

## **Diclofenac does not interact with codeine metabolism in vivo: A study in healthy volunteers**

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## **Abstract**

### **Background**

Previously, we have demonstrated a marked inhibition of codeine glucuronidation in human liver tissue homogenate by diclofenac. Therefore, we aimed to investigate whether diclofenac inhibits glucuronidation of codeine also *in vivo* in healthy volunteers.

### **Methods**

In a randomised, placebo-controlled, double-blind, cross-over study, 12 healthy volunteers received a single dose of 100mg codeine phosphate plus 50mg diclofenac sodium or codeine phosphate plus placebo. Serum concentrations of codeine and its metabolites as well as urinary excretion were analysed using mass spectrometry. Side effects were recorded and analgesic efficacy was determined using the cold pressure test.

### **Results**

A single dose of diclofenac did not alter the formation of codeine-6-glucuronide in healthy volunteers. Metabolic clearance of codeine to morphine was not affected by diclofenac. In terms of side effects, both treatments were well tolerated. Diclofenac did not significantly influence the analgesic effects of codeine in the cold pressure test.

### **Conclusions**

In contrast to recent *in vitro* data, a single oral dose of diclofenac did not alter glucuronidation of codeine in healthy volunteers.

## Background

The weak opioid codeine is widely used in the management of pain. Various studies have demonstrated a synergistic analgesic effect of an opioid-NSAID combination[1-4], particularly if repeated doses are given.[5-7] The synergistic effect is thought to be caused by the known different pharmacodynamic mechanisms, opioids acting via opioid receptors in the central nervous system, NSAIDs affecting the synthesis of prostaglandins due to inhibition of the enzyme cyclooxygenase. NSAIDs have also been postulated to display additional antinociceptive effects in the central nervous system.[8] Moreover, a synergistic effect is also possible by pharmacokinetic interactions between both kinds of drugs: NSAIDs may decrease renal excretion of the pharmacologically active metabolite M-6-G (morphine-6-glucuronide) following morphine administration.[9] Previously, we have demonstrated a marked inhibition of codeine glucuronidation in human liver tissue homogenate by diclofenac ( $K_i$  of  $7.9\mu\text{M}$ ).[10] Others have shown [11] a more than 50% inhibition of glucuronidation of the structural analogue dihydrocodeine by  $50\mu\text{M}$  diclofenac in vitro. Codeine is predominantly metabolised by glucuronidation to C-6-G (codeine-6-glucuronide). Minor metabolic pathways include N-demethylation to norcodeine and O-demethylation to morphine.[12,13] The latter depends on the polymorphically expressed CYP2D6.[14-16] There is increasing evidence, that the analgesic effect of codeine is mediated by its O-demethylated metabolite morphine [17,18] and that the glucuronidated metabolite M-6-G possesses even more analgesic potency as morphine itself.[19] In humans, analgesic activity of C-6-G has not been reported; however, antinociceptive responses after intracerebroventricular administration has been reported in rats.[20]

Since *in vitro* findings may not necessarily be of clinical relevance, we aimed to investigate whether diclofenac inhibits glucuronidation of codeine also *in vivo* in healthy volunteers in terms of pharmacokinetics, analgesic efficacy and side effects.

## **Methods**

### **Subjects**

Twelve healthy male volunteers, median age 31 years (range 26-42 years), median weight 77 kg (range 63-95kg), median BMI 23.9kg/m<sup>2</sup> (range 19.9-28.4kg/m<sup>2</sup>) participated in the study. One subject had to be replaced because of additional drug intake during the study.

The study had been approved by the ethics committee of the local medical board (Landesärztekammer Baden Württemberg) according to the Declaration of Helsinki (1996 Sommerset). All volunteers gave their written informed consent prior to study inclusion.

The volunteers were healthy according to history, physical examination and laboratory tests, had no history of drug abuse and did not take any medication. The volunteers had previously been genotyped for CYP2D6 by allele-specific PCR [21,22] and all were predicted as extensive metabolisers (EM). In addition, the subjects were phenotyped with respect to sparteine oxidation polymorphism with a single dose of 100mg sparteine sulphate and were classified according to the metabolic ratio (MR) of sparteine and its 2- and 5-dehydrometabolite.[23] In order to limit the effect of variability of CYP2D6 in regard to metabolic capacity, only volunteers with a MR<1 were included in the study, except one subject showing a MR of 2.8 (intermediate metaboliser).

## **Study design**

The study was designed as a randomised, placebo-controlled, double-blind, cross-over trial. Randomisation was performed using the computer program Sampsize 2.0 (Blackwell Science Ltd., Machin, Campbell, Fayers, Pinol).

Each volunteer received on the study days 1 and 8 in random order either 100mg codeine phosphate + 50mg diclofenac sodium **or** 100mg codeine phosphate + placebo. Codeine phosphate was provided as tablets (Codeinum Phosphoricum forte Compretten<sup>®</sup>, Glaxo Welcome GmbH/Cascan GmbH, Hamburg, Germany), diclofenac sodium and placebo were provided as identically appearing capsules (manufactured by Contract Pharma GmbH&Co KG, Murr, Germany).

## **Blood and urine sampling**

On study days 1 and 8 the following identical procedure was performed: Blood samples were taken before drug administration as well as 0.25, 0.5, 0.45, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 35 and 36 hours after drug administration. Blood samples were centrifuged after 30 min. and serum was stored at -20°C until analysed. Urine was collected before drug administration, from 0-12, 12-24 and 24-36 hours after drug administration. An aliquot was stored at -20°C until analysed.

## **Determination of analgesic effect**

The analgesic effects of codeine with and without simultaneous application of diclofenac or placebo were assessed using the cold pressure test as previously described.[17] Briefly, the cold pressure test apparatus consisted of temperature-controlled water baths of 35±0.5°C and 1.0±0.5°C (ice-water bath). The nondominant forearm was placed into the warm-water bath for exactly 2 minutes.

