

# A Randomized, Controlled Trial Validates a Peripheral Supra-Additive Antihyperalgesic Effect of a Paracetamol–Ketorolac Combination

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**Abstract:** The combination of paracetamol with non-steroidal anti-inflammatory drugs (NSAIDs) is widely used; however, the nature and mechanism of their interaction are still debated. A double-blind, pharmacokinetic/pharmacodynamic, randomized, cross-over, placebo-controlled study was carried out in human healthy volunteers. The aim was to explore the existence of a positive interaction between paracetamol 1 g and ketorolac 20 mg administered intravenously on experimental pain models in human beings. The effects of the paracetamol–ketorolac combination were compared with similar doses of respective single analgesic and to placebo on the sunburn model (UVB-induced inflammation), cold pain tolerance and the nociceptive flexion reflex. The kinetics of the plasma concentrations of paracetamol and ketorolac were measured using 2D-liquid chromatography-mass spectrometry. Thirteen volunteers were screened, and 11 completed the study. Ketorolac significantly decreased primary hyperalgesia to heat stimuli compared with paracetamol ( $p < 0.014$ ). The combination performed better than paracetamol ( $p < 0.001$ ) and placebo ( $p < 0.042$ ), increasing heat pain threshold by 1.5°C. The combination radically reduced primary hyperalgesia to mechanical stimulation (39%) compared with placebo ( $p < 0.002$ ) and single agents (paracetamol  $p < 0.024$  and ketorolac  $p < 0.032$ ). The combination also reduced, slightly although significantly, the intensity of pain (10%) for the cold pressor test (*versus* placebo:  $p < 0.012$ , paracetamol:  $p < 0.019$  and ketorolac  $p < 0.004$ ). None of the treatments significantly affected the central models of pain at this dosage level. No pharmacokinetic interactions were observed. These results suggest a supra-additive pharmacodynamic interaction between paracetamol and ketorolac in an inflammatory pain model. The mechanism of this interaction could mainly rely on a peripheral contribution of paracetamol to the effect of NSAIDs.

Multimodal analgesia involves a combination of different classes of analgesics to improve pain relief and reduce the adverse effects of drugs [1]. The combination of paracetamol with non-steroidal anti-inflammatory drugs (NSAIDs) is widely used in clinical practice although it has been demonstrated to be effective in pain management only in few clinical studies [2–4]. The putative rationale underlying this association is their different site and/or mode of action (central *versus* peripheral, serotonin and/or cannabinoid systems *versus* prostaglandin synthesis) [5]. The mechanism of action of paracetamol is yet to be fully elucidated [6]. Much evidence supports the hypothesis of a central analgesic effect [7], while the other evidence supports a peripheral effect [8]. The prostaglandin H<sub>2</sub> synthase (PGHS) [9], the serotonergic system [10,11] and/or the cannabinoid system [12,13] have been proposed as potential targets. NSAIDs essentially act by inhibiting prostaglandin biosynthesis at the site of the inflammation [14]. However, some NSAIDs also exhibit a central action [15]. Experimental data suggest that indomethacin [16], ibuprofen [17] and ketoprofen [18] are centrally act-

ing NSAIDs, while ketorolac [19] or acetylsalicylic acid [20] are mainly peripherally acting agents. The central effect of the former could be because of pharmacokinetic factors and the ability of one drug to enter the central nervous system (CNS) [15]. Indomethacin, ketoprofen [21] and ibuprofen [22] are rapidly detected in the cerebrospinal fluid after a systemic administration, whereas acetylsalicylic acid is not [21].

There are various methods available in human experimental pain research to quantitatively assess various aspects of pain [23], and these are useful tools to characterize the analgesic effects of drugs [24]. Models provoking primary and secondary hyperalgesia are relevant and mimic clinical pain, which is often associated with signs of primary and secondary hyperalgesia. Primary and secondary hyperalgesia can be induced by burn injury, capsaicin, freeze injury or ultraviolet irradiation [25]. The sunburn model, in which UVB irradiation induces stable primary and secondary hyperalgesia [26], was chosen in our study because such stability provides an ideal setting for pharmacokinetic/pharmacodynamic studies. The primary area of hyperalgesia induced by UVB irradiation is explained by the sensitization of peripheral nociceptor terminals and peripheral pain hypersensitivity, while the secondary hyperalgesia involves a central mechanism of sensitization [27].

