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Research article

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 by diclofenac in human liver tissue homogenate. We therefore 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 singe of 100 mg codeine phosphate plus 50 mg diclofenac sodium or codeine phosphate plus placebo. Over a 36 hour period serum concentrations of codeine and its metabolites as well as urinary excretion were analysed using LC-mass spectrometry. Side effects were recorded and analgesic efficacy was determined using the cold pressor test (0–6 h).

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 pressor test.

Conclusions: In contrast to recent in vitro data, a single oral dose of diclofenac did not alter the 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 via a pharmacokinetic interaction between the two classes of drugs: NSAIDs may decrease the renal excretion of the pharmacologically active metabolite of morphine M-6-G (morphine-6-glucuronide).[9] We have previously demonstrated a marked inhibition of codeine glucuronidation in human liver tissue homogenate

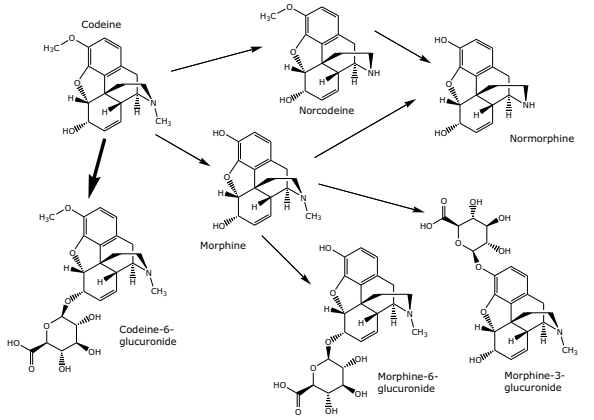

Figure 1
The chemical structures of codeine and its major metabolites in humans.

by diclofenac (K_i of 7.9 μM).[10] Others have shown [11] a more than 50% inhibition of the glucuronidation of the codeine structural analogue, dihydrocodeine, by 50 μM diclofenac in vitro. Codeine is predominantly metabolised by glucuronidation to C-6-G (codeine-6-glucuronide) (Figure 1). Minor metabolic pathways include N-demethylation to norcodeine and O-demethylation to morphine. [12,13] The latter is catalysed by 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 greater analgesic potency than morphine itself.[19] In humans, the analgesic activity of C-6-G has not been reported; however, antinociceptive responses after intracerebroventricular administration have been reported in rats. [20] Since in vitro findings may not necessarily be of clinical relevance, we aimed to investigate whether diclofenac inhibits codeine glucuronidation *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 63–95 kg), median BMI 23.9 kg/m^2 (range 19.9–28.4 kg/m^2) participated in the study. One subject had to be replaced because of additional drug intake during the study.

The study was 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 regular medication. The volunteers had previously been genotyped for CYP2D6 by allele-specific PCR [21,22] and all were predicted to be extensive metabolisers (EM). In addition, the subjects were phenotyped with respect to sparteine oxidation polymorphism with a single dose of 100 mg sparteine sulphate and were classified as EMs according to the metabolic ratio (MR) of sparteine and its 2- and 5-dehydrometabolite.[23] In order to limit the effect of the variability of CYP2D6 on metabolic capacity, volunteers with a MR<1 were generally included in the study, with one subject having 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 100 mg codeine phosphate + 50 mg diclofenac sodium **or** 100 mg codeine phosphate + placebo. Codeine phosphate was provided as tablets (Codeinum Phosphoricum forte Compretten®, Glaxo Welcome GmbH/Cascan GmbH, Hamburg, Germany), diclofenac sodium and placebo were provided as capsules of identical appearance (manufactured by Contract Pharma GmbH & Co KG, Murr, Germany).

Blood and urine sampling

On study days 1 and 8 the following identical procedures were carried out: 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 and from 0–12, 12–24 and 24–36 hours after drug administration. The volume was measured and an aliquot of each collection was stored at -20°C until analysed.

