ORIGINAL ARTICLE

Codeine did not increase analgesic efficacy of coxibs in contrast to that of paracetamol or ibuprofen: isobolographic analysis in mice

Martin Janovský, Miloslav Kršiak

Department of Pharmacology, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic

Correspondence to:	Martin Janovský, PharmD. Department of Pharmacology, 3rd Faculty of Medicine, Charles University, Ruská 87, 100 34 Prague 10, Czech Republic. TEL: +420 267102405; FAX: +420 267102461; E-MAIL: m.janovsky@gmail.com

Abstract

OBJECTIVES: There is good evidence that opioids can potentiate analgesic activity of some older non-opioid analgesics (such as paracetamol or ibuprofen) but it is not known whether this also holds true for newer non-opioid analgesics that selectively inhibit cyclooxygenase 2 (coxibs). This study was undertaken to determine the nature of the interaction between codeine and celecoxib or etoricoxib in peritoneal irritation-induced visceral pain in mice. For comparison, interactions of codeine with paracetamol and ibuprofen were also tested using the same method.

MATERIAL AND METHODS: A small volume of a weak acetic acid (0.6%) was injected into the peritoneal cavity and the number of writhes (contractions of abdominal muscles) was counted. All drugs were given orally. Their interaction was characterized using isobolographic analysis.

RESULTS: Codeine, etoricoxib, celecoxib, ibuprofen and paracetamol all independently produced dose-dependent suppression of writhing. The isobolographic analysis carried out using equipotent dose ratios showed that the interactions between codeine and etoricoxib or celecoxib were sub-additive or additive, respectively. This was in contrast to combinations of codeine with ibuprofen or paracetamol, which were supra-additive. Interaction indexes γ, determined as a ratio between experimental and theoretical ED50 values of the mixture, were as follows: 2.7 for codeine + etoricoxib, 0.62 for codeine + celecoxib, 0.43 for codeine + ibuprofen and 0.33 for codeine + paracetamol.

CONCLUSIONS: These and other results suggest that opioids do not seem to potentiate analgesic effects of selective COX-2 inhibitors, in contrast to nonselective COX inhibitors or paracetamol.

Abbreviations:

COX	- cyclooxygenase	NMRI	- Naval Medical Research Institute
ED	- effective dose	SEM	- standard error of the mean
IASP	- International Association for the Study of Pain	VÚFB	- Pharmacy and Biochemistry Research Department

INTRODUCTION

Higher analgesic efficacy of combinations of codeine or other opioids with paracetamol has been reported in a number of clinical studies and several large meta-analysis reviews (Edwards *et al.* 2002; Gaskell *et al.* 2009; Moore *et al.* 1997; Toms *et al.* 2009). Opioids are combined in clinical practice not only with paracetamol, but also with other non-opioid analgesics. It has been reported that combinations of codeine with ibuprofen have analgesic efficacy which is superior to ibuprofen alone in the treatment of postoperative pain and for treatment of arthrosis (McQuay *et al.* 1989; McQuay *et al.* 1992; Quiding *et al.* 1992). However, combinations of opioids with ibuprofen or other nonselective inhibitors of cyclooxygenase 1 and 2 (COX-1 and COX-2), lack the gastrointestinal safety of combinations of opioids with paracetamol.

Gastrointestinal toxicity, which is probably due to COX-1 inhibition (Vane & Botting 1998), has been substantially reduced in selective COX-2 inhibitors (coxibs). Recently, coxibs have been used in the treatment of pain and certain inflammatory disorders. Therefore, it would be logical to combine opioids with selective COX-2 inhibitors in the hope of achieving higher analgesic efficacy without increasing the risk of gastrointestinal toxicity. Since reports on the analgesic efficacy of combinations of opioids with coxibs, in pre-clinical experiments, are limited to a single coxib – rofecoxib (Deciga-Campos *et al.* 2003; Garcia-Hernandez *et al.* 2007; Satyanarayana *et al.* 2004), which has been withdrawn from the market, and since clinical studies are lacking altogether, we have, in the present study, tested interactions between codeine and two commonly used coxibs – celecoxib and etoricoxib. The interactions were evaluated using isobolographic analysis in a model of visceral pain (acetic acid-induced peritoneal irritation) in mice. For a comparison, interactions of codeine with paracetamol and ibuprofen were also tested using the same method.