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This study aimed at experimentally assessing in healthy volunteers: (i) the peripheral and central analgesic efficacy of a combination of paracetamol with ketorolac administered intravenously; (ii) to compare it with single agents and respective placebo. According to the hypothesis, the combination would result in an increased pharmacodynamic effect on an inflammatory model of pain.

### Materials and Methods

**Study design.** This pharmacokinetic/pharmacodynamic randomized study of different treatments included a four-session cross-over design. As per the Latin square cross-over design, the volunteers were allocated to one of the four possible treatment sequences. The Latin square ensured that each volunteer received all treatments and that each treatment pair (e.g. placebo followed by paracetamol) occurred at the same frequency [28]. The four study arms were as follows: (i) combined intravenous (iv) paracetamol 1 g and iv ketorolac 20 mg; (ii) iv paracetamol 1 g; (iii) iv ketorolac 20 mg; and (iv) placebo. An independent pharmacist prepared a concealed allocation schedule by randomization of these sequences, in blocks of four, in a consecutive number series. The enrolled volunteers were assigned in turn to the next consecutive number, and the corresponding series of study drugs were dispensed. The volunteers and the investigator were blindfolded.

The study was approved by the local Ethics Committee and the Swiss Agency for Therapeutic Products (Swissmedic) and was conducted in accordance with Good Clinical Practice.

**Study drugs.** Drugs were given intravenously as a 100 ml solution plus a 0.66 ml solution in syringe to maintain a double-blinding, double-dummy design. The 100 ml ready-to-use solution contained 1 g paracetamol (Perfalgan®, Bristol-Myers Squibb, Baar, Switzerland) or placebo (sodium chloride 0.9%) and was administered as a 10-min. iv infusion. The 0.66 ml solution in syringe contained 20 mg ketorolac (Tora-Dol®, Hoffmann-La Roche, Reinach, Switzerland) or placebo (sodium chloride 0.9%) and was administered by iv bolus.

**Volunteers.** In this study, twelve healthy, pain-free, non-smoking, skin type III, 20 to 50-year-old, male volunteers were recruited. Skin type III has been described by Fitzpatrick as individuals presenting a slightly tender burn 24 hr after an initial sun exposure (i.e. 45–60 min. of noon exposure in northern latitudes in the early summer) and a moderate tan at 7 days [29].

The exclusion criteria involved contraindications to paracetamol and NSAIDs and intake of other analgesics and skin diseases. After adequate verbal and written information, written informed consent was obtained.

**Pain models. UVB model.** The sunburn inflammation was induced as described previously [30]. The volunteers were exposed to twice the minimal erythema doses of UVB light (UV801KL; Waldmann, Villingen-Schwenningen, Germany) on a 3 × 3 cm area on the glabrous part of the non-dominant forearm. A different area was irradiated during each study session. The minimal erythema dose was estimated according to the skin type of the volunteers. It was seen that erythema and localized hyperalgesia developed within hours in all the selected volunteers, reaching a maximum 20 hr after the irradiation and remained stable for at least 30 hr [26].

**Area of secondary hyperalgesia to pinprick.** This area was determined on the skin surrounding the erythema using a rigid von Frey hair (462 mN) [31]. The borders of hyperalgesia were determined by stimulating along eight linear paths, which started outside the injury, and by moving towards the erythema until a change in sensation was experienced. The points were marked on a transparent sheet. Subsequently, a photocopy was made, and the area was cut and

weighed on an analytical balance. The area was calculated according to the weight by the use of standards of 9 and 25 cm<sup>2</sup>.

**Heat pain threshold.** The heat pain threshold was defined as the lowest temperature producing pain and was used to reflect the sensitivity to heat stimulation in the primary hyperalgesic area. It was assessed using a 9-cm<sup>2</sup> Peltier thermode (Medoc Advanced Medical Systems, Ramat-Yishai, Israel) placed in contact with the erythematous skin [32]. The skin adaptation temperature was 32°C, the rate of temperature change was 1°C/sec., and the maximum temperature was 50°C.

**Mechanical pain threshold.** The mechanical pain threshold was defined as the lowest pressure producing pain and was used to reflect the sensitivity to mechanical stimulation. It was assessed using an electronic von Frey device (Bioseb, Id-Tech Bioseb, Chaville, France) as described previously [33]. An increasing punctuate pressure was applied up to the point of detection of mechanical pain threshold. Each mechanical pain threshold value was averaged from three separate consecutive measurements at different points in the test area. Three skin zones were evaluated: zone of primary hyperalgesia, zone of secondary hyperalgesia and the control skin zone (contralateral side). The zone of primary hyperalgesia was the area within the injury site, whereas the zone of secondary hyperalgesia was in adjacent areas [26].

