Caffeine Accelerates Absorption and Enhances the Analgesic Effect of Acetaminophen

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The aim of this study was to determine the analgesic effect of acetaminophen compared to a combination of both caffeine and acetaminophen or caffeine alone using tonic and phasic pain stimulation. Twenty-four subjects were treated orally with 1000 mg acetaminophen, 130 mg caffeine, and a combination of both in a 4-way crossover, double-blind, placebo-controlled study. Pharmacokinetics and analgesic effects were assessed by means of an experimental pain model based on pain-related cortical potentials after phasic stimulation of the nasal mucosa with CO_2 and based on pain ratings after tonic stimulation with dry air. Analgesic effects of acetaminophen and acetaminophen plus caffeine but not caffeine alone caused a significant reduction of pain-related cortical potentials beginning 30 minutes after medication. The combination demonstrated an enhanced effect throughout the observation time up to 3 hours. Caffeine accelerated acetaminophen absorption, indicated by enhanced early AUCs. Significant analgesic effects of the combination on tonic pain ratings were found throughout the observation time as compared to acetaminophen and placebo. In this study, caffeine enhanced and prolonged the analgesic activity of acetaminophen.

Keywords: *Acetaminophen; paracetamol; caffeine; pain measurement; evoked potentials*

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Acetaminophen (paracetamol) is a nonopioid, non–NSAID (nonsteroidal anti-inflammatory drug) analgesic that is widely used as an over-the-counter drug for the treatment of mild to moderate pain. The mechanism of action is not yet clarified but may involve inhibition of cerebral prostaglandin synthesis[1-3] and central serotonergic or opioidergic pathways.[4-6] Caffeine is a common adjuvant to analgesic drugs.[7,8] The mechanism of its putative analgesia-enhancing effect remains unclear. Caffeine is thought to produce competitive antagonism at adenosine receptors,[9,10] induce changes of the pharmacokinetics of acetaminophen,[11,12] and induce changes in mood,[13] all of which may lead to enhancement of the analgesic action of acetaminophen.

The aim of this study was to investigate the analgesic activity of 1000 mg acetaminophen compared with caffeine alone and the combination of both using an experimental human pain model. To define caffeine actions, subjective and objective pain assessments and pharmacokinetic evaluations were included in this study. The pain model used is based on evoked cortical potentials and pain ratings following specific stimulation of nasal nociceptors with gaseous carbon dioxide (CO_2),[14,15] and it is also based on pain ratings after tonic painful stimulation of the nasal mucosa with dry air of controlled flow and temperature.[16] This model has been applied successfully to quantify the activity of several nonopioid[15,17-23] and opioid analgesics.[15,24-26]

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METHODS

Subjects

The study was approved by the ethics committee of the University of Erlangen-Nuremberg. It was conducted according to the Declaration of Helsinki on biomedical research involving human subjects (Somerset West amendment). Twenty-four subjects were enrolled (12 men, 12 women, aged 18-45 years [median, 26 years]), all within ±20% of their ideal body weight (men: median weight, 75 kg; range, 67-86 kg; women: median weight, 57 kg; range, 51-66 kg). All were found to be healthy by routine clinical examination and laboratory tests at the beginning and end of the study. Oral contraceptives were the only medication allowed during the study.

Protocol

This was a single-center, 4-way crossover, randomized, placebo-controlled, double-blind study. Each subject received a single dose of 1000 mg acetaminophen (Panadol), 130 mg caffeine, 1000 mg acetaminophen plus 130 mg caffeine (Panadol Extra), or placebo, taken with 150 mL of water, after fasting for 6 hours. Subjects remained fasted but were allowed to drink water during the study period. The washout periods between drug administrations were at least 6 days.

On each study day, participants were subjected to 5 periods of phasic pain lasting for 20 minutes each and applied at baseline and at 20, 40, 90, and 180 minutes after receiving medication. During a stimulus period, 16 stimuli of 60% v/v CO_2 and 32 stimuli of 70% v/v CO_2 were delivered to the left nostril in a randomized sequence. The reason for using 2 concentrations was to reduce response bias, giving the subjects a choice to discriminate between different painful intensities. The volunteers also received 4 periods of tonic pain delivered to the right nostril (dry air applied to the nasal mucosa for 16 minutes) at baseline and at 60, 110, and 200 minutes after medication. During the study period, volunteers remained seated in an air-conditioned chamber. "White noise" (50 dB SPL) was applied continuously by headphones to mask distracting noises. Subjects were familiarized with the actual experimental conditions in a training session prior to the actual experiments.

Phasic and Tonic Pain Stimulation of the Nasal Mucosa

For painful stimulation, tonic and phasic stimuli were applied heterotopically to the left and right nasal cavities (ie, long-lasting pain was induced in the right nostril, while short pulses of painful stimuli were delivered to the left).[27] Phasic pain with gaseous CO_2 stimuli (duration 200 ms, stimulus rise time below 20 ms, interstimulus interval approximately 20 s) was applied to the left nasal mucosa by means of a device (OM4, Burghart Instruments, Wedel, Germany) that allows for painful stimulation without concomitant alteration of mechanical or thermal conditions at the mucosa.[28,29] The stimuli produced a sharp stinging pain and are used to obtain cortical pain-related evoked potentials from electroencephalogram (EEG) data.