MATERIAL AND METHODS

Animals

Random bred male NMRI mice (weight 28 to 30 g) obtained from VÚFB Konárovice (Czech Republic) were used in all experiments. The animals were housed under standard laboratory conditions (in a temperature-controlled room ($21 \pm 1\,°C$) with a 12-h light/dark cycle). Animals were fed standard rodent chow (Pelet, St1; VELAZ, Czech Republic) and had free access to water throughout the experiment.

All experiments were reviewed and approved by the Committee for Protection of Laboratory Animals, Third Faculty of Medicine, Charles University, and were concordant with the requirements of the IASP (International Association for the Study of Pain) Committee for Research and Ethical Issues (Zimmermann 1983).

Drugs and chemicals

In all experiments, drugs were administered as oral suspensions. The suspensions were prepared by dispersing the drug in 0.9% saline with addition of Tween 80 (Sigma-Aldrich, Inc., Sokolovská 100/94, Prague, Czech Republic). Control groups received 0.9% saline orally, as a placebo. Paracetamol, ibuprofen and codeine were obtained from Sigma-Aldrich, Inc., as a source of etoricoxib and celecoxib, Arcoxia® tablets (Merck & Co., Inc.) and Celebrex® capsules (Pfizer, Inc.), respectively, were used.

Measurement of analgesic efficacy

An algesiometric test of acute abdominal pain (writhing test) was used in all experiments. An intraperitoneal injection of a weak solution of acetic acid in mice produces writhes, characterized by contraction of the abdominal musculature followed by an extension of the hind limbs. This model of acute visceral pain has been routinely used for many years to evaluate antinociceptive effects of drugs (Collier *et al.* 1968). In the present experiment, mice were injected intraperitoneally with 0.6% acetic acid ($0.1\,ml/10\,g$). The number of writhes per minute was counted at 5-minute intervals, starting 5 minutes after acetic acid or saline administration. The analgesic effect was evaluated as the number of writhes in a 20 minute period and was determined using two experienced observers blinded to the treatment. Codeine (10 to 30 mg/kg), etoricoxib (0.5 to 4 mg/kg), celecoxib (2 to 12 mg/kg), ibuprofen (10 to 90 mg/kg), and paracetamol (150 to 300 mg/kg), or saline were administered orally 15 min before acetic acid injection (n = 6/group).

Isobolographic analysis

The interactions of the non-opioid analgesic agents with a weak opioid, codeine, were evaluated using isobolographic analysis, as described by Tallarida (Tallarida *et al.* 1989). First, the analgesic potencies of the individual drugs were measured and from the log dose-response curves, linear regression lines were constructed and ED_{50} values were determined using least-squares linear regression analysis. Subsequently, a theoretical value for the additive ED_{50} ($ED_{50\ add}$) of mixtures of individual non-opioid analgesics with codeine was obtained from the calculation:

$$ED50_{add} = \frac{ED50_{non\text{-}opioid}}{P1 + (R \times P2)}$$

In this calculation, R is the potency ratio of the non-opioid analgesic relative to codeine, P1 is the proportion of the non-opioid analgesic and P2 the proportion of codeine in the total mixture (Miranda *et al.* 2002). This value lies close to the line of additivity of the isobologram, connecting the ED_{50} value of the non-opioid analgesic plotted on the abscissa with the ED_{50}

of codeine on the ordinate. Combinations of two full agonists with variable relative potencies were used in our study. In this case, the line of additivity, as well as the position of the theoretical additive point on the isobologram, is only approximate. This issue is discussed in detail by Tallarida (Tallarida 2006).