**Nociceptive flexion reflex.** The nociceptive flexion reflex was assessed by electromyography (Nicolet Viking IV; Nicolet, Madison, Wisconsin) as described previously [34]. The nociceptive flexion reflex was determined in response to single electrical stimulations (series of 30–40) of a fixed duration (0.5 msec.) randomly applied transcutaneously on the sural nerve with variable intensities (0–100 mA) and under a constant current stimulator voltage (100 µV) (Nicolet Viking IV, Nicolet Madison, Wisconsin, USA). The nociceptive flexion reflex was recorded by electromyography on the ipsilateral biceps femoris. The nociceptive flexion reflex threshold was defined as the intensity of current eliciting 50% of positive responses. This was determined by fitting the data to the equation of Hill using the Marquardt algorithm. The subjective pain thresholds in response to experimental electrical stimulation were evaluated simultaneously with the nociceptive flexion reflex using a numerical rating scale (0–10).

**Cold pressor test.** The cold pressor test was used, as described previously [34], to assess the pain tolerance using a bath in which the water was maintained at ~0°C with a stirring device ('in-house' device). The volunteers were instructed to place their dominant hand until they felt 'the maximal bearable pain'. The pain tolerance was defined as the time interval between immersion and withdrawal of the hand. A cut-off time of 120 sec. was set for safety reasons. Additionally, the volunteers were also asked to continually rate the pain intensity on an electronic visual analogue scale (Douleur MS-DOS; Gillequin, Geneva, Switzerland). The pain intensity was analysed at 10 and 15 sec. of immersion.

**Measurements.** Volunteers were familiarized with the test procedures prior to the commencement of the study. The four study sessions were conducted at least a week apart. Before the UVB irradiation, the heat pain threshold was measured to assess the extent of hyperalgesia, by comparing pre- and post-irradiation values. The sunburn inflammation was induced 20 hr before each study session. The sensory tests were performed in a standardized sequence: (i) mechanical pain threshold; (ii) area of secondary hyperalgesia; (iii) heat pain threshold; (iv) nociceptive flexion reflex; (v) cold pressor test, at baseline and 1, 2, 4 and 6 hr after drug administration. The UVB-related tests were also assessed at 24 hr after drug administration, representing 44 hr after UVB irradiation (20 hr after irradiation + 24 hr after drug administration). The blood samples were taken at baseline and 0.25, 0.5, 1, 2, 4, 6 and 24 hr after drug administration.

**Pharmacokinetic profile in plasma.** The blood samples (12 ml) were drawn into EDTA tubes. The plasma concentrations of paracetamol and ketorolac enantiomers were determined by 2D-liquid chromatography-mass spectrometry as described elsewhere [35]. Pharmacokinetic data analysis was performed using WinNonlin® 5.1 (Pharsight Corporation, Mountain View, CA, USA).

**Statistical analysis.** The primary end-point was fixed as a change in heat pain threshold in the area of primary hyperalgesia. This end-point was used to calculate the sample size. On the basis of previous variance estimates, it was calculated that twelve volunteers would provide an 80% probability of detecting a 1.5°C difference between the treatments ( $\alpha = 0.05$ , two-sided). The secondary outcome was changes in mechanical pain threshold, in the area of secondary hyperalgesia to pinprick, in the nociceptive flexion reflex and in the cold pain tolerance. The changes in the pain threshold were calculated by the difference between the post-treatment and the baseline values.

Statistical analysis was performed using SPSS 11.0 (SPSS Inc, Chicago, IL, USA). All results were expressed as the mean  $\pm$  S.D. The trapezoidal method was used to calculate the area under the drug effect–time curves (AUEC) from 0 to 6 hr. The AUECs were compared using Friedman analysis of variance and a subsequent post hoc test (Wilcoxon signed rank test). For the cold pressor test, the changes in pain intensity were compared using Friedman analysis of variance and a subsequent post hoc test (Wilcoxon signed rank test). The statistical significance was defined as  $p \leq 0.05$ .

## Results

### Volunteers.

Thirteen volunteers were screened for this study. Two volunteers withdrew prior to the commencement of the study. One volunteer withdrew for unknown reasons, while the other one withdrew for medical reasons. Eleven volunteers successfully completed the study. The participants were 26.0  $\pm$  9.8 years old.

### Adverse events.

**Sunburn.** None of the volunteers reported any pain during the UVB irradiation or any spontaneous pain associated with the erythema. Further, no blisters were observed.