Tonic painful stimulation was induced into the right nostril by means of a dry air stream of controlled temperature (32°C), flow (8 $L \cdot min^{-1}$), and humidity (20% relative humidity) for a period of 16 minutes. Participants reported a dull or burning pain that reached its steady state within a few minutes.[16,30]

Trigeminal Event-Related Potentials

The EEG was recorded from 5 positions of the international 10/20 system[31] (Cz, C3, C4, Fz, and Pz) referenced to linked earlobes (A1+A2; SIR EEG amplifier, Roettenbach, Germany). Possible eye blink artifacts were monitored from an additional site (Fp2/A1+A2). Stimulus-linked EEG segments of 2048 ms duration were sampled with a frequency of 250 Hz (band pass, 0.16-70 Hz). After analog-to-digital conversion (MIO 16X, National Instruments, Austin, Tex), the EEG segments were stored on a Macintosh computer. These records were averaged separately for each recording position and stimulus concentration, discarding all eye blink–contaminated records. By this procedure, trigeminal event-related potentials (ERPs) were obtained in response to the painful CO_2 stimuli. Base-to-peak amplitudes P1, N1, and P2; the peak-to-peak amplitudes P1N1 and N1P2; and the latencies of P1, N1, and P2 were measured relative to stimulus onset.[28,29]

Measurement of Phasic and Tonic Pain Intensity

Volunteers rated phasic pain intensity using a visual analog scale (VAS) within 3 to 4 seconds of receiving a phasic stimulus compared with pain intensity from a standard stimulus (70% v/v CO_2, intensity defined as 100 estimation units [EU]) presented at the beginning of each trial day.

Subjects also rated pain intensity every 30 seconds during the 16-minute tonic pain stimulation period, with the scale's left-hand end indicating "no pain" (0 EU) and the right-hand end indicating "unbearable" pain (100 EU). Because tonic pain

reached its steady state after 8 minutes, estimates of the second half of the 16-minute stimulation period were analyzed.[16]

Background Electroencephalogram and Tracking Performance

The spontaneous background EEG was obtained during phasic and tonic pain sessions as an indicator of arousal effects. Periods of 4096 ms were sampled from all recording positions with a sampling frequency of 125 Hz (band pass, 0.16-70 Hz). The EEG data segments contaminated by eye blinks or muscle artifacts were excluded from analysis. The data were submitted to frequency analysis (fast Fourier transformation [FFT]) and averaged for each recording position. The resulting power spectra were divided into 6 frequency bands: delta (0.98-3.4 Hz), theta (3.4-7.6 Hz), $alpha_1$ (7.6-9.0 Hz), $alpha_2$ (9.0-13.2 Hz), $beta_1$ (13.2-20.0 Hz), and $beta_2$ (20.0-30.0 Hz).

To detect changes in vigilance, subjects performed a simple tracking task on a video screen during intervals between phasic stimuli and between tonic pain estimations. Using a joystick, they had to keep a small square inside a larger one that moved randomly. The fraction of time the subjects could keep the small square inside the larger one was used to determine the "actual tracking performance."[15]

Blood Sampling and Analytical Procedures

Blood samples (10 mL) were drawn through an intravenous catheter and collected into EDTA tubes before and 20, 40, 60, and 90 minutes and 2, 2.5, 3, 3.5, 4, 5, and 6 hours after administration of the drug. After centrifugation at ~1200*g*, plasma was separated, and the samples were immediately frozen at –25°C. Acetaminophen and caffeine plasma concentrations were analyzed by high-performance liquid chromatography (HPLC) with UV detection. Acetaminophen was analyzed as published before.[2,32] Caffeine plasma samples were prepared by adding 50 µL perchloric acid and 50 µL internal standard stock solution (1 µg 8-Chlorotheophylline/mL distilled water) to 1 mL plasma followed by the addition of 100 µL ammonium sulfate buffer and 5 mL diethylether. After centrifugation, the organic layer was transferred into a glass tube and evaporated under a nitrogen stream. The residue was dissolved in 200 µL of mobile phase. Analytes were separated using a reversed-phase column (125/4 Nucleodur C18 Pyramid, Macherey-Nagel, Düren, Germany) and a C18 precolumn insert. The mobile phase consisted of a 95:4:1 (v/v) mixture of 50 mM sodium acetate (pH 4.0), acetonitrile, and tetrahydrofurane. The flow rate was 1.5 mL/min. UV detection was set at 273 nm. The intraday and interday assay precision and accuracy for a low (50 ng/mL), medium (250 ng/mL), and high concentration (1000 ng/mL) of caffeine were not more than 13.5% (low), 9.5% (medium), and 9.6% (high concentration), respectively. The reliable lower limit of quantification was 0.5 µg/mL for acetaminophen and 50 ng/mL for caffeine according to Shah et al.[33]

Pharmacokinetics Analysis

Descriptive data analysis was performed using the software package WinNonlin Professional (Version 3.3, Pharsight Corp, Mountain View, Calif). Peak plasma concentrations (C_{max}) and time to peak concentration (t_{max}) were obtained from individual plasma data. Each $AUC_{0\text{-}t}$ was calculated using the linear trapezoidal method for ascending concentrations and the log-trapezoidal method for descending concentrations.[34]

For bioequivalence tests, the software SAS for Windows (Version 8.2, SAS Institute Inc, Cary, NC) was used for statistical evaluation. A linear mixed-effects model was used to analyze the logarithmically transformed (natural log) AUC and C_{max} for acetaminophen using PROC MIXED in SAS. The residual variance from the model was used to construct confidence intervals for the difference between the test product and the reference treatment. These were back-transformed (antilogged) to give point estimates and confidence intervals for the ratio of the treatment least squares means.

t_{max} for acetaminophen was analyzed nonparametrically by the Wilcoxon sign rank tests. Median differences between formulations are presented with 95% confidence intervals (CIs) for the median difference based on the Hodges-Lehman 1-sample estimate. Bioequivalence for acetaminophen for C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ was determined if the 90% CI for the treatment mean ratio lay completely within the range from 0.80 to 1.25. For early AUCs, 95% confidence intervals were constructed to look for differences between treatments.