Determination of analgesic effect

The analgesic effects of codeine with and without simultaneous administration of diclofenac or placebo were assessed using the cold pressor test as previously described.[17] Briefly, the cold pressor 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 20 mmHg below the diastolic blood pressure and the eyes were covered with eye-patches. Subjects placed their forearm in a fixed position with the fingers wide

apart into the cold-water bath (for a maximum time period of 2 min). They were instructed to clearly indicate the time of the first pain sensation as well as the time 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 the pain threshold. The pain tolerance is defined as the time from immersion of the forearm into the cold-water bath until the time of intolerable pain. A training session was carried out before the study to screen out volunteers with a pain tolerance of less than 15 sec or more than 120 sec and to familiarise the volunteers with the study procedure. The pain experiments were conducted on study days before drug administration (10–15 min before drug administration) and 1, 1.5, 2, 2.5, 3, 3.5, 4 and 6 hours after drug administration. Subsequently, the changes from baseline were determined and for each study day the area under the pain threshold change versus time curve and the area under the pain tolerance change versus time curve were calculated.

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 zero h (baseline, before drug intake), 2, 4 and 6 hours after drug intake. The values were corrected for the baseline measurement at t = 0 h.

Analytical measurement of codeine and its metabolites

Codeine and its metabolites were determined using HPLC-electrospray mass spectrometry analogous to a previously published method[24] with minor modifications. Samples (1.0 ml of plasma, or 100 µl of urine diluted with 900 µl of water) were spiked with the deuterated internal standards (10 µl of standard mix with 50 pmol/µl codeine-G-glucuronide-d_3, 5 pmol/µl codeine, 10 pmol/µl morphine-3-glucuronide-d_3, 5 pmol/µl morphine-6-glucuronide-d_3 and 1 pmol/µl 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 (Merck, Darmstadt, Germany) endcapped analytical column (125 × 3 mm I.D., 5 µm 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 less than 12 %.

Analytical measurements of diclofenac

Following the addition of 100 µl of a 10 µg/ml solution of 4'-methoxydiclofenac in water for internal standardisation and 2 ml 10 mM phosphate buffer pH 6.5 to 1 ml of serum, samples were extracted by an automated solid phase extraction procedure using an ASPEC XL (Gilson, Villiers Ie Bel, France). Samples were applied on Bakerbond SPE™ C_{18} extraction columns (3 ml, 500 mg) which were equilibrated with 3 ml methanol and 3 ml of phosphate buffer. The columns were washed with 2 ml of phosphate buffer and 2 ml of phosphate buffer: methanol 6:4 (v/v) and eluted with phosphate buffer: methanol 2:8 (v/v). The solvents were evaporated and the residue was resuspended in 150 µl of the mobile phase. A 100 µl 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 5 µm 125 × 4.6 mm column equipped with a guard column filled with the same material (Bischoff, Leonberg, Germany) using 8 mM tetrabutylammonium bromide in 10 mM phosphate buffer pH 6.5:acetonitrile:tetrahydrofuran 65:30:5 (v/v/v) at a flow rate of 1 ml/min. Diclofenac and the internal standard 4'-methoxydiclofenac were detected at 282 nm 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 2000 ng/ml (r^2 ranging from 0.9993 to 1). Inter-assay variability (n = 9) was 14.2, 8.3, and 7.8% for 30, 300, and 2000 ng/ml, respectively. Accuracy was within 10% at these concentrations.

Pharmacokinetic evaluation

Standard noncompartmental analysis for calculations using serum concentration-time data was performed using 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 [pmol 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 $\ln(2)/\lambda_z$, the terminal elimination rate constant λ_z was determined from the slope of the regression line of In(concentration) vs time

$AUC_{0-\infty}$- area under the serum concentration curve [pmol ml^{-1}*h], calculated by the trapezoidal rule, the segment to infinity was calculated from the last concentration measured by dividing the last concentration measured by the elimination rate constant (λ_z)

ratio AUC' s- $AUC_{0-\infty}$ codeine/$AUC_{0-\infty}$ 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 calculated using dose/$AUC_{0-\infty}$ [ml min^{-1}]

$CL_{cod\rightarrow c\text{-}6\text{-}G}$- metabolic clearance of codeine to C-6-G calculated as Ae C-6-G/$AUC_{0-\infty}$ codeine [ml min^{-1}]