Fifteen seconds before transferring the forearm into the cold-water bath, a blood pressure cuff was inflated to 20mmHg below the diastolic blood pressure and the eyes were covered with eye-patches. Subjects placed their forearm in a constant position with the fingers wide apart into the cold-water bath (for a maximum time period of 2 min.) and were instructed to clearly indicate the time point of the first pain sensation and the time point of intolerable pain, at which the forearm was removed from the cold-water bath. The time from the immersion of the forearm into the cold-water bath to the first pain sensation was measured, and is defined as pain threshold. The pain tolerance is defined as the time from immersion of the forearm into the cold-water bath until to the time point of intolerable pain. A training session was carried out before the study to screen out volunteers with a pain tolerance less than 15 sec and more than 120 sec and to familiarise the volunteers with the study procedure. The experiments were conducted on study days 1 and 8 before drug administration and 1, 1.5, 2, 2.5, 3, 3.5, 4 and 6 hours after drug administration. Subsequently, for each study day the area under the pain threshold and the area under the pain tolerance were calculated and corrected for the baseline at  $t = 0h$ .

### **Determination of adverse events**

Adverse events were evaluated by the following method: The volunteers were required to list each symptom (fatigue, headache, dizziness, blurred vision, nausea, itching, exanthema and any other symptom) on a visual analogue scale (VAS) rated from 0 (not present) to 10 (most severe) at 0h (baseline, before drug intake), 2, 4 and 6 hours after drug intake. The values were corrected to baseline at  $t = 0h$ .

## **Analytical measurements of codeine and its metabolites**

Codeine and its metabolites were determined with HPLC-electrospray mass spectrometry analogous to a previously published method[24] with minor modifications. Samples (1.0 ml of plasma, or 100  $\mu$ l of urine diluted with 900  $\mu$ l of water) were spiked with the deuterated internal standards (10  $\mu$ l of standard mix with 50 pmol/ $\mu$ l codeine-6-glucuronide- $d_3$ , 5 pmol/ $\mu$ l codeine, 10 pmol/ $\mu$ l morphine-3-glucuronide- $d_3$ , 5 pmol/ $\mu$ l morphine-6-glucuronide- $d_3$  and 1 pmol/ $\mu$ l morphine- $d_3$ ) and extracted automatically using end-capped  $C_2$  solid-phase extraction columns. For the determination of the conjugates of norcodeine and normorphine, urine was hydrolysed[25] prior to extraction. The mobile phases used for HPLC were: (A) 1 % acetic acid in water and (B) 1 % acetic acid in acetonitrile. HPLC separation was achieved on a LiChrospher 100 RP-18 end-capped analytical column (125 $\times$ 3mm I.D., 5 $\mu$ m particle size) at a flow rate of 0.5 ml/min using a linear gradient from 8 % B to 40 % B in 8 min. The mass spectrometer (HP 1100 MSD, Hewlett-Packard, Waldbronn, Germany) was operated in the selected ion monitoring mode using the respective  $MH^+$  ions,  $m/z$  476 for codeine-6-glucuronide,  $m/z$  300 for codeine,  $m/z$  303 for codeine- $d_3$ ,  $m/z$  286 for norcodeine,  $m/z$  462 for morphine-3-glucuronide and morphine-6-glucuronide and  $m/z$  465 for the deuterated morphine glucuronides,  $m/z$  286 for morphine,  $m/z$  289 for morphine- $d_3$  and  $m/z$  272 for normorphine. The limits of quantification achieved with this method were 0.5 pmol/ml for the morphine-glucuronides and morphine, 2 pmol/ml for codeine, normorphine and norcodeine and 5 pmol/ml for codeine-6-glucuronide with coefficients of variation better than 12 %.

## **Analytical measurements of diclofenac**

Following the addition of 100µl of a 10µg/ml solution of 4'-methoxydiclofenac in water for internal standardisation and 2ml 10mM phosphate buffer pH 6.5 to 1ml of serum, samples were extracted by an automated solid phase extraction procedure using an ASPEC XL (Gilson, Villiers le Bel, France). After extraction with Bakerbond spe<sup>TM</sup> C<sub>18</sub> columns (3ml, 500mg) they were subsequently rinsed with 3ml methanol and 3ml of the phosphate buffer samples were applied on the columns and washed with 2ml of phosphate buffer and 2ml of phosphate buffer:methanol 6:4 (v/v). The analytes were eluted with phosphate buffer:methanol 2:8 (v/v), the solvents evaporated and the residue resuspended in 150µl of the mobile phase. A 100µl aliquot was injected on a HPLC-system consisting of an autosampler SIL 9A, a solvent delivery system LC 9A and an UV-detector (Shimadzu, Duisburg, Germany). Separation was performed on a Waters Spherisorb ODS1 5µm 125x4.6mm column equipped with a guard column filled with the same material (Bischoff, Leonberg, Germany) with 8mM tetrabutylammonium bromide in 10mM phosphate buffer pH 6.5:acetonitrile:tetrahydrofuran 65:30:5 (v/v/v) at a flow rate of 1ml/min. Diclofenac and the internal standard 4'-methoxydiclofenac were detected at 282nm at a retention time of 11.0 and 12.4 min, respectively. Recovery was between 85 and 100.5%. Calibration curves were linear over a concentration range from 10 to 2000ng/ml ( $r^2$  ranging from 0.9993 to 1). Inter-assay variability was 14.2, 8.3, and 7.8% for 30, 300, and 2000ng/ml, respectively. Accuracy was better than 10% for these concentrations.