Pharmacokinetic-Pharmacodynamic Interrelation

Relationships between acetaminophen plasma concentrations and effects on evoked potentials estimates were assessed by means of compartmental pharmacokinetic-pharmacodynamic (PK-PD) modeling. Effects on

evoked potentials were modeled as differences between posttreatment measurements and baseline values of the statistically preselected PD parameters.

Using the software package WinNonlin Professional (Version 3.3), simultaneous fitting of PK and PD data was introduced. Models for PK and PD data were chosen with respect to residual plots (observed minus predicted values versus time), the Akaike information criterion (AIC), parameter precision, condition numbers, or F tests.

Inspecting individual plots representing plasma concentration versus effect data suggested hysteresis for data collected after administration of the test formulation with caffeine but not after the reference formulation without caffeine. In the case of hysteresis loops, an additional effect compartment was linked to the pharmacokinetic model.[35]

Data obtained after administration of both formulations were pooled separately for further evaluation. Concentration-effect relationship was best described by the following linear model:

$$E = E_0 + m \cdot C,$$

where E is the analgesic effect, E_0 is the effect at baseline, m is the slope of the relation, and C is the plasma or effect site concentration. Power models (ie, exponent $\neq$ 1) and E_{max} models were also tried but were rejected because they contained more parameters without improving the fit.

Discriminatory in vitro dissolution tests[36] and deconvolution of in vivo plasma concentrations of the test and reference formulations were performed to explore absorption differences.

Pharmacodynamic Analysis

Statistical evaluations and analyses were performed using SPSS 10.0 for Windows (SPSS Inc, Chicago, Ill). Phasic pain data after stimulation with 60% v/v CO_2 were used as primary efficacy variables because the weaker concentration of CO_2 showed significant effects in previous studies investigating nonopioid analgesics.[15,20] The parameters of the evoked potentials, power spectrum of EEG, tracking performance, and pain intensity after drug administration (phasic pain at 30, 50, 100, and 190 minutes and tonic pain at 72, 122, and 212 minutes) for each visit were transferred to absolute differences with respect to baseline values. Each parameter was analyzed in an analysis of variance for repeated measures (general linear model [GLM]), with within-subject factors of drug and time. Within this model, contrasts were calculated to test the null hypotheses of identical analgesic effects after administration of 1000 mg acetaminophen, its combination with 130 mg caffeine, and caffeine alone compared to placebo, provided that the GLM yielded significant drug effects (α = .05). Where appropriate to show differences other than to placebo, paired *t* tests were added.

RESULTS

Twenty-four subjects completed the study. There were 5 adverse event reports of mild to moderate headache: 3 with 1000 mg acetaminophen, 1 with 1000 mg acetaminophen plus 130 mg caffeine, and 1 with placebo.

Pain-Related Trigeminal Potentials

At recording positions Cz and Pz, we observed significant drug effects in the repeated-measures analysis of variance for amplitude P2 after stimulation of the nasal mucosa with 60% v/v CO_2 (Cz: $P < .05$, F = 2.9 and Pz: $P < .05$, F = 3.6). Within-subject contrasts showed significant reductions in pain-related ERPs (amplitude P2) at these recording positions for acetaminophen (at 30 minutes after medication; Cz: $P < .01$, F = 8.0; Pz: F = 10.4) and for acetaminophen with caffeine (at 30 minutes; Cz: $P < .05$, F = 7.1; Pz: F = 5.9 and at 190 minutes; Pz: F = 7.4) compared with placebo (for *P* values at recording position Pz, see Figure 1).

There were no further consistent and overall drug effects visible in the evoked potentials at recording positions Fz, C3, or C4 after stimulation of the nasal mucosa with 60% v/v CO_2.

Pain Intensity

There was no statistically significant effect of study medication on phasic pain intensity estimates.

Repeated-measures analysis identified significant drug effects for tonic pain estimates ($P < .001$, F = 7.0). There were statistically significant reductions in pain intensity at all time points after tonic pain stimulation following administration of acetaminophen with caffeine (72 minutes after medication: F = 11.4; at 122 minutes: F = 10.4; and at 212 minutes: F = 8.8; see Figure 2 for *P* values). The differences in pain intensity recorded between acetaminophen with caffeine and acetaminophen alone were also statistically significant (paired *t* tests: at 72 minutes: $P < .05$, $T = -2.7$; at 122 minutes: $P < .05$, $T = -2.7$; at 212 minutes: $P < .01$, $T = -3.0$; compare Figure 2).