**Study medications.** Both paracetamol and ketorolac were well tolerated. Mild pain at the injection site (ketorolac  $N = 3$  and placebo  $N = 1$ ) and epigastralgia (ketorolac  $N = 2$ ) were observed. The outcome was spontaneously favourable in all cases.

### Characteristics of UVB-induced hyperalgesia.

The UVB irradiation caused reproducible skin inflammation in all volunteers, which was evident by visible erythema (fig. 1). In addition, the peripheral sensitization and inflammation translated into primary hyperalgesia with an increased sensitivity to heat and mechanical stimulation, observed as a statistically significant decrease in the heat pain threshold from 46.6  $\pm$  1.1°C (pre-irradiation values) to 40.1  $\pm$  1.7°C ( $p < 0.001$ ) and a statistically significant decrease in the mechanical pain threshold from 198.8  $\pm$  76.5 g to 74.7  $\pm$  34.8 g, representing a decrease of 62  $\pm$  14% ( $p < 0.002$ ). Furthermore, the UVB irradiation also resulted in the devel-

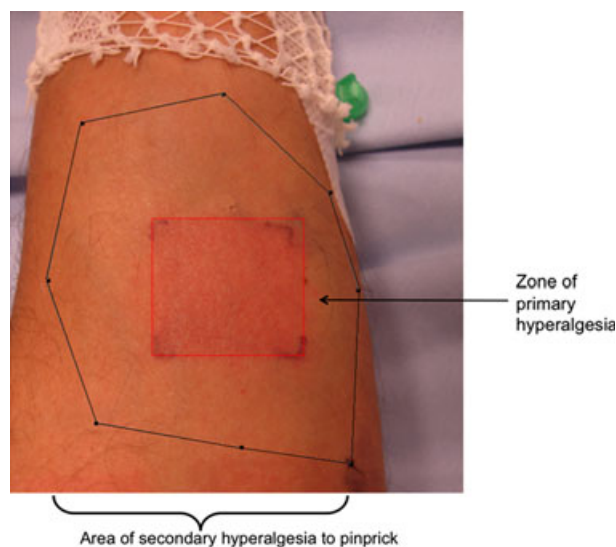


Fig. 1. Zones of primary and secondary hyperalgesia induced by UVB. The primary hyperalgesia was limited to the area of injury (highlighted square), and the secondary hyperalgesia developed in undamaged skin surrounding the injury.

opment of an area of secondary hyperalgesia of 21.3  $\pm$  11.3 cm<sup>2</sup>, measured 20 hr after the irradiation (fig. 1). However, two of the eleven volunteers did not develop hyperalgesia to pinprick outside the irradiated site during one of the four study sessions. Allodynia in this area of secondary hyperalgesia was documented by a decrease in the mechanical pain threshold from 198.8  $\pm$  76.5 g to 129.9  $\pm$  58.8 g (33  $\pm$  27%). The primary hyperalgesia to thermal stimuli and the primary and secondary hyperalgesia to mechanical stimuli remained remarkably stable throughout the study day (20–26 hr after exposure) and lasted up to 44 hr after UVB exposure (representing 20 hr after exposure + 24 hr after drug administration). The area of secondary hyperalgesia also remained remarkably stable throughout the day. However, a significant shrinking from 21.3  $\pm$  11.3 cm<sup>2</sup> to 17.7  $\pm$  11.4 cm<sup>2</sup> after 44 hr ( $p < 0.017$ ) was observed.

### Peripheral effect.

The effect of the treatments on thermal primary hyperalgesia is presented in fig. 2. The paracetamol–ketorolac combination produced a greater antihyperalgesic effect to heat stimulation than placebo ( $p < 0.042$ ) and paracetamol alone ( $p < 0.001$ ). The combination seemed to produce a better effect than ketorolac, increasing heat pain threshold from baseline by an additional 0.4°C (1.5°C versus 1.1°C increase), but the difference between the combination and ketorolac was not significant ( $p = 0.52$ ). Ketorolac alone produced a greater antihyperalgesic effect than paracetamol ( $p < 0.014$ ).

