobolographic analysis, using the acetic acid writhing test. The isobolographic analysis indicates a synergistic interaction between the coadministration paracetamol with tramadol which was not modified by the non-selective opioid antagonist naltrexone. This association could be of clinical significance in the treatment of pain due to a reduction of doses and adverse effects [38].

In these preclinical studies, the tapentadol-ketorolac and tapentadol with diclofenac combinations were tested in the acetic acid contortion test in mice, the isobolographic analysis, indicates that the systemic co-administration of these drugs is synergistic, indicating a possible opioid modulation in the efficacy of the NSAIDs [39,40]

The pharmacotherapy of neuropathic pain with monotherapy has been associated with limited efficacy and dose-related adverse effects, therefore a combination of drugs could be increasing the analgesia. Thus, in a model of peripheral neuropathy, the association of ketorolac with tramadol exerted a synergistic analgesic activity. The possible mechanism

of this synergistic analgesic effects of opioids and NSAIDs may be related with the opioid inhibition of GABAergic synaptic transmission, which is potentiated by COX inhibitors due to the enzymatic conversion to pain-relieving 12-lipoxygenase products. The results provide a probable alternative for the management of neuropathic pain [41].

The administration of the combination of tramadol with paracetamol exhibits synergistic antinociceptive effects by suppressing neuropathic pain, induced in a surgical model, and inflammatory pain by a CFA pain assay. The synergy of the association was significantly antagonized by the non-selective opioid antagonist naloxone and not modified by the adrenergic antagonist atipamezole, or the serotonin antagonist granisetron. The findings suggest that the synergistic antinociceptive effects of the association tramadol with paracetamol appear to be mediated by the MOR opioid receptor and partially by the accumulation of the active metabolite of tramadol M1 (O-desmethyltramadol), since the contribution of the noradrenaline and serotonin reuptake inhibitor properties of tramadol may be relatively smaller than that of MOR opioid receptor activation [42].

5 Conclusion

This review highlights the synergistic activity of the drugs most used in pain management. Initially, they are used in isolation, but due to the rebelliousness of pain, their association with so-called multimodal analgesia has increased, especially in its synergistic division. This association has spread mainly NSAIDs and opioids because their existence is due to the explanation that this effect lies in very different mechanisms of action ascribed to each of the components of the co-administration. The mechanism by which the antinociceptive and anti-inflammatory activity of NSAIDs exceeds their traditional inhibitory effect on COX-1, COX-2, and COX-3, for which it has been proposed to add different mechanisms at the molecular level to explain the synergizing action, among which the following should be mentioned:

- Inhibition of nuclear factor kappa β, TNF-α, IL-1β, i-NOS, L-selectin, PG-keto reductase, β2-integrin, matrix-metallo-proteinase, lactoferrin, transthyretin.
- Activation of monoaminergic, serotonergic, cholinergic, opioidergic, endocannabinoid systems and others [43-47].

The synergy reported in this review a consequence of the coadministration of opioids demonstrates a complex activation of the MOR, DOR, or KOR opioid receptors, which is accompanied by other several potential molecular mechanisms such as

- The upregulated activities of both the PLA (2)-AA-12 lipoxygenase and cAMP / PKA pathways, and inhibition of GABA.
- Inhibition of IPSCs (inhibitory postsynaptic currents) by β-arrestin 1 and 2 and c-Src (proto-oncogene tyrosine kinase). Inhibition of GABA release by activation of a voltage-gated K+ channel. Inhibition of glutamatergic synaptic transmission.
- Activation of astrocytic MOR with glutamate release at Schaffer Collateral-CA1 synapses.
- Close voltage-gated Ca+2 channel [48-50].

Combination therapy of opioid agonists with NSAIDs makes it possible to increase analgesic efficacy and reduce the adverse effects of both components. This synergistic effect would make it possible to increase the therapeutic profile of the association agents. Consequently, with this modality of multimodal analgesia, a greater investigation of the mechanisms of action of the participants in the association is necessary, mainly at the molecular level, which would allow better pain therapy.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest.

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Author's contributions

All the authors enumerated, have direct and substantial contribution to the work and approved the version submitted for revision.

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