Mixtures of codeine with each non-opioid analgesic were tested in fixed-ratio combinations involving fractions of their respective ED_{50} values, as follows: 1/2, 1/4, 1/8, and 1/16 (n = 9/group). Analgesic potencies of the mixtures were measured and from their log dose-response curves, experimental values of ED_{50} ($ED_{50\ exp}$) were determined. The theoretical and experimental values for the ED_{50} of the mixtures were compared using a modified t-test and a significant difference was considered to be present when the value of "t" was higher than "T", in this test. On the isobologram, when the experimentally determined ED_{50} point of the mixture fell under the line of additivity, the interaction was considered to be synergistic.

Interaction index γ

The value of the interaction index γ allows quantitative characterization of the intensity of the interaction between two drugs. It is determined as the quotient of the experimental and theoretical ED_{50} values of the mixture (Tallarida 2002):

$$\gamma = \frac{ED50_{experimental}}{ED50_{theoretical}}$$

The lower the γ value, the stronger the observed interaction between the two drugs.

Statistics

The Student's t-test was used to evaluate statistical significance and *p*-values less than 0.5 were considered to be significant. Results are presented as corresponding ED_{50} values with standard errors of the mean (SEM). Linear regression lines were plotted using SigmaPlot software, version 8.0 (SPSS, Inc.), isobolographic calculations were performed using PharmToolsPro software, version 1.1.2 (The McCary Group, Inc.), based on the book "Drug Synergism and Dose-Effect Data Analysis" by Tallarida (Tallarida 2000).

RESULTS

Analgesic activity of non-opioid analgesics and codeine in the writhing test

All drugs were administered orally at four doses and exhibited dose-dependent analgesic efficacy. The ED_{50} values obtained from the least-squares linear regression analysis, were 10.5 ± 2.29 mg/kg (codeine), 3.27 ± 1.47 mg/kg (etoricoxib), 11.58 ± 3.68 mg/kg (celecoxib), 58.13 ± 5.32 mg/kg (ibuprofen), and 225.36 ± 1.02 mg/kg

(paracetamol). The linear regressions lines are shown in Figure 1 A–E.

Interactions between non-opioid analgesics and codeine

For the mixture of etoricoxib and codeine, the experimental $ED_{50\ exp}$ value was 18.13 ± 7.5 mg/kg. The value of the theoretical $ED_{50\ add}$ for this combination was determined to be 6.72 ± 1.11 mg/kg. On the isobologram (Figure 2), the experimental combination point lies above the line of additivity and the values of $ED_{50\ add}$ and $ED_{50\ exp}$ are significantly different suggesting that the interaction between etoricoxib and codeine is sub-additive.

The $ED_{50\ exp}$ value of the mixture of celecoxib and codeine determined experimentally from the dose-response curve of their ED_{50} fractions, in the writhing test, was 6.74 ± 0.83 mg/kg. The theoretically determined value of the $ED_{50\ add}$ of the mixture of celecoxib and codeine was 10.87 ± 1.12 mg/kg. Though the experimental point lies under the line of additivity in the isobologram (Figure 3), the difference between the values of $ED_{50\ add}$ and ED_{50exp} is not significant, suggesting an additive interaction between these two drugs.

For the mixture of ibuprofen and codeine, the $ED_{50\ exp}$ value was 14.95 ± 2.62 mg/kg. This value is lower than the theoretical $ED_{50\ add}$ of the mixture, which was 34.60 ± 2.92 mg/kg. On the isobologram (Figure 4), the experimental point lies below the line of additivity and the difference between the values of $ED_{50\ add}$ and $ED_{50\ exp}$ is significant, suggesting that the interaction between ibuprofen and codeine is synergistic.