The effect of the treatments on mechanical primary hyperalgesia is presented in fig. 3. The paracetamol–ketorolac combination produced a greater antihyperalgesic effect to mechanical stimulation than placebo ( $p < 0.002$ ) and each of the analgesics given alone (paracetamol:  $p < 0.024$  and ketorolac:  $p < 0.032$ ). This antihyperalgesic effect lasted for more than 6 hr. The combination increased mechanical pain

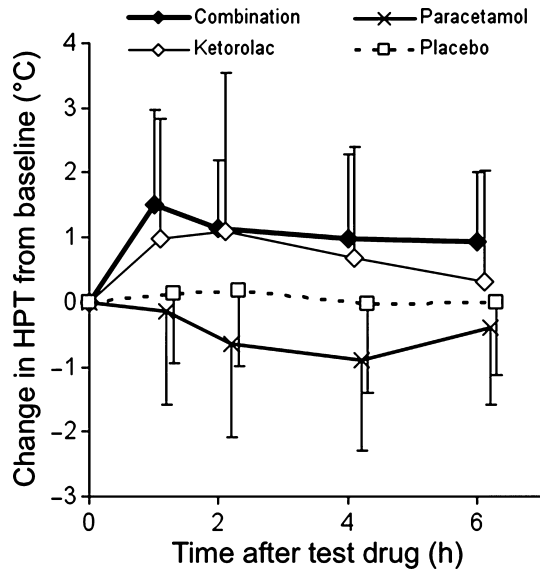


Fig. 2. Change from baseline in the heat pain threshold (HPT), measured in the area of primary hyperalgesia before drug administration and at 1, 2, 4 and 6 hr after drug administration. Data are expressed as means  $\pm$  S.D.;  $N = 11$ . The paracetamol-ketorolac combination was significantly superior to placebo ( $p < 0.042$ ) and paracetamol alone. Ketorolac was superior to paracetamol alone ( $p < 0.014$ ).

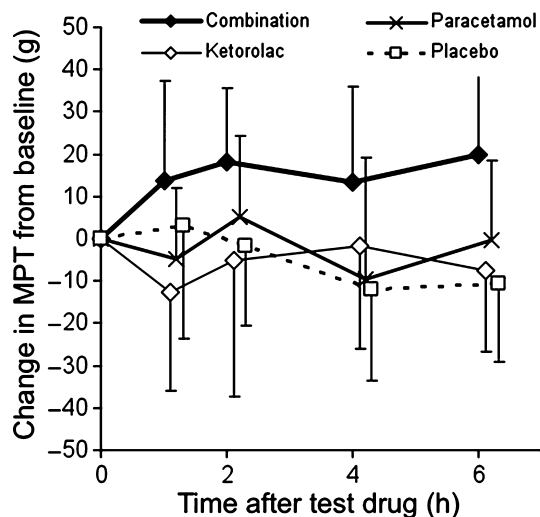


Fig. 3. Change from baseline in the mechanical pain threshold (MPT), measured in the area of primary hyperalgesia before drug administration and at 1, 2, 4 and 6 hr after drug administration. Data are expressed as means  $\pm$  S.D.;  $N = 11$ . The paracetamol-ketorolac combination was superior to placebo ( $p < 0.002$ ) and each of analgesics alone (paracetamol:  $p < 0.024$  and ketorolac:  $p < 0.032$ ).

threshold by up to 39% (18 g) from baseline. Conversely, neither paracetamol nor ketorolac alone produced any significant antihyperalgesic effect to mechanical stimulation compared with placebo ( $p = 0.578$  and  $p = 0.320$ , respectively).

No analgesic effect was observed within the control skin on the contralateral arm.

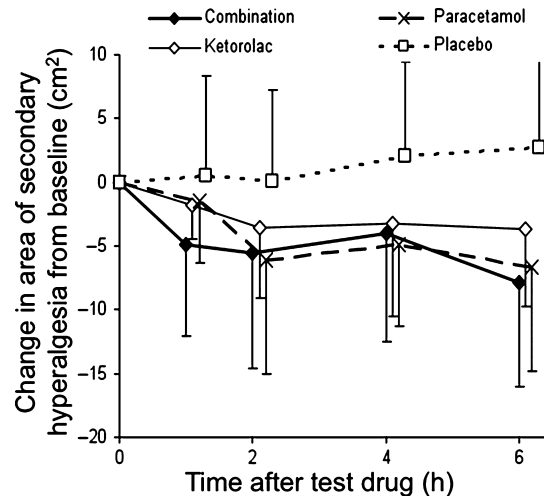


Fig. 4. Change from baseline in the area of secondary hyperalgesia to pinprick, measured before drug administration and at 1, 2, 4 and 6 hr after drug administration. Data are expressed as means  $\pm$  S.D.;  $N = 11$ . Hyperalgesic areas were not significantly different between the treatment groups.