## Pharmacokinetic evaluation

Standard noncompartmental analysis for calculations using serum concentration-time data was performed with TOPFIT 2.0 (Gustav Fischer Verlag 1993)

The following pharmacokinetic parameters were determined from serum concentration-time data and urine concentration data for codeine (cod), codeine-6-glucuronide (C-6-G), norcodeine (NC), morphine (morph), morphine-3-glucuronide (M-3-G), morphine-6-glucuronide (M-6-G) and normorphine (NM):

$C_{max}$	peak serum concentration [ $\mu\text{mol ml}^{-1}$ ], obtained from the visual inspection of the serum concentration-time curves
$t_{max}$	time to reach peak serum concentrations [h], obtained from the visual inspection of the serum concentration-time curves
$t_{1/2}$	terminal phase half-life [h] calculated according to $\ln(2)/\lambda_z$
$AUC_{0-\infty}$	area under the serum concentration curve [ $\mu\text{mol ml}^{-1}\cdot\text{h}$ ], calculated by the trapezoidal rule, the segment to infinity was determined from the last concentration measured divided by the elimination rate constant
ratio AUC`s	AUC codeine/AUC morphine
Ae	cumulative amount of codeine, C-6-G, norcodeine, norcodeine-glucuronide, morphine, M-3-G, M-6-G, normorphine and normorphine-glucuronide [% of dose] excreted in urine.
CLo	apparent oral clearance of codeine determined by dose/AUC [ $\text{ml min}^{-1}$ ]
$CL_{cod \rightarrow C-6-G}$	metabolic clearance of codeine to C-6-G calculated as $Ae_{C-6-G}/AUC_{codeine}$ [ $\text{ml min}^{-1}$ ]

$CL_{\text{cod} \rightarrow \text{NC}}$	metabolic clearance of codeine to norcodeine calculated as $(Ae_{\text{norcodeine}} + Ae_{\text{norcodeine-glucuronide}})/AUC_{\text{codeine}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{cod} \rightarrow \text{morph}}$	metabolic clearance of codeine to morphine calculated as $(Ae_{\text{morphine}} + Ae_{\text{M-3-G}} + Ae_{\text{M-6-G}} + Ae_{\text{normorphine}} + Ae_{\text{normorphine-glucuronide}})/AUC_{\text{codeine}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{morph} \rightarrow \text{M-3-G}}$	metabolic clearance of morphine to M-3-G calculated as $Ae_{\text{M-3-G}}/AUC_{\text{morphine}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{morph} \rightarrow \text{M-6-G}}$	metabolic clearance of morphine to M-6-G calculated as $Ae_{\text{M-6-G}}/AUC_{\text{morphine}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{morph} \rightarrow \text{NM}}$	metabolic clearance of morphine to normorphine calculated as $(Ae_{\text{normorphine}} + Ae_{\text{normorphine-glucuronide}})/AUC_{\text{morphine}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{ren cod}}$	renal clearance of codeine calculated as $Ae_{\text{codeine}}/AUC_{\text{codeine}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{ren morph}}$	renal clearance of morphine calculated as $Ae_{\text{morphine}}/AUC_{\text{morphine}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{ren C-6-G}}$	renal clearance of C-6-G calculated as $Ae_{\text{C-6-G}}/AUC_{\text{C-6-G}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{ren NC}}$	renal clearance of norcodeine calculated as $Ae_{\text{norcodeine}}/AUC_{\text{norcodeine}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{ren M-3-G}}$	renal clearance of M-3-G calculated as $Ae_{\text{M-3-G}}/AUC_{\text{M-3-G}}$ [ $\text{ml min}^{-1}$ ]
$CL_{\text{ren M-6-G}}$	renal clearance of M-6-G calculated as $Ae_{\text{M-6-G}}/AUC_{\text{M-6-G}}$ [ $\text{ml min}^{-1}$ ]

$CL_{\text{ren NM}}$  renal clearance of normorphine calculated as  $Ae \text{ normorphine} / AUC$   
normorphine [ $\text{ml min}^{-1}$ ]

### **Statistical analysis**

In this cross over study, 12 subjects were required to have a 80% power of detecting a potentially clinical relevant difference in pharmacokinetic parameters of 30% with a variability of 25% at the 5% significance level. Pharmacokinetic data are presented as mean  $\pm$  95% CI. Pharmacokinetic data of codeine and its metabolites after administration of codeine + placebo vs. codeine + diclofenac are compared by Wilcoxon matched pairs test. In addition, AUC and  $C_{\text{max}}$  of codeine and C-6-G were investigated in terms of bioequivalence: Point estimates (geometric means) and 90% CI were given for the ratios test/reference (test: pharmacokinetic parameters under consideration after codeine + diclofenac, reference: pharmacokinetic parameters under consideration after codeine + placebo). Test was considered bioequivalent to reference if 90% CI of AUC ratios were within 0.80 - 1.25 and  $C_{\text{max}}$  ratios were within 0.75-1.34.

Adverse events are presented in two different manners: 1.) The number of subjects reporting *any* side effect after administration of codeine + placebo vs. codeine + diclofenac were compared with chi square test. 2.) At different time points (2, 4 and 6h after drug administration) VAS-rated mean sum scores of all reported side effects after administration of codeine + placebo vs. codeine + diclofenac were calculated; the mean sum scores are compared using paired Wilcoxon matched pairs test at the different time points.

The area under the pain threshold and the area under the pain tolerance (corrected to baseline at  $t = 0\text{h}$ ) are presented as mean  $\pm$  SD and are compared using Wilcoxon matched pairs test.

A p value of less than 0.05 was considered significant.

## Results

### Pharmacokinetic data

Similar serum concentrations of codeine and its metabolite C-6-G were observed after treatment with codeine + placebo vs. codeine + diclofenac (Figure 1). Peak serum concentrations ( $C_{\text{max}}$ ) of codeine (764 pmol ml<sup>-1</sup>; 602-924 95% CI vs. 821 pmol ml<sup>-1</sup>; 663-980 95% CI) and C-6-G (5263 pmol ml<sup>-1</sup>; 4738-5788 95% CI vs. 5621 pmol ml<sup>-1</sup>; 5167-6074), did not differ significantly between the two different treatments, as well as the time to attain peak serum concentrations ( $t_{\text{max}}$ ) and the terminal half-life  $t_{1/2}$  (Table 1).