The $ED_{50\ exp}$ value of the paracetamol-codeine mixture was 38.42 ± 4.40 mg/kg. The value of theoretical $ED_{50\ add}$ for this combination was 117.34 ± 1.26 mg/kg. On the isobologram, the experimental combination point lies under the line of additivity and the difference between the values of $ED_{50\ add}$ and $ED_{50\ exp}$ was statistically significant, suggesting a synergistic interaction between these two drugs. The isobologram of the interaction between paracetamol and codeine is shown in Figure 5.

Interaction index γ

The values of the interaction indexes for the individual combinations decrease as follows:

$\gamma = 2.7\ _{COD+ETO} > \gamma = 0.62\ _{COD+CEL} > \gamma = 0.43\ _{COD+IBU} > \gamma = 0.33\ _{COD+PAR}$

Thus, the strongest synergy was produced by the combination of paracetamol and codeine.

DISCUSSION

The interactions between codeine and etoricoxib or celecoxib were additive or sub-additive, respectively, in the present isobolographic study. This is in contrast to

1A

1B

1C

1D

1E
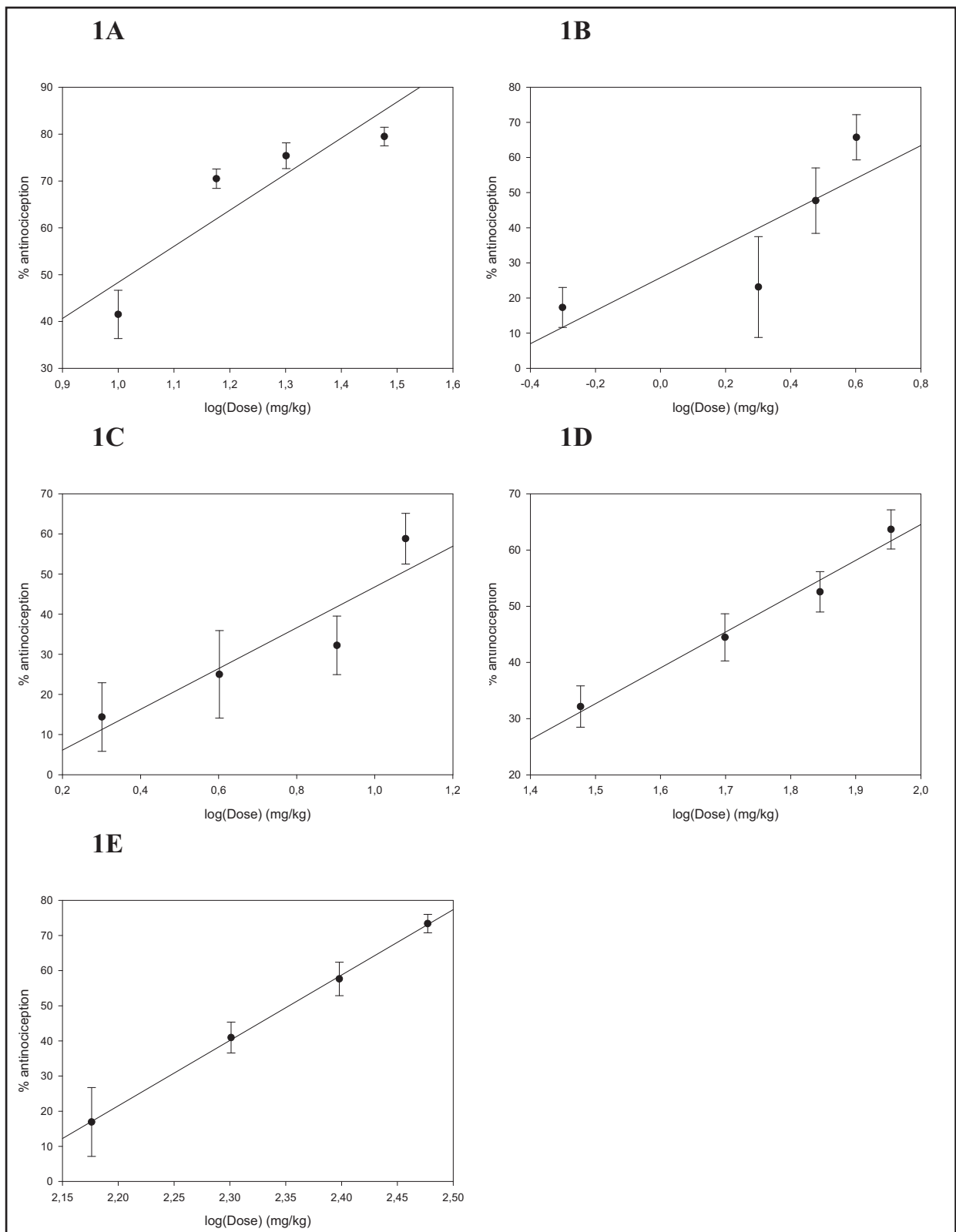
Fig. 1. The ED_{50} values ± SEM were determined from their individual linear regression lines. The dose-response curve of codeine (10.5 ± 2.29 mg/kg) is presented in 1A, etoricoxib (3.27 ± 1.47 mg/kg) in 1B, celecoxib (11.58 ± 3.68 mg/kg) in 1C, ibuprofen (58.13 ± 5.32 mg/kg) in 1D and paracetamol (225.36 ± 1.02 mg/kg) in 1E.