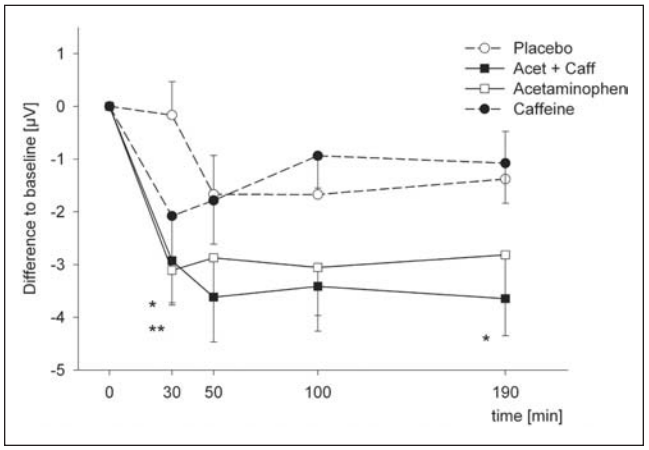

*Figure 1. Effects of the study medications on pain-related evoked potentials after phasic stimulation with 60% v/v CO_2. A significant reduction of amplitude P2 could be shown 30 minutes after administration of acetaminophen and its combination with caffeine at recording position Pz. There was also a significant reduction in amplitude P2 at 190 minutes after application of the combination drug (mean and SEM; n = 24). Contrasts to placebo with *P < .05 and **P < .01.*

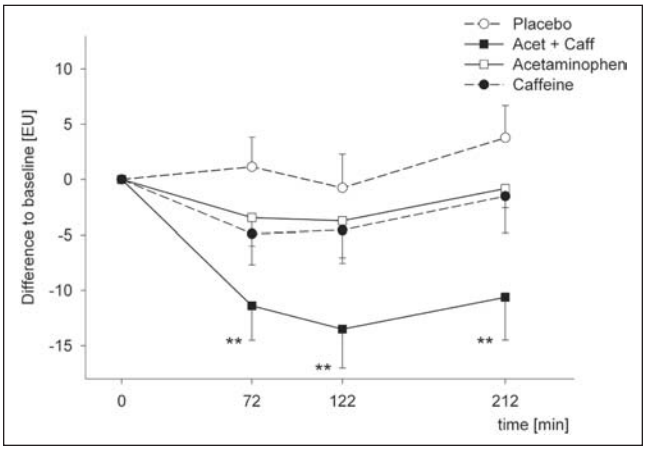

*Figure 2. Effects of the study medications on tonic pain ratings (EU, estimation units from the visual analog scale). A significant reduction of estimates could be shown during the entire observation time after administration of acetaminophen plus caffeine (mean and SEM; n = 24; contrasts to placebo with **P < .01). This effect on tonic pain was also significant compared to acetaminophen alone (*t *tests).*

Treatments with acetaminophen alone or caffeine alone as compared to placebo did not result in significant changes in pain intensity after tonic pain stimulation.

Tracking Performance and Background EEG (FFT)

Tracking performance was significantly improved following administration of both caffeine formulations during phasic and tonic pain stimulation. The effect following 60% v/v CO_2 phasic pain was more sustained ($P < .05$, $F = 3.1$), lasting for up to 110 minutes, than following 70% v/v CO_2 phasic pain ($P < .05$, $F = 3.0$), lasting 30 minutes. Improvement in tracking performance during tonic pain was sustained up to 212 minutes with the caffeine formulations (factor drug: $P < .001$, $F = 8.0$; see Figure 3). No changes in tracking performance compared with placebo were observed for acetaminophen alone.

There was a significant decrease ($P < .05$) in power density at all recording positions and in all frequency bands after drug administration, from 25 to 195 minutes during phasic pain and from 60 to 200 minutes during tonic pain, with both caffeine-containing medications compared to placebo. The difference was visible in the lower frequency bands (delta and theta) and most pronounced in the $beta_1$ frequency band and in the frontal recording position Fz. The decrease in power density was also seen for the total power, indicating a shift to higher frequency bands (>20 Hz). This EEG effect is in line with an arousal elicited by caffeine. No differences in power density were observed between acetaminophen and placebo.

Plasma Concentration, Bioequivalence, and Dissolution Tests

Table I gives a summary of the pharmacokinetic data comparing both acetaminophen formulations. The slightly faster rate of absorption of acetaminophen after administration of the combination formulation was reflected in an increase of early AUC values (Figure 4). The 2 formulations were bioequivalent for the extent of absorption ($AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$). C_{max} was not bioequivalent as the upper limit of the 90% CI was just outside the defined range. There was no significant difference between treatments for t_{max}. A significant difference between formulations was seen in favor of the combination formulation for $AUC_{0\text{-}20\ min}$. The $AUC_{0\text{-}40\ min}$ was also numerically much greater for the caffeine-containing formulation but did not reach statistical significance (Table I).