The AUCs of codeine did not differ between the two treatments (Table 2). In addition, bioequivalence could be stated for AUCs and  $C_{\text{max}}$  of codeine and C-6-G between the two treatments: Point estimates of the test/reference ratios and 90% CI were 1.051 (0.985-1.137) for AUC codeine, 1.058 (1.024-1.096) for AUC of C-6-G, 1.09 (0.972-1.312) for  $C_{\text{max}}$  of codeine and 1.071 (1.015-1.142) for  $C_{\text{max}}$  of C-6-G. However, a small but significant increase of the AUC of C-6-G (+ 5.5%) was observed in subjects after codeine + diclofenac (Table 2).

In terms of morphine formation, the ratios of AUC codeine/AUC morphine did not reveal a significant difference (53.4; 10.5-96.4 95% CI vs. 45.2; 16.4-74.0 95% CI) between the two treatments.

Peak serum concentrations ( $C_{max}$ ) of morphine (22.4 pmol ml<sup>-1</sup>; 12.3-32.5 95% CI vs. 24.3 pmol ml<sup>-1</sup>; 13.7-35.0 95% CI) and M-6-G (63.4 pmol ml<sup>-1</sup>; 38.7-88.1 95% CI vs. 68.8 pmol ml<sup>-1</sup>; 41.2-96.5 95% CI) did not differ significantly between the two different treatments, as well as the time to attain peak serum concentrations ( $t_{max}$ ) and the terminal half-life  $t_{1/2}$  (Table 1). AUC of morphine did not differ between the two treatments (Table 2). A small but significant increase of the AUC of M-6-G (+10.8%) was observed in subjects after codeine + diclofenac (Table 2). Serum concentration-time curves of morphine, M-3-G and M-6-G are displayed in Figure 2. No significant changes were observed in either the apparent oral clearance of codeine (CL<sub>o</sub>) (1805 ml min<sup>-1</sup>; 1498-2112 95% CI vs. 1700 ml min<sup>-1</sup>; 1470-1929 95% CI), metabolic clearance of codeine to C-6-G, norcodeine and morphine, of morphine to M-3-G, M-6-G and normorphine or renal clearance of codeine and its metabolites after the two different treatments (Tables 3 and 4).

The total amount excreted of codeine and its metabolites was 81.3% of dose (76.3-86.3 95% CI) vs. 84.9% of dose (81.8-88.0 95% CI) and did not differ between the two treatment groups. The urinary recoveries of codeine and its metabolites are listed in Table 5.

Diclofenac was detected in the serum of all subjects after codeine + diclofenac treatments with mean peak serum concentrations of 4.4nmol/ml (3.77-5.06 95% CI) after 1.73h (1.21-2.26 95% CI).

### **Pharmacodynamic data and adverse events**

The area under the pain threshold-time as well as the area under the pain tolerance-time did not differ significantly after administration of codeine + placebo vs. codeine + diclofenac (8.68 ± 12.7 vs. 4.87 ± 9.82 s\*h and 23.3 ± 57.5 vs. 13.9 ± 35.6 s\*h,

respectively). The time courses of pain threshold and pain tolerance are displayed in Figure 3.

Both codeine + placebo and codeine + diclofenac were well tolerated by the volunteers. Only minor side effects occurred and no rating higher than 5 was reported on a VAS scale. The number of subjects having reported any adverse event from VAS 1-5 rated from 0 (not present) to 10 (most severe) at 2, 4 and 6 hours after drug intake is displayed in table 6 and did not differ significantly between the two treatments. Also, no significant differences could be observed concerning the mean sum scores of all reported side effects at the given time points (Table 7).

## **Discussion**

The possible pharmacokinetic interaction between codeine and diclofenac could have clinical implications: One could have speculated that by inhibiting codeine glucuronidation other metabolic pathways of codeine, especially O-demethylation to morphine may then be favoured in extensive metabolizers of CYP2D6, resulting in elevated morphine serum levels with in consequence higher analgesic potency, and, possibly also increased adverse effects. A direct influence of diclofenac on O-demethylation of codeine to morphine via interaction with CYP2D6 has recently been excluded.[26]

Considering our previous in vitro data[10], it was important to verify the inhibition of codeine glucuronidation by diclofenac in vivo after application of commonly used doses.

However, after single dose administration of codeine + diclofenac to healthy volunteers we did not observe major changes in the pharmacokinetics of codeine and

its metabolites compared to codeine + placebo. In this study, codeine and diclofenac were given in a dose commonly used for clinical treatment of pain. In addition, no significant differences in the ratios AUC codeine/ AUC morphine were detected after the two different treatments, implicating that the formation of morphine out of codeine remained unaffected. A slight increase of the AUCs of C-6-G (+5.5%) and M-6-G (+10.8%) after the diclofenac containing regimen was observed, reaching statistical significance ( $p < 0.05$ ). To our opinion, these minor changes are not of clinical relevance after single dose administration. Nevertheless, the observed non significant decrease of renal clearance especially of M-6-G in the diclofenac containing regimen might play a role during chronic treatment with opioids .

In this study, we did not investigate a possible influence of codeine on the pharmacokinetics of diclofenac because it could recently be demonstrated that codeine did not influence the relative bioavailability of diclofenac in vivo.[27]

The pharmacokinetic results obtained from this study do not confirm our previous in vitro data with human liver tissue homogenates, which revealed a marked inhibition of codeine-6-glucuronidation caused by diclofenac ( $K_i$  of  $7.9\mu\text{M}$  in a given concentration range of  $100 - 10000\mu\text{M}$  codeine and  $0.5 - 100\mu\text{M}$  diclofenac).[10]

Although UGT (UDP-glucuronosyltransferase) 2B7, known as the UGT catalysing codeine-6-glucuronidation, is known to be expressed in the intestine,[28] we did not favour a potential interaction of codeine-6-glucuronidation at the level of the small intestine because of a very low intrinsic clearance compared to liver tissue in vitro.[10]

The pharmacokinetic data of codeine and its metabolites obtained in our study are supported by investigators of other groups.[12,13,29] The codeine peak serum

concentrations observed in our study ranged from 517 to 1481 pmol ml<sup>-1</sup> and therefore did not reach the concentrations used in vitro.