$CL_{cod\rightarrow NC}$- metabolic clearance of codeine to norcodeine calculated as (Ae norcodeine + Ae norcodeine-glucuronide)/$AUC_{0-\infty}$ codeine [ml min^{-1}]

$CL_{cod\rightarrow morph}$- metabolic clearance of codeine to morphine calculated as (Ae morphine + Ae M-3-G + Ae M-6-G + Ae normorphine + Ae normorphine-glucuronide)/ $AUC_{0-\infty}$ codeine [ml min^{-1}]

$CL_{morph\rightarrow M\text{-}3\text{-}G}$- metabolic clearance of morphine to M-3-G calculated as Ae M-3-G/$AUC_{0-\infty}$ morphine [ml min^{-1}]

$CL_{morph\rightarrow M\text{-}6\text{-}G}$- metabolic clearance of morphine to M-6-G calculated as Ae M-6-G/$AUC_{0-\infty}$ morphine [ml min^{-1}]

$CL_{morph\rightarrow NM}$ metabolic clearance of morphine to normorphine calculated as (Ae normorphine + Ae normorphine-glucuronide)/$AUC_{0-\infty}$morphine [ml min^{-1}]

$CL_{ren\ cod}$- renal clearance of codeine calculated as Ae codeine/$AUC_{0-\infty}$ codeine [ml min^{-1}]

$CL_{ren\ morph}$- renal clearance of morphine calculated as Ae morphine/$AUC_{0-\infty}$ morphine [ml min^{-1}]

$CL_{ren\ C\text{-}6\text{-}G}$- renal clearance of C-6-G calculated as Ae C-6-G/$AUC_{0-\infty}$ C-6-G [ml min^{-1}]

$CL_{ren\ NC}$- renal clearance of norcodeine calculated as Ae norcodeine/$AUC_{0-\infty}$ norcodeine [ml min^{-1}]

$CL_{ren\ M\text{-}3\text{-}G}$- renal clearance of M-3-G calculated as Ae M-3-G/$AUC_{0-\infty}$ M-3-G [ml min^{-1}]

$CL_{ren\ M\text{-}6\text{-}G}$- renal clearance of M-6-G calculated as Ae M-6-G/$AUC_{0-\infty}$ M-6-G [ml min^{-1}]

$CL_{ren\ NM}$- renal clearance of normorphine calculated as Ae normorphine/$AUC_{0-\infty}$ normorphine [ml min^{-1}]

Statistical analysis

In this cross over study, 12 subjects were required to ensure a 80% power of detecting a potentially clinically relevant difference in pharmacokinetic parameters of 30% with a variability of 25% at the 5% significance level for α. Pharmacokinetic data are presented as mean ± 95% CI. Pharmacokinetic data of codeine and its metabolites after administration of codeine + placebo vs. codeine + diclofenac are compared using the Wilcoxon matched pairs test. In addition, AUC and C_{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 log transformed AUC ratios were within 0.80–1.25 and C_{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 using the chi square test. 2.) At different time points (2, 4 and 6 h 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 the paired Wilcoxon matched pairs test at the different time points. The area under the pain threshold versus time curve and the area under the pain tolerance versus time curve (corrected to baseline at t = 0 h) are presented as mean ± SD and are compared using Wilcoxon matched pairs test. A p value of less than 0.05 was considered significant.

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.
C_{max} codeine [pmol/ml]	764 (602–924)	821 (663–980)	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.
C_{max} C-6-G [pmol/ml]	5263 (4738–5788)	5621 (5167–6074)	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.
C_{max} morphine [pmol/ml]	22.4 (12.3–32.5)	24.3 (13.7–35.0)	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.
C_{max} M-6-G [pmol/ml]	63.4 (38.7–88.1)	68.8 (41.2–96.5)	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^{-1}*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.