In vitro dissolution tests revealed a faster dissolution for the reference acetaminophen compared with the test formulation, including caffeine (Figure 5A). The deconvolution of averaged in vivo plasma concentrations for both formulations indicated a higher

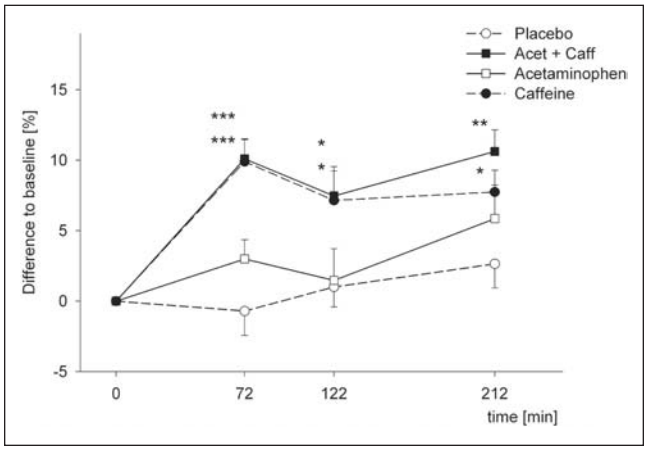

*Figure 3. Effects of the study medications on changes in tracking performance. The volunteers were exposed to tonic pain. All caffeine-containing medication showed significant enhanced performance compared to placebo during the observation time of 3.5 hours (mean and SEM; n = 24). Contrasts to placebo with *P < .05, **P < .01, and ***P < .001.*

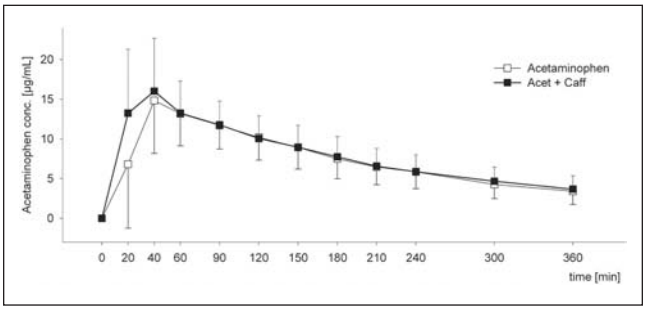

Figure 4. Acetaminophen plasma concentrations after oral administration of 1000 mg acetaminophen (white squares) and 1000 mg acetaminophen plus 130 mg caffeine tablets (black squares) to healthy volunteers (mean and SD; n = 24). Faster rate of absorption after administration of the caffeine formulation was reflected by increased early AUCs.

and faster absorption rate for acetaminophen in combination with caffeine (Figure 5B).

Pharmacokinetic-Pharmacodynamic Interrelations

The amplitude P2 at recording position Pz was chosen as an effect measure for PK-PD analysis. Similar reductions in P2 amplitude were observed for both acetaminophen formulations over 200 minutes when acetaminophen concentrations were above 8 µg/mL.

Phasic pain parameters of the PK-PD linked model are given in Table II for naive averaged and pooled data sets. Clockwise hysteresis was present between the acetaminophen plasma concentration after administration of the caffeine formulation and the amplitude P2 at recording positions Pz and Cz. The equilibration half-life between plasma and the effect site was 12 (± 4.1) and 19 (± 5.7) minutes for the reduction in amplitude P2 at recording position Cz and Pz,

Table I Summary of Results From Analysis of Pharmacokinetic Variables for Acetaminophen Comparing Both Formulations

Pharmacokinetic Variable	Least Squares Means: Acetaminophen Plus Caffeine	Least Squares Means: Acetaminophen[a]	Point Estimate[b]	CI for Point Estimate (*P* Value)
C_{max}, mg/L[c]	18.9	17.1	110.7	$95.7, 128.2^{90}$
$AUC_{0\text{-}90\ min}$, mg·h/L[c]	16.9	14.5	116.3	$95.5, 141.7^{90}$
$AUC_{0\text{-}t}$, mg·h/L[c]	46.7	43.7	106.7	$100.6, 113.2^{90}$
$AUC_{0\text{-}\infty}$, mg·h/L[c]	57.4	53.1	108.1	$101.4, 115.4^{90}$
t_{max}, h	0.67[d]	0.67[d]	–0.17[e]	$-0.43, 0.16^{95}$ (.3861)
$AUC_{0\text{-}20\ min}$, mg·h/L[c]	1.4	0.7	187.2	$100.7, 348.0^{95}$ (.0476)
$AUC_{0\text{-}40\ min}$, mg·h/L[c]	5.3	3.7	145.8	$86.4, 246.1^{95}$ (.1495)
$AUC_{0\text{-}60\ min}$, mg·h/L[c]	10.2	8.1	125.9	$87.4, 181.5^{95}$ (.2049)

The 90, 95 superscripts indicate the size of the confidence interval (CI); *P* value presented for 95% CI.
a. Reference mean.
b. Difference in least squares mean of logged data and antilogged to represent treatment mean as a ratio of the reference mean, except for t_{max}.
c. Analysis based on logarithmically transformed data; back-transformed results are presented.
d. Medians are presented.
e. Hodges-Lehman estimator for the median difference.