Kirkwood et al. could demonstrate a more than 50% inhibition of glucuronidation of the structural analogue dihydrocodeine with 50µM diclofenac.[11] In our study, mean peak serum concentrations of diclofenac were 4.4nmol/ml (range 3.01 - 6.21 nmol/ml) on average and were in the same range as reported by Davies et al.[30] Mean t<sub>max</sub> of diclofenac was 1.73h, so peak serum concentrations of diclofenac occurred delayed to t<sub>max</sub> of C-6-G and M-6-G (table 1). In addition, diclofenac is known to be highly bound to serum protein (>99.7%).[30] Therefore it is likely that diclofenac, at least after administration of a common single dose, does not achieve serum levels fast and high enough to inhibit codeine glucuronidation in vivo. On the other hand, it is unlikely that a multiple dose regimen of diclofenac (e.g. 50mg tid) would have led to cumulation of diclofenac serum levels due to the short elimination half-life of diclofenac (mean t<sub>1/2</sub> 0.97h in our study). Therefore, to our opinion, also during steady-state diclofenac will not alter codeine glucuronidation in vivo.

Concerning codeine glucuronidation, our pharmacokinetic results demonstrate that in vivo conditions do not necessarily reflect the in vitro conditions.

In terms of side effects, both codeine + placebo as well as codeine + diclofenac were well tolerated by the volunteers in our study. Only minor side effects occurred and no rating higher than 5 was reported on a VAS scale.

There are several studies demonstrating the benefit of the combination of NSAIDs plus opioids in comparison to opioids alone in the treatment of postsurgical pain, pain induced by arthrosis and chronic pain in cancer patients.[1-7] In one study, after a single dose of 100mg diclofenac rectally given to postsurgical patients receiving morphine PCA, a significant decrease in hourly morphine consumption and

plasma morphine and morphine-6-glucuronide were observed after administration of diclofenac, but with a lag time of more than 5 hours.[31]

In our study, we used the cold pressure test for analysing the analgesic effects of opioids because it was proven to be sensitive to the effects of codeine and morphine in several studies.[17,18] In contrast, this test is not appropriate to investigate the efficacy of NSAIDs alone.[32] In our study, the area under the pain threshold and the area under the pain tolerance did not differ significantly in the given time window after the two treatments. In case of a pharmacokinetic interaction between codeine and diclofenac leading to higher serum levels of morphine and M-6-G, an increase in pain threshold and pain tolerance would have been postulated.

Because we could exclude a pharmacokinetic interaction between codeine and diclofenac, the synergistic analgesic effects in the above mentioned studies are likely to result solely from the different pharmacodynamic mode of actions. Most postsurgical patients and many cancer patients have an inflammatory component within their pain, which responds to cyclooxygenase inhibition. Furthermore, NSAIDs have been reported to have a specific effect in malignant bone pain.[33]

## **Conclusions**

A single dose of diclofenac did not alter glucuronidation of codeine in healthy volunteers which is in contrast to recent in vitro data. The formation of morphine out of codeine was not affected. The combination of codeine and diclofenac was well tolerated.

## **List of abbreviations used**

CI:	confidence interval
MR:	metabolic ratio
NSAIDs:	non steroidal anti-inflammatory drugs
C-6-G:	codeine-6-glucuronide
M-6-G:	morphine-6-glucuronide
UGT:	UDP-glucuronosyltransferase

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## **Declaration of competing interests**

none declared

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## Figure legends

### Fig. 1

Serum concentration time profile of codeine and codeine-6-glucuronide after administration of codeine + placebo vs. codeine + diclofenac (Mean, SD, n= 12)

- codeine + placebo
- ◆ codeine + diclofenac

### Fig. 2

Serum concentration time profile of morphine, morphine-3- and morphine-6-glucuronide after administration of codeine + placebo vs. codeine + diclofenac (Mean, SD, n= 12)

- codeine + placebo
- ◆ codeine + diclofenac

### Fig. 3A

Pain threshold after administration of codeine + placebo vs. codeine + diclofenac, corrected to baseline at t = 0 (Mean, SD, n= 12)

- codeine + placebo
- codeine + diclofenac

### Fig. 3B

Pain tolerance after administration of codeine + placebo vs. codeine + diclofenac, corrected to baseline at t = 0 (Mean, SD, n= 12)

- codeine + placebo
- codeine + diclofenac

Table 1: Time to attain peak serum concentrations ( $t_{max}$ ) and terminal half-life of codeine and its metabolites (n=12).

	codeine + placebo mean (95% CI)	codeine + diclofenac mean (95% CI)	p level
$t_{max}$ codeine [h]	0.69 (0.51-0.87)	0.71 (0.51-0.91)	n.s.
$t_{1/2}$ codeine [h]	3.37 (3.16-3.59)	3.43 (3.24-3.62)	n.s.
$t_{max}$ C-6-G [h]	1.13 (0.89-1.35)	1.10 (0.87-1.34)	n.s.
$t_{1/2}$ C-6-G [h]	3.54 (3.39-3.68)	3.54 (3.36-3.73)	n.s.
$t_{max}$ morphine [h]	0.63 (0.45-0.80)	0.67 (0.53-0.81)	n.s.
$t_{1/2}$ morphine [h]	8.78 (5.97-11.59)	8.54 (6.74-10.61)	n.s.
$t_{max}$ M-6-G [h]	1.5 (1.37-1.64)	1.35 (1.06-1.65)	n.s.
$t_{1/2}$ M-6-G [h]	7.77 (7.40-8.13)	8.10 (7.57-8.63)	n.s.

Table 2: Area under the serum concentration time curve ( $AUC_{0-\infty}$ ) of codeine and its metabolites (n=12).

[pmol ml <sup>-1</sup> *h]	codeine + placebo mean (95% CI)	codeine + diclofenac mean (95% CI)	p level
AUC codeine	2416 (2025-2807)	2515 (2176-2855)	n.s.
AUC C-6-G	29550 (26190-32910)	31120 (28150-34090)	< 0.05
AUC norcodeine	342 (257-428)	338 (228-448)	n.s.
AUC morphine	94.5 (52.9-136)	92.1 (53.8-131)	n.s.
AUC M-3-G	2137 (1298-2976)	2259 (1427-3091)	n.s.
AUC M-6-G	425 (254-596)	471 (298-644)	< 0.05
AUC normorphine	540 (396-684)	549 (387-710)	n.s.