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 2). Peak serum concentrations (C_{max}) of codeine (764; 602–924 pmol ml^{-1} vs. 821; 663–980 pmol ml^{-1}) and C-6-G (5263; 4738–5788 pmol ml^{-1} vs. 5621; 5167–6074 pmol ml^{-1}), did not differ significantly between the two different treatments, nor did the time to attain peak serum concentrations (t_{max}) and the terminal half-life $t_{1/2}$ (Table 1). The concentrations of codeine and its metabolites in the one intermediate metaboliser (MR = 2.8) were within the range of the concentrations of the other volunteers with MR<1, however as expected the metabolites formed via O-demethylation (morphine, morphine glucuronides and normorphine) were the lowest or second lowest observed.

The AUCs of codeine did not differ between the two treatments (Table 2). In addition, the two treatment, based on AUCs and C_{max} of codeine and C-6-G, could be considered bioequivalent: Point estimates of the test/reference ratios and 90% CI were 1.007 (0.998–1.017) for AUC codeine, 1.004 (1.001–1.008) for AUC of C-6-G, 1.015 (0.990–1.042) for C_{max} of codeine and 1.008 (1.001–1.016) for C_{max} of C-6-G. However, a small but significant increase in 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^{-1}; 12.3–32.5 95% CI vs. 24.3 pmol ml^{-1}; 13.7–35.0 95% CI) and M-6-G (63.4 pmol ml^{-1}; 38.7–88.1 95% CI vs. 68.8 pmol ml^{-1}; 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

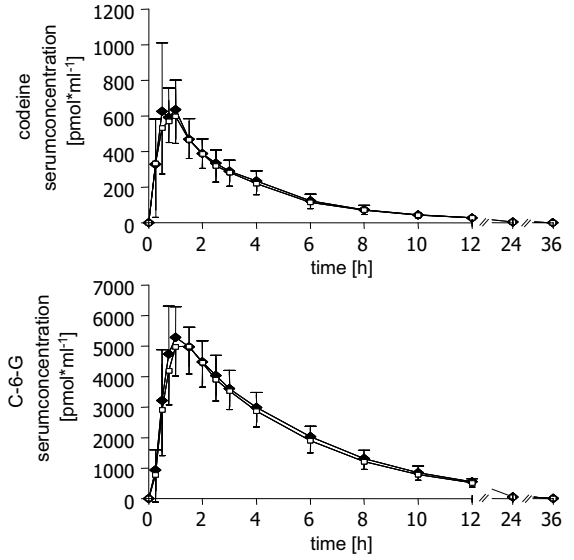

Figure 2
Serum concentration time profile of codeine and codeine-6-glucuronide after administration of codeine + placebo vs. codeine + diclofenac (Mean, SD, n= 12) □ codeine + placebo ♦ codeine + diclofenac

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 after codeine + diclofenac (Table 2). Serum concentration-time curves of morphine, M-3-G and M-6-G are displayed in Figure 3.

No significant differences were observed in either the apparent oral clearance of codeine (CLo) (1805 ml min^{-1}; 1498–2112 95% CI vs. 1700 ml min^{-1}; 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 between the two treatments (Tables 3 and 4). The total amount excreted of codeine and its metabolites excreted 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 with mean peak serum concentration of 4.4 nmol/ml (3.77–5.06 95% CI) after 1.73 h (1.21–2.26 95% CI) (see Table 6).

Pharmacodynamic data and adverse events

The area under the pain threshold-time as well as the area under the pain tolerance-time curves 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 4.

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 reporting any adverse event from VAS rated from 0 (not present) to 10 (most severe) at 2, 4 and 6 hours after drug intake is displayed in table 7 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 8).

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 and a greater analgesic effect, and, possibly also increased adverse effects. A direct influence of diclofenac on O-demethylation of codeine to morphine via interaction with CYP2D6 has been excluded.[26]

Considering our previous in vitro data[10], it was important to verify whether codeine glucuronidation was inhibited by diclofenac in vivo after administration of a commonly used dose.

However, after single dose administration of codeine + diclofenac in 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, indicating that the formation of morphine from codeine remained unaffected. A slight increase in the AUCs of C-6-G (+5.5%) and M-6-G (+10.8%) after the diclofenac containing regimen was observed, reaching statistical significance ($p < 0.05$). This might be a result of inhibition of renal excretion of both glucuronides by diclofenac or its glucuronide. In our opinion, these minor changes are not of clinical relevance after single dose administration. Nevertheless, the observed non significant decrease in renal clearance especially of M-6-G in the diclofenac containing regimen, might play a role during chronic treatment with opioids.