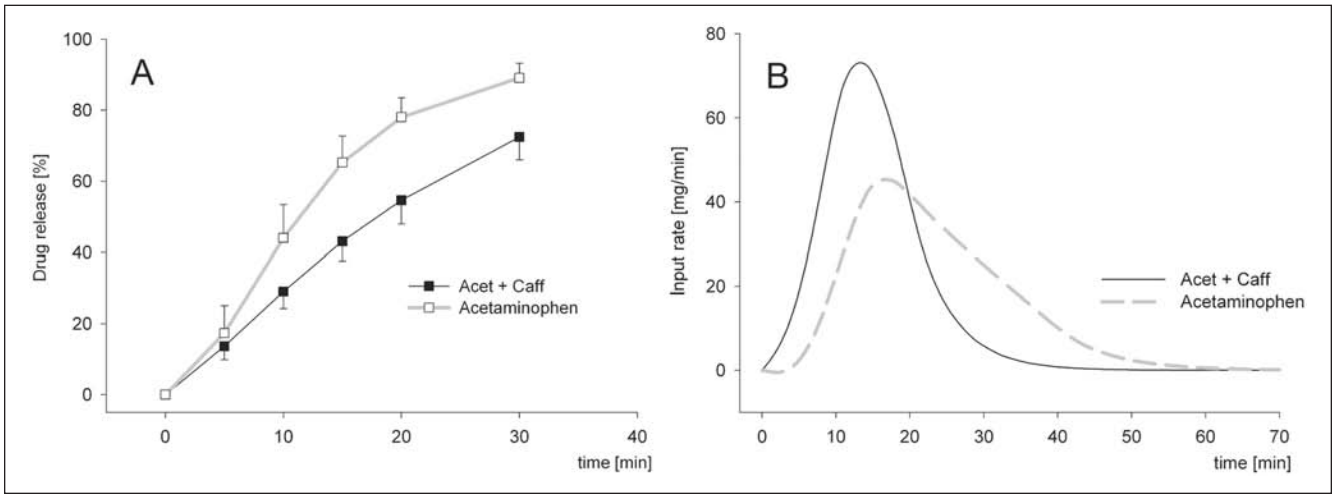

Figure 5. (A) In vitro dissolution and (B) in vivo deconvolution data for both acetaminophen formulations. (A) In vitro dissolution test (USP paddle, 0.05 M HCl, 30 rpm; n = 5, mean ± SD) revealed slower acetaminophen release from the combination tablet (Acet + Caff). (B) However, the in vivo deconvolution data from averaged acetaminophen plasma values demonstrate the increased rate of appearance of acetaminophen in plasma seen for the combination formulation.

respectively. The relationship between acetaminophen concentrations at effect site and effects on PD parameters were best described by a linear model. Fixing slope (m) to 0 significantly worsened the fit of all selected PD parameters. This further supports the statistical significance of the relation between acetaminophen plasma concentrations and the observed changes in EP amplitudes. Results from the naive pooled data set are shown in Figure 6 for predicted PK and PD data.

Table II Parameters of Pharmacodynamic Modeling of Acetaminophen-Induced Changes in Amplitude P2 of Pain-Related Evoked Potentials

	Values of the PK-PD Model: Naive Averaged Data Set			
	Slope m, μV/(μg/mL)		k_{e0}, min^{-1}	
PD Parameters at Recording Position	**Estimated Values (and SEE)**	**Variability (CV in %)**	**Estimated Values (and SEE)**	**Variability (CV in %)**
P2 at Pz (Acetaminophen)	–0.258 (0.029)	11.40	—	—
P2 at Pz (Acet + Caff)	–0.335 (0.035)	10.70	0.0369 (0.0112)	30.26
P2 at Cz (Acetaminophen)	–0.241 (0.032)	13.37	—	—
P2 at Cz (Acet + Caff)	–0.280 (0.027)	9.78	0.0599 (0.0213)	35.57
	Values of the PK-PD Model: Naive Pooled Data Set			
	Slope m, μV/(μg/mL)		k_{e0}, min^{-1}	
PD Parameters at Recording Position	**Estimated Values (and SEE)**	**Variability (CV in %)**	**Estimated Values (and SEE)**	**Variability (CV in %)**
P2 at Pz (Acetaminophen)	–0.257 (0.035)	13.55	—	—
P2 at Pz (Acet + Caff)	–0.335 (0.055)	16.35	0.0371 (0.0172)	46.39
P2 at Cz (Acetaminophen)	–0.240 (0.036)	14.93	—	—
P2 at Cz (Acet + Caff)	–0.280 (0.047)	16.88	0.0590 (0.0359)	60.84

Phasic pain values of the pharmacokinetic-pharmacodynamic (PK-PD) linked models using naive averaged (top) and naive pooled (bottom) data for both acetaminophen formulations (n = 24). SEE, standard error of estimate; CV%, coefficient of variation in percentage (Acetaminophen = formulation 1000 mg acetaminophen, Acet + Caff = formulation 1000 mg acetaminophen plus 130 mg caffeine).

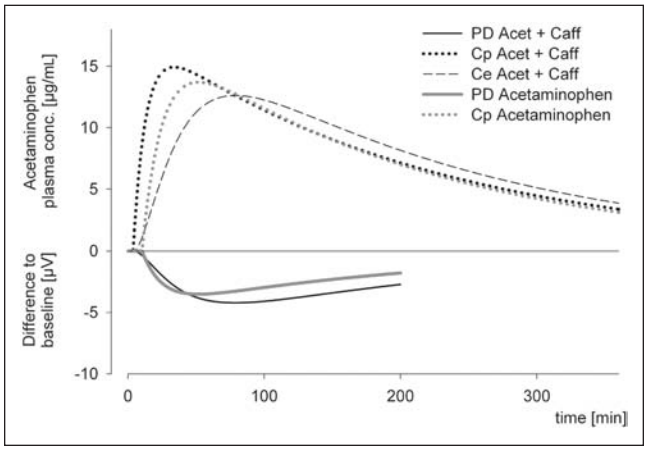

Figure 6. Phasic pain pharmacokinetic-pharmacodynamic modeling results using naive pooled data sets for recording position Pz and amplitude P2 based on pharmacodynamic (PD) parameters as shown in Table II. Data collected after administration of acetaminophen alone and with caffeine are presented in gray and black, respectively. Predicted PD data are depicted in solid lines and predicted plasma PK data in dotted lines. According to the hysteresis loop, effect site concentration Ce was used for the formulation with caffeine (dashed line = effect site concentration of acetaminophen in the presence of caffeine).