Table 3: Partial metabolic clearances of codeine and morphine after administration of 100 mg codeine phosphate (n=12).

Cl <sub>met</sub> [ml min <sup>-1</sup> ]	codeine + placebo mean (95% CI)	codeine + diclofenac mean (95% CI)	p level
codeine → C-6-G	1134 (882-1385)	1104 (928-1279)	n.s.
codeine → morphine	174 (118-230)	176 (126-226)	n.s.
codeine → norcodeine	102 (69.1-136)	97.1 (76.2-118)	n.s.
morphine → M-3-G	2631 (1227-4036)	2550 (1527-3573)	n.s.
morphine → M-6-G	702 (362-1042)	692 (446-938)	n.s.
morphine → normorphine	3090 (431-5749)	2600 (1456-3744)	n.s.

Table 4: Renal clearances of codeine and metabolites after administration of 100 mg codeine phosphate (n=12).

Cl <sub>ren</sub> [ml min <sup>-1</sup> ]	codeine + placebo mean (95% CI)	codeine + diclofenac mean (95% CI)	p level
codeine	66.5 (54.6-78.3)	64.2 (53.6-74.9)	n.s.
C-6-G	88.6 (76.4-101)	86.6 (78.6-94.5)	n.s.
norcodeine	220 (187-253)	233 (208-259)	n.s.
morphine	117 (72.4-162)	114 (83.6-144)	n.s.
M-3-G	88.2 (76.1-100)	86.2 (77.1-95.4)	n.s.
M-6-G	122 (108-136)	114 (101-127)	n.s.
normorphine	123 (106-141)	133 (115-152)	n.s.

Table 5: Urinary excretion of codeine and metabolites after administration of 100 mg codeine phosphate (n=12).

Ae (% of dose)	codeine + placebo mean (95% CI)	codeine + diclofenac mean (95% CI)	p level
codeine	3.74 (3.33-4.15)	3.89 (3.10-4.68)	n.s.
C-6-G	62.2 (57.0-67.5)	64.7 (60.7-68.7)	n.s.
norcodeine	1.76 (1.35-2.18)	1.86 (1.36-2.36)	n.s.
norcodeine-glucuronide	3.87 (3.01-4.73)	4.01 (3.02-5.00)	n.s.
morphine	0.22 (0.13-0.32)	0.23 (0.14-0.33)	n.s.
M-3-G	4.40 (2.81-5.99)	4.66 (3.10-6.22)	n.s.
M-6-G	1.21 (0.77-1.64)	1.31 (0.83-1.78)	n.s.
normorphine	1.56 (1.91-1.93)	1.73 (1.34-2.11)	n.s.
normorphine-glucuronide	2.30 (1.80-2.79)	2.50 (1.99-3.01)	n.s.

Table 6: Number of subjects reporting any adverse event

	codeine + placebo	codeine + diclofenac
fatigue	10	8
headache	4	1
dizziness	6	5
blurred vision	3	3
nausea	2	2
itching	0	0
exanthema	0	0
others	0	0

Table 7: Mean of sum scores of all adverse events

time after drug administration	codeine + placebo mean $\pm$ SD	codeine + diclofenac mean $\pm$ SD
2 h	4.3 $\pm$ 4.4	2.3 $\pm$ 2.1
4 h	1.6 $\pm$ 1.5	1.3 $\pm$ 1.7
6 h	2.9 $\pm$ 2.8	1.7 $\pm$ 1.7