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

Cl_{met} [ml min^{-1}]	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 metabilites after administration of 100 mg codeine phosphate (n = 12).

Cl_{ren} [ml min^{-1}]	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.

In this study, we did not investigate a possible influence of codeine on the pharmacokinetics of diclofenac because it has been previously demonstrated that codeine does 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 non-competitive inhibition of codeine-6-glucuronidation caused by diclofenac (K_i of 7.9 μM in a concentration range of 100–10000 μM codeine and 0.5–100 μM diclofenac).[10] Although UGT (UDP-glucuronosyltransferase) 2B7, the UGT catalysing codeine-6-glucuronidation, morphine-6-glucuronidation and diclofenac glucuronidation [28], is known to be expressed in the intestine,[29] we did not favour a potential interaction of codeine-6-glucuronidation at the level of the small intestine because of its very low intrinsic clearance compared to liver tissue in vitro.[10]

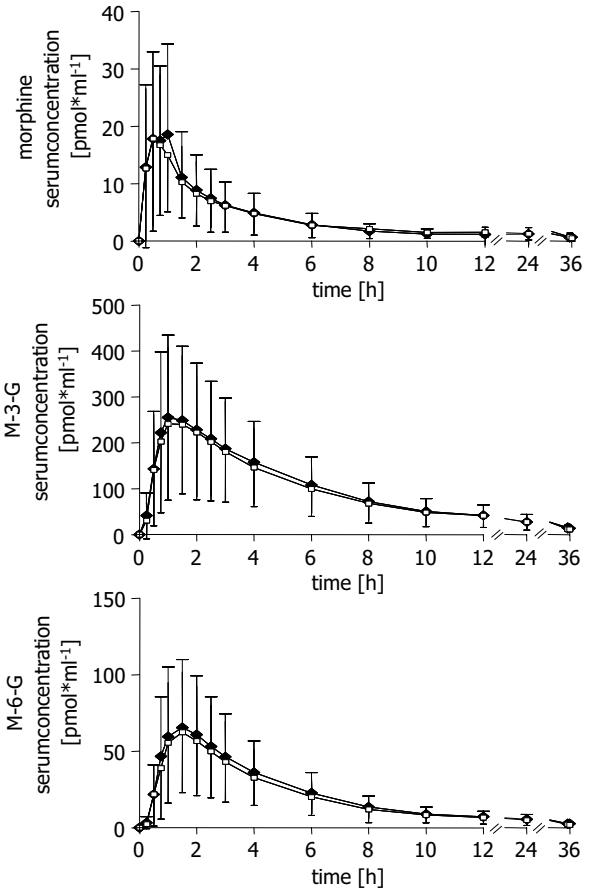

Figure 3
Serum concentration time profile of morphine, morphine-3- and morphine-6-glucuronide after administration of codeine + placebo vs. codeine + diclofenac (Mean, SD, n= 12) □ codeine + placebo ♦ codeine + diclofenac