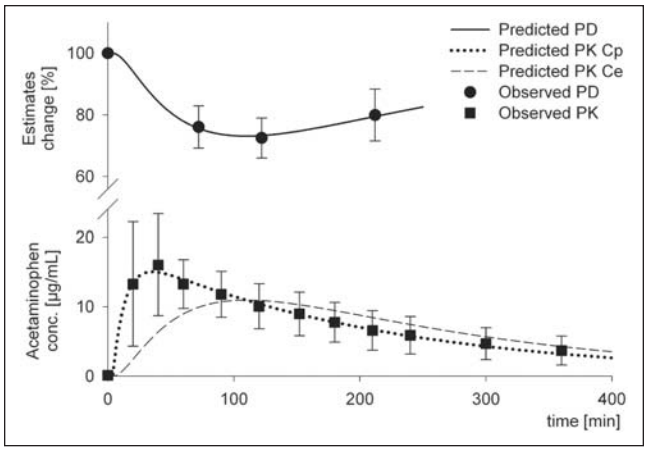

Figure 7. Pharmacokinetic-pharmacodynamic (PK-PD) modeling results using naive averaged data sets for tonic pain estimates after administration of acetaminophen plus caffeine. Upper part: predicted (solid line) and observed mean reduction in estimates (circles with error bars for SEM). Lower part: observed (squares with error bars for SD) and predicted plasma (Cp) and effect site (Ce) concentration of acetaminophen.

Pharmacokinetic/pharmacodynamic results for tonic pain were obtained by the same linear model using naive averaged PD data from the acetaminophen plus caffeine formulation. The predicted and observed data for tonic pain are shown in Figure 7. Modeling parameters were as follows: slope m = –2.46 (± 0.07)%/(µg/mL) and k_{e0} = 0.0194 (± 0.0013) min^{-1}. The estimated equilibration half-life between plasma and effect site was 36 (± 2.4) minutes.

DISCUSSION

Caffeine-containing over-the-counter analgesics still comprise a major fraction of this market. Many experts and consumers appear to favor such combinations.[37] We explored the combined effect of acetaminophen plus caffeine in a model of experimental pain. It allows for constant measurement of drug concentrations and vigilance as well as pain-related evoked potentials and subjective estimates of perceived pain.[14,15] We could show that caffeine enhances early absorption of acetaminophen and increases vigilance and arousal. In addition, the combination of acetaminophen plus caffeine was significantly superior compared to acetaminophen or caffeine alone in reducing perceived tonic pain throughout the whole measurement period (ie, between 1 and almost 4 hours). The same long-lasting effect was observed in objective parameters (ie, pain-related evoked potentials).

Several clinical studies have indicated that caffeine may enhance the analgesic activity of cyclooxygenase inhibitors in patients.[38-41] On the other hand, all attempts to show a direct analgesic effect of caffeine in human studies have been unsuccessful.[42,43] Recently, some hypoalgesic activity of caffeine was described in humans suffering from exercise-induced muscle pain[44,45] or ischemic muscle pain.[46] These effects could result, however, from an increased blood supply to the musculature and may not reflect true analgesic activity. A few years ago, we showed that caffeine enhances the analgesic activity of propyphenazone in our experimental pain model.[47] This was unexpected and had to be taken as a preliminary finding because at that time, we did not have a "caffeine-only" control group. We did, however, monitor the pharmacokinetics in all volunteers and found no significant acceleration of propyphenazone absorption.[47] We concluded that the analgesic effect could not result from pharmacokinetic effects. It remained unexplained.

In our actual experiments, the caffeine treatment per se showed no antinociceptive or analgesic effect, but it increased the effect of acetaminophen. The observation from the present study appears noteworthy for 2 reasons.

1. The pharmacokinetic data show the "acetaminophen plus caffeine" and the "acetaminophen-only"

formulations to be bioequivalent for acetaminophen with respect to the bioavailability (later AUCs). However, the early AUC values and the peak plasma concentration (C_{max} value) for acetaminophen were higher when acetaminophen was given together with caffeine. A higher rate of absorption of acetaminophen from the caffeine formulation has been demonstrated in previous studies.[11,12,48] The reasons include enhanced blood flow in the gastrointestinal mucosa and possible decrease in early clearances. Both drugs are substrates of cytochrome P450 1A2.[49,50] Because caffeine weakly inhibits the enzyme,[51,52] this might contribute to the higher values of the early AUCs of acetaminophen in the combination drug. It is unlikely to contribute much because the total AUC values of acetaminophen alone as compared to acetaminophen plus caffeine were bioequivalent. The elevated C_{max} values and the higher early AUCs of acetaminophen could explain the slightly increased antinociceptive effects of acetaminophen plus caffeine at early time points. In addition, we observed an increased antinociceptive effect at later time points. A correlate for this was found in our PK-PD analysis (amplitude P2). Here we observed a prolonged persistence of effect site concentrations for acetaminophen if caffeine is present (see Figure 6). This long-lasting effect was also reflected in significantly reduced amplitude P2 at 190 minutes postdose when comparing acetaminophen plus caffeine and placebo (see Figure 1). In the present study, the amplitude of ERPs was decreased by 40% with regard to placebo after administration of acetaminophen plus caffeine. Compared with other analgesics investigated with the same model, the magnitude of effect of the combination drug was superior to that produced by 800 mg ibuprofen (28%)[17] or 1000 mg acetylsalicylic acid (28%)[15] and is comparable with that observed after administration of 1200 mg azapropazone (35%),[20] 14 mg intravenous morphine (40%),[25] or 0.2 mg fentanyl (32%).[53]