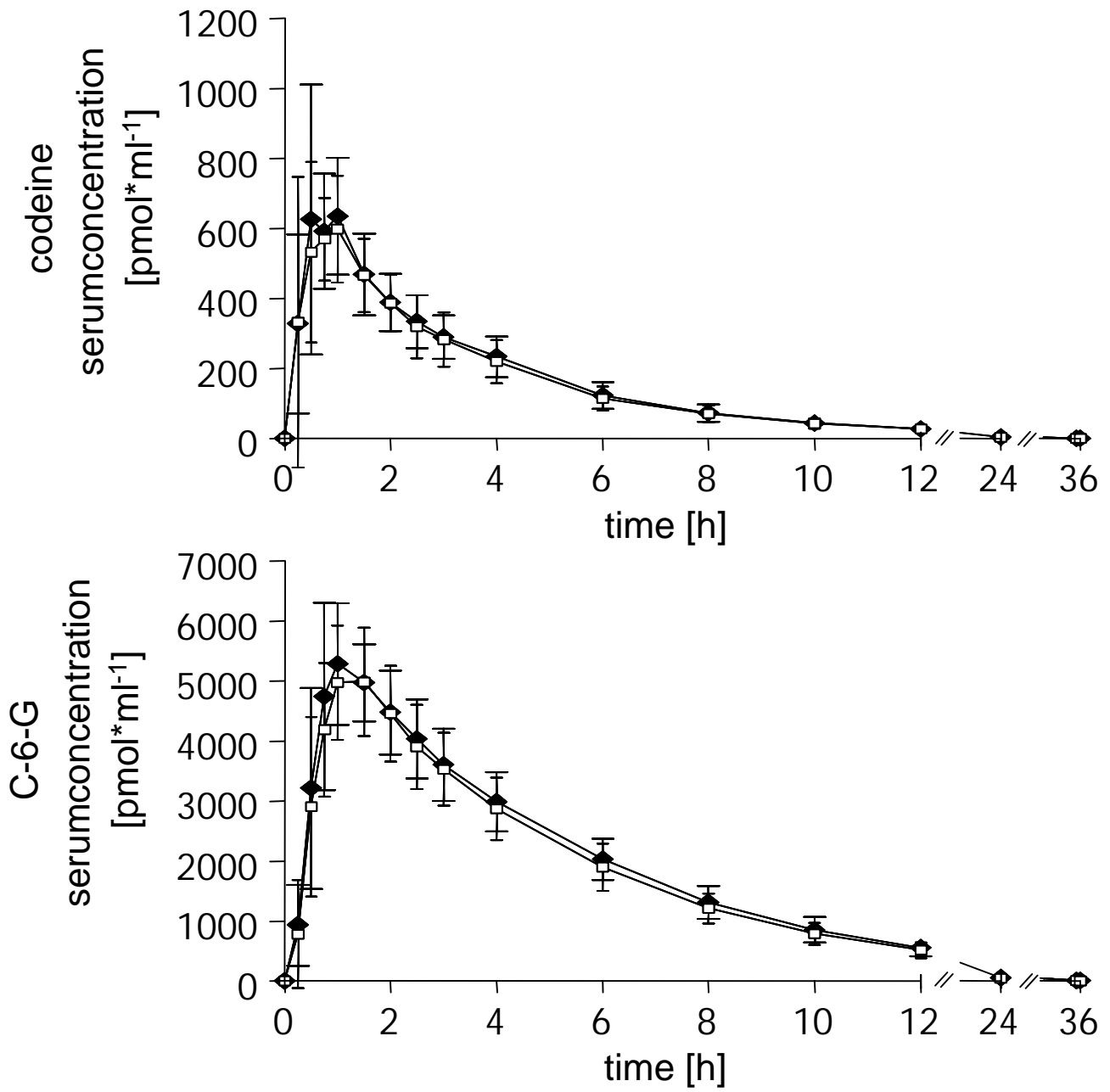


Figure 1

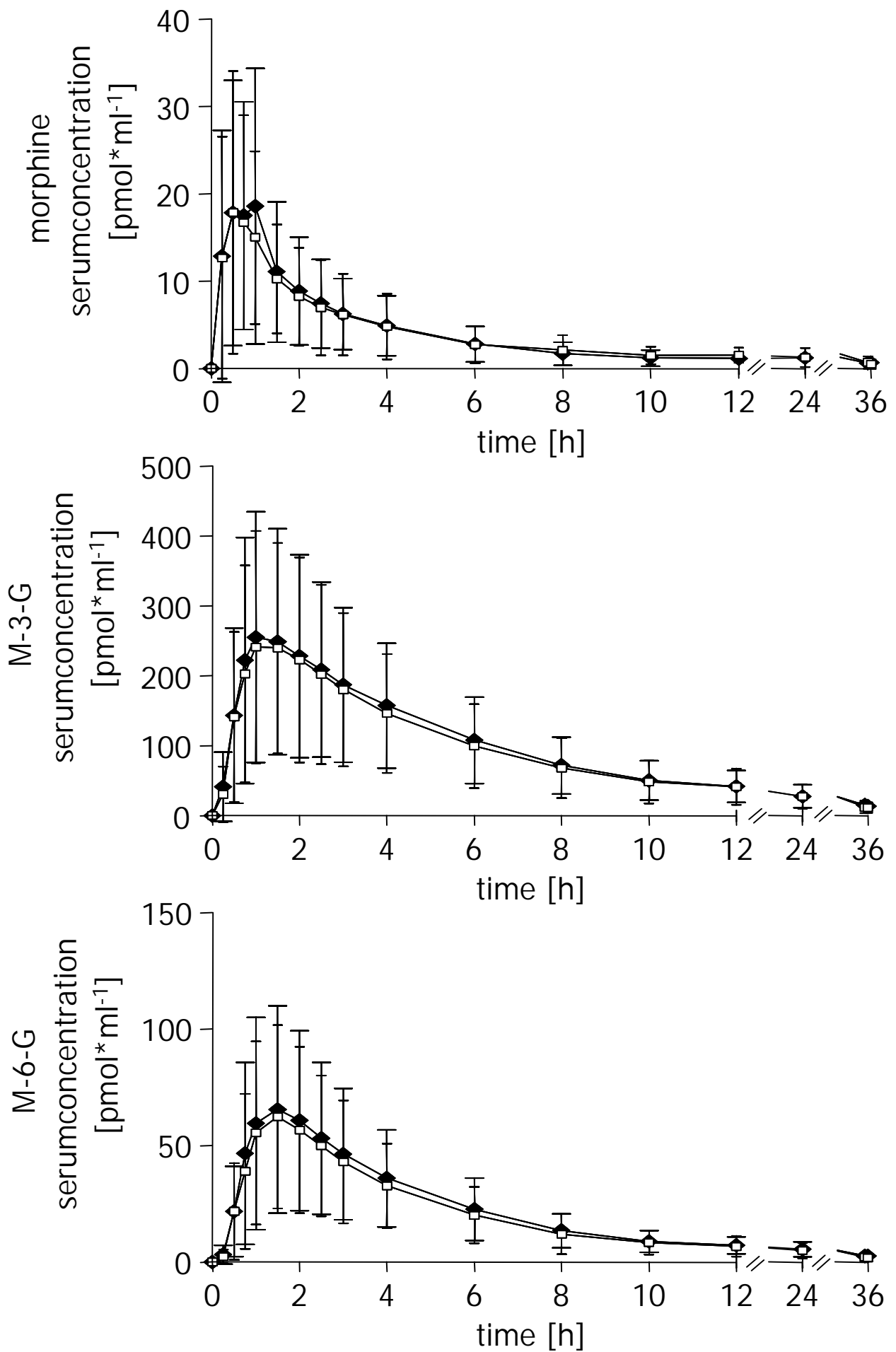