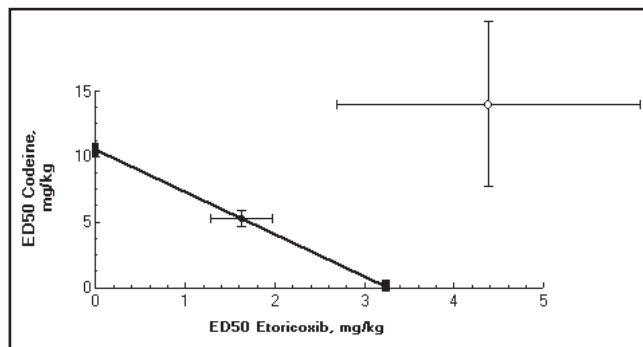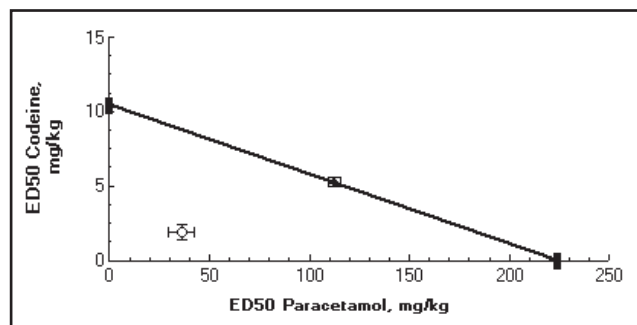
Fig. 2. Isobologram of the etoricoxib+codeine combination. The line of additivity connects the ED_{50} of etoricoxib on the x-axis with the ED_{50} of codeine on the y-axis. Filled circle corresponds to the theoretical $ED_{50\,add} \pm$ SEM and open circle corresponds to the experimental $ED_{50\,exp} \pm$ SEM.
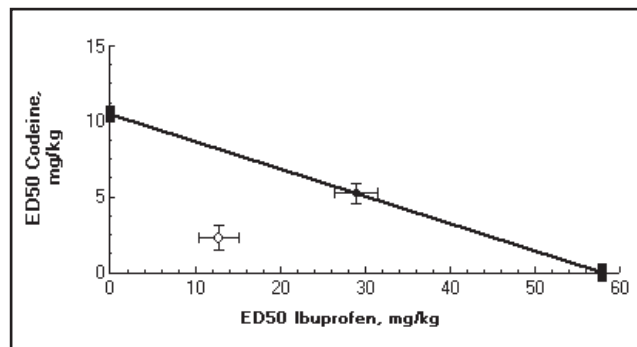
Fig. 4. Isobologram of the ibuprofen+codeine combination. The line of additivity connects the ED_{50} of ibuprofen on the x-axis with the ED_{50} of codeine on the y-axis. Filled circle corresponds to the theoretical $ED_{50\,add} \pm$ SEM and open circle corresponds to the experimental $ED_{50\,exp} \pm$ SEM.
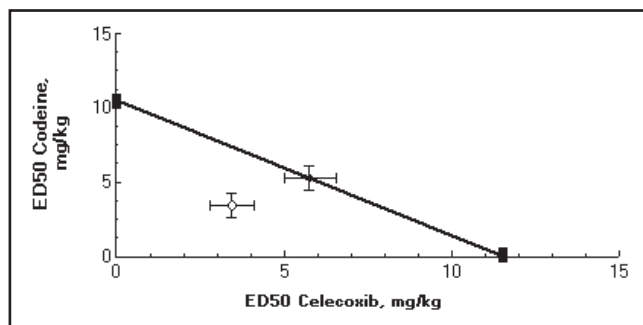
Fig. 3. Isobologram of the celecoxib+codeine combination. The line of additivity connects the ED_{50} of celecoxib on the x-axis with the ED_{50} of codeine on the y-axis. Filled circle corresponds to the theoretical $ED_{50\,add} \pm$ SEM and open circle corresponds to the experimental $ED_{50\,exp} \pm$ SEM.