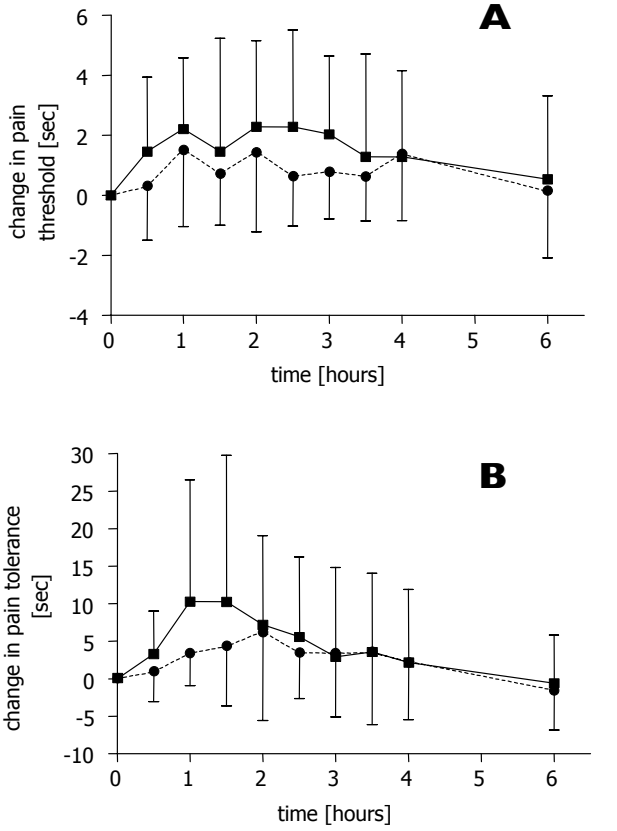

Figure 4
Pain threshold after administration of codeine + placebo vs. codeine + diclofenac, corrected to baseline at t = 0 (Mean, SD, n= 12) ▪ codeine + placebo • codeine + diclofenac. **B** Pain tolerance after administration of codeine + placebo vs. codeine + diclofenac, corrected to baseline at t = 0 (Mean, SD, n= 12) ▪ codeine + placebo • codeine + diclofenac

The pharmacokinetic data for codeine and its metabolites obtained in our study are supported by the data obtained by other investigators.[12,13,30] The codeine peak serum concentrations observed in our study ranged from 517 to 1481 pmol ml^{-1} and therefore did not reach the concentrations used in our previous in vitro investigation.

Kirkwood et al. could demonstrate a more than 50% inhibition of the glucuronidation of the codeine structural analogue dihydrocodeine by 50 μM diclofenac. [11] In our study, mean peak serum concentrations of diclofenac were 4.4 nmol/ml (range 3.01–6.21 nmol/ml) and were in the same range as reported by Davies et al.[31] Mean t_{max} of diclofenac was 1.73 h, so peak serum concentrations of diclofenac were later than the t_{max} of C-6-G and M-6-G (table 1). In addition, diclofenac is known to be highly bound to serum protein (>99.7%).[31] Therefore it is likely that diclofenac, at least after administration of a common single dose, does not achieve serum levels fast enough and high enough to inhibit codeine glucuronidation in vivo. Furthermore, it is unlikely that a multiple dose regimen of diclofenac (e.g. 50 mg tid) would lead to the cumulation of diclofenac serum levels due to the short elimination half-life of diclofenac (mean $t_{1/2}$ 0.97 h in our study). Therefore, in our opinion, steady-state administration of diclofenac will also not alter codeine glucuronidation in vivo. However, the data obtained in this study cannot exclude a pharmacokinetic / pharmacodynamic interaction at steady-state. Concerning codeine glucuronidation, our pharmacokinetic results demonstrate that in vitro conditions studied did not necessarily reflect the in vivo conditions at therapeutic doses. 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

Table 6: Pharmacokinetic parameters of diclofenac after oral administration of 50 mg diclofenac sodium together with 100 mg codeine phosphate (n = 12).

	mean (95% CI)
C_{max} [nmol/ml]	4.42 (3.77–5.06)
t_{max} [h]	1.73 (1.21–2.26)
AUC [h nmol/ml]	5.63 (4.77–6.50)
$t_{1/2}$ [h]	0.97 (0.68–1.26)

Table 7: 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 8: Mean of sum scores of all adverse events

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

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 100 mg 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.[32]

In our study, we used the cold pressor test for analysing the analgesic effects of opioids because it has been 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.[33] In our study, the area under the pain threshold versus time curve and the area under the pain tolerance versus time curve did not differ significantly after the two treatments over the time intervals studied. 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. A placebo part of the study was omitted because the primary objective was the assessment of a pharmacokinetic interaction.

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 to their pain, which responds to cyclooxygenase inhibition. Furthermore, NSAIDs have been reported to have a specific effect in malignant bone pain. [34]

Conclusions

A single dose of diclofenac does not alter the glucuronidation of codeine in healthy volunteers, which is in contrast to recent in vitro data. The formation of morphine from 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

Competing interests

None declared

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