2. The significant increase of analgesic activity of the combination documented for tonic pain compared to placebo and acetaminophen alone lasted for the whole time period from 1 to 3.5 hours. Such long-lasting effects on tonic pain ratings were observed previously only after treatment with the sustained-release preparation of 100 and 200 mg tramadol.[26] In our actual study, we were able to describe this longer lasting effect by compartmental PK-PD modeling (see Figure 7). We observed quite different equilibration half-life times as compared to phasic pain (19 vs 36 minutes), suggesting that different effect compartments are involved for the processing of tonic and phasic pain. These different compartments may reflect the involvement of C and A_{delta} nerve fibers. On a physiological basis, phasic pain is thought to be mediated by A_{delta} fibers, whereas tonic pain also includes C fiber activation and represents inflammatory pain on subjective ratings.[27,54] The effect on tonic pain was significantly higher not only compared to placebo but also compared to acetaminophen alone, which further supports a combination or additive effect of acetaminophen if caffeine is present. We have to conclude, therefore, that caffeine causes a significantly enhanced and sustained antinociceptive effect of acetaminophen at least in this model of experimental pain. The question remains by what molecular mechanism(s) this effect might be mediated.

Caffeine is generally regarded as a weak phosphodiesterase inhibitor but also as an adenosine receptor antagonist. The phosphodiesterase inhibition becomes prominent in humans only after fairly high caffeine doses (ie, above 1000 mg[55-57]) and is unlikely at the dose of caffeine given in our experiments (130 mg). The same holds true for the contention that caffeine may stimulate the production of nitric oxide (NO) by activating the cyclic guanosine monophosphate (GMP) pathway: NO might have antinociceptive activity.[58,59] Again, the dose of caffeine given in our experiments appears too small to produce this effect. In addition, most authors suggest that NO released in the central nervous system rather acts proalgesic than antialgesic.[60,61] Consequently, this explanation is not very convincing either.

Other mechanisms may be involved. For example, it has been speculated that cholinergic mechanisms initiated by caffeine may contribute to antinociceptive activity.[62] Again, solid evidence is lacking. Central nervous system (CNS) stimulants such as caffeine have been shown to increase the release of neurotransmitters such as serotonin and noradrenaline in animals,[63,64] but high doses (50 mg/kg) had to be administered.

The antagonistic effects at adenosine receptors are seen at lower doses compared to the inhibitory action of caffeine on phosphodiesterase. They appear more likely to contribute to the antinociceptive effect observed. Still, recent evidence rather hints at an antinociceptive effect of adenosine agonists[65] and not of adenosine antagonists as caffeine. On the other hand, animal studies from the same authors show antinociceptive effects of caffeine in the hot-plate and formalin test.[63] This discrepancy may be explained by different actions of adenosine activators and inhibitors on presynaptic and postsynaptic receptors and the site of action in the CNS.[62,66]

A new interpretation may come from recent observations from our group. It seems possible that caffeine activates inhibitory (antihyperalgesic) glycinergic transmission.[67] Recently, Yang and colleagues[68] have found adenosine to suppress glycinergic transmission via presynaptic A1 receptors in the substantia gelatinosa of rats. This would imply that caffeine could enhance the inhibitory glycinergic transmission and thus enhance antinociception. Indeed, inhibitors of PGE_2 production have been shown by our group to act along this line to antagonize hyperalgesia by blocking PGE_2 production, which curbs glycinergic inhibition of nociception in the spinal cord postsynaptically.[69-71]

Such synergistic effects were described in primary rat microglial cells.[72] Also, the cyclooxygenase inhibitory activity of acetaminophen may be underestimated in the past, as shown by recent data.[73-76] This would imply a specific synergistic effect of caffeine with cyclooxygenase inhibitors on the central and/or spinal level, which we have observed here.

Unfortunately, our human pain model is not suited to elucidate the molecular mechanisms that might be contributing to the sustained antinociception observed with the acetaminophen plus caffeine combination. However, in view of our results concerning the effects of cyclooxygenase inhibitors on glycine, it appears worthwhile to investigate if caffeine has an effect on glycinergic inhibition. Such animal experiments are in progress at present. If so, it would explain our observation, and this could also help researchers to understand why common wisdom has always advocated coadministration of caffeine plus inhibitors of cyclooxgenases.

In conclusion, in this study, we provide evidence of a sustained antinociception-enhancing effect of caffeine when given with acetaminophen. Similar results have been observed before with propyphenazone plus caffeine. These observations may not have a conclusive mechanistical explanation at present, but the assumption that caffeine may enhance glycinergic inhibition of nociception appears attractive. More research is needed to add proof to this contention. If this contention turns out to be true, such an effect of caffeine would explain why caffeine is reportedly effective as an enhancer of analgesia produced by a weak inhibition of prostaglandin production in the CNS by, for example, aspirin (low doses), phenazone derivates, and acetaminophen. Moreover, it would hint at a new action to enhancing the antinociceptive activity of cyclooxygenase inhibitors.

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