Fig. 5. Isobologram of the paracetamol+codeine combination. The line of additivity connects the ED_{50} of paracetamol on the x-axis with the ED_{50} of codeine on the y-axis. Filled circle corresponds to the theoretical $ED_{50\,add} \pm$ SEM and open circle corresponds to the experimental $ED_{50\,exp} \pm$ SEM.

the combinations of codeine with paracetamol or ibuprofen, which were supra-additive.

Coxibs have been used in clinical practice for about a decade but we were unable to find any report regarding their routine use in combination with opioids or any clinical evidence suggesting higher efficacy when used in combination with opioids. This is in contrast to combinations of opioids with paracetamol or ibuprofen, which are frequently used clinically, with strong evidence of supra-additivity (Edwards *et al.* 2002; Gaskell *et al.* 2009; McQuay *et al.* 1989; Moore *et al.* 1997; Toms *et al.* 2009).

The present results are in agreement with preclinical experiments testing analgesic efficacy of combinations of opioids with another coxib – rofecoxib, which has now been withdrawn from the market. Only additive or sub-additive antinociceptive effects were found in tramadol / rofecoxib combinations when tested in a rat model of arthritic pain (Garcia-Hernandez *et al.* 2007). Isobolographic analysis in acetic acid-induced writhing, in mice, showed synergistic or supra-additive interactions between naproxen, a noselective COX inhibitor, and tramadol, however, not between rofecoxib and tramadol (Satyanarayana *et al.* 2004). An earlier study

reported that though three combinations of rofecoxib with morphine exhibited potentiation of antinociceptive effects, nine other combinations showed only additive antinociceptive effects in a rat model of arthritis (Deciga-Campos *et al.* 2003).

In summary, the present results and those of other preclinical studies, together with clinical experience suggest that opioids do not seem to potentiate analgesic effects of selective COX-2 inhibitors, which is in contrast to the potentiation seen with nonselective COX inhibitors or paracetamol. This discrepancy may have both theoretical and practical meaning. From a theoretical perspective, it may be important to find the cause of this discrepancy in terms of the mechanism of analgesic action of these drugs. The clinical implication is that, regrettably and unexpectedly, opioids do not seem to potentiate the analgesic effects of selective COX-2 inhibitors.