Figure 2

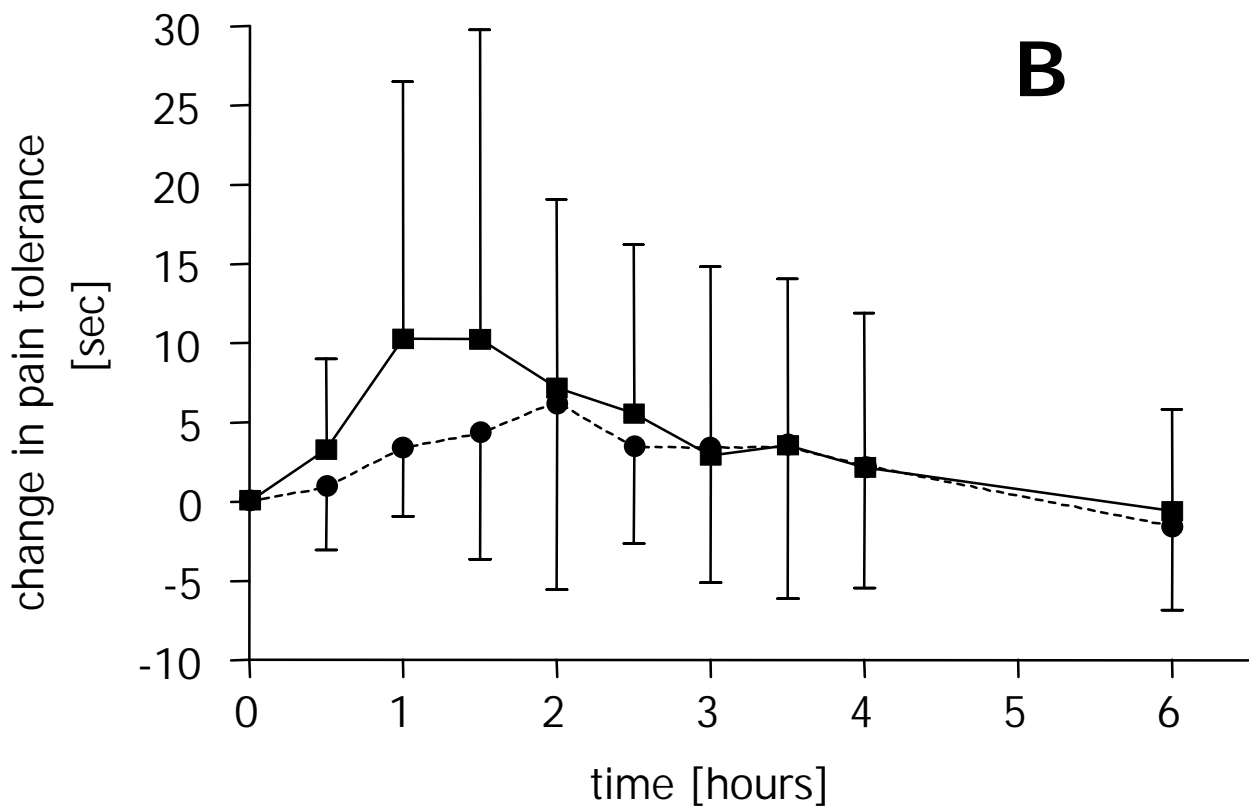
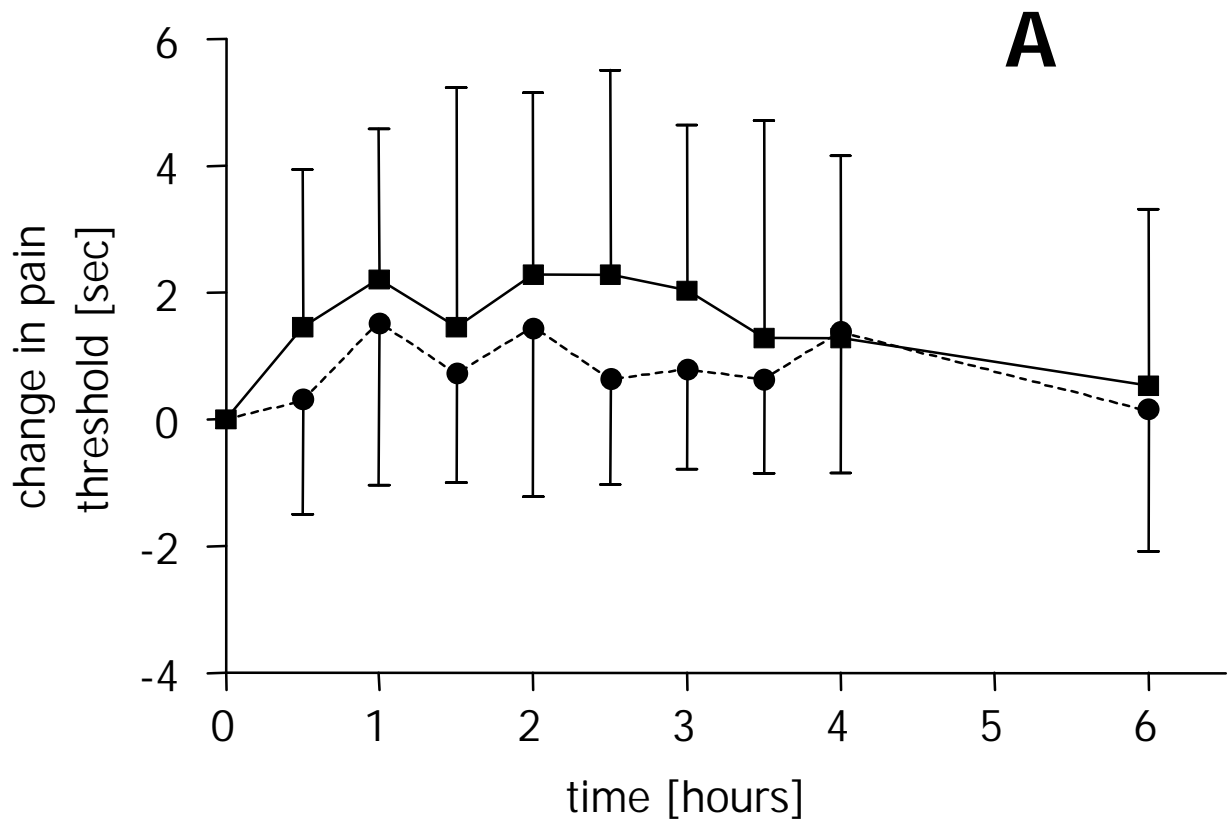


Figure 3