ACKNOWLEDGEMENTS

School of Medicine, Philadelphia, Pennsylvania), for his help with isobolographic analysis calculations. We also thank Lenka Horáková, Markéta Dvořáková, Blanka Mairychová, and Eva Šulcová for their assistance with experimental elements of this study.

REFERENCES

1 Collier HO, Dinneen LC, Johnson CA, Schneider C (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother. **32**: 295–310.

2 Deciga-Campos M, Lopez UG, Reval MI, Lopez-Munoz FJ (2003). Enhancement of antinociception by co-administration of an opioid drug (morphine) and a preferential cyclooxygenase-2 inhibitor (rofecoxib) in rats. Eur J Pharmacol. **460**: 99–107.

3 Edwards JE, McQuay HJ, Moore RA (2002). Combination analgesic efficacy: individual patient data meta-analysis of single-dose oral tramadol plus acetaminophen in acute postoperative pain. J Pain Symptom Manage. **23**: 121–130.

4 Garcia-Hernandez L, Deciga-Campos M, Guevara-Lopez U, Lopez-Munoz FJ (2007). Co-administration of rofecoxib and tramadol results in additive or sub-additive interaction during arthritic nociception in rat. Pharmacol Biochem Behav. **87**: 331–340.

5 Gaskell H, Derry S, Moore RA, McQuay HJ (2009). Single dose oral oxycodone and oxycodone plus paracetamol (acetaminophen) for acute postoperative pain in adults. Cochrane Database Syst Rev.CD002763.

6 McQuay HJ, Carroll D, Guest P, Juniper RP, Moore RA (1992). A multiple dose comparison of combinations of ibuprofen and codeine and paracetamol, codeine and caffeine after third molar surgery. Anaesthesia. **47**: 672–677.

7 McQuay HJ, Carroll D, Watts PG, Juniper RP, Moore RA (1989). Codeine 20 mg increases pain relief from ibuprofen 400 mg after third molar surgery. A repeat-dosing comparison of ibuprofen and an ibuprofen-codeine combination. Pain. **37**: 7–13.

8 Miranda HF, Sierralta F, Pinardi G (2002). Neostigmine interactions with non steroidal anti-inflammatory drugs. Br J Pharmacol. **135**: 1591–1597.

9 Moore A, Collins S, Carroll D, McQuay H (1997). Paracetamol with and without codeine in acute pain: a quantitative systematic review. Pain. **70**: 193–201.

10 Quiding H, Grimstad J, Rusten K, Stubhaug A, Bremnes J, Breivik H (1992). Ibuprofen plus codeine, ibuprofen, and placebo in a single- and multidose cross-over comparison for coxarthrosis pain. Pain. **50**: 303–307.

11 Satyanarayana PS, Jain NK, Singh A, Kulkarni SK (2004). Isobolographic analysis of interaction between cyclooxygenase inhibitors and tramadol in acetic acid-induced writhing in mice. Prog Neuropsychopharmacol Biol Psychiatry. **28**: 641–649.

12 Tallarida RJ (2000). Drug Synergism and Dose Effect Data Analysis.: Chapman & Hall/CRC.

13 Tallarida RJ (2002). The interaction index: a measure of drug synergism. Pain. **98**: 163–168.

14 Tallarida RJ (2006). An overview of drug combination analysis with isobolograms. J Pharmacol Exp Ther. **319**: 1–7.

15 Tallarida RJ, Porreca F, Cowan A (1989). Statistical analysis of drug-drug and site-site interactions with isobolograms. Life Sci. **45**: 947–961.

16 Toms L, Derry S, Moore RA, McQuay HJ (2009). Single dose oral paracetamol (acetaminophen) with codeine for postoperative pain in adults. Cochrane Database Syst Rev.CD001547.

17 Vane JR, Botting RM (1998). Mechanism of action of nonsteroidal anti-inflammatory drugs. Am J Med. **104**: 2S–8S.

18 Zimmermann M (1983). Ethical guidelines for investigations of experimental pain in conscious animals. Pain. **16**: 109–110.