#### Effects on cold pain tolerance.

Two hours after drug administration, the combination produced a significant, albeit a slight, decrease from baseline in pain intensity measured during the cold pressor test, of  $10 \pm 12.7\%$  in comparison with placebo ( $p < 0.012$ ) and single agents (paracetamol  $p < 0.019$  and ketorolac  $p < 0.004$ ). However, none of the analgesics taken alone or in combination affected the immersion time significantly.

#### Effects on secondary hyperalgesia and the nociceptive flexion reflex.

All analgesics tended to decrease the area of secondary hyperalgesia in comparison with placebo. However, the trend was not significant ( $p < 0.234$ ) (fig. 4). It was seen that neither paracetamol, ketorolac nor the combination produced any effect on the nociceptive flexion reflex.

#### Pharmacokinetics.

The mean plasma concentration-*versus*-time profiles of paracetamol and ketorolac enantiomers are presented in fig. 5. Their pharmacokinetic data were best described using a 2-compartment model. Further, they were comparable between the different arms (combination *versus* single agent) (table 1).

## Discussion

The present study aimed to assess the existence of an additive effect of a combination of paracetamol with ketorolac on experimental pain models and to explore the putative site of the interaction. It was observed that the combination significantly decreased UVB-induced heat and mechanical primary hyperalgesia, reflecting a peripheral effect. Additionally, the combination seemed to be superior to ketorolac or paracetamol used separately. Regarding pain tolerance, the combination in comparison with individual analgesic signifi-

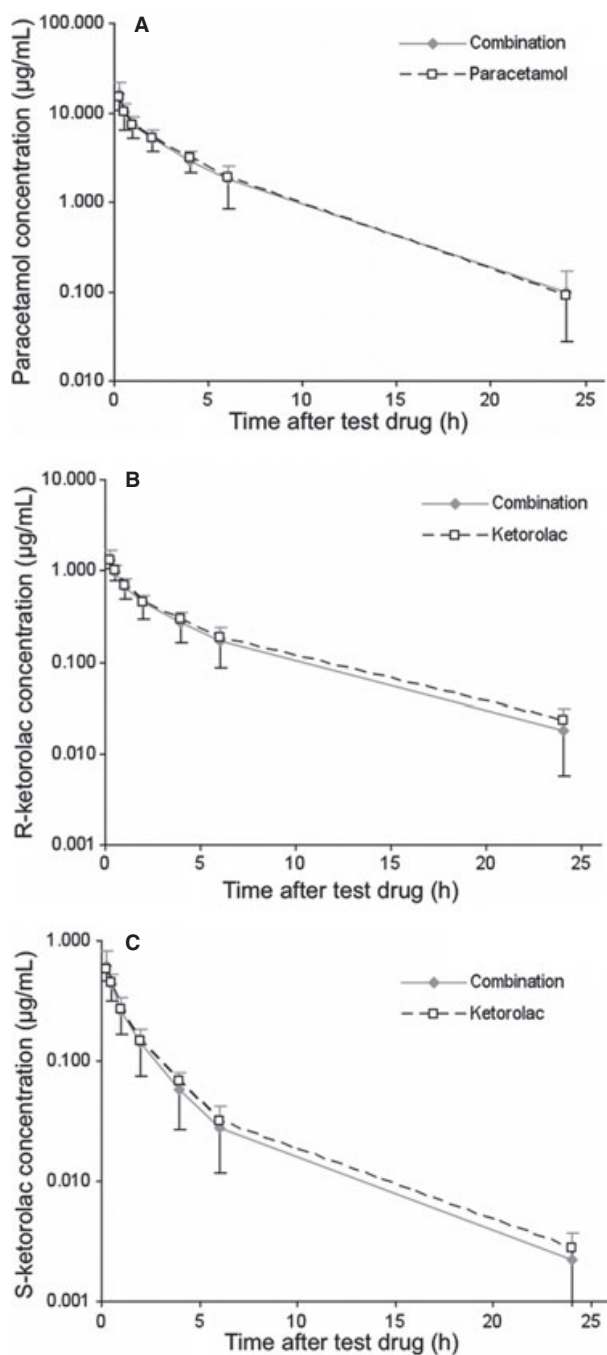


Fig. 5. Pharmacokinetic profiles of paracetamol and ketorolac enantiomers in plasma (semi-logarithmic scale). In (A), the paracetamol concentrations were obtained in the combination and paracetamol arms; in (B) and (C), the R-ketorolac and S-ketorolac concentrations were obtained in the combination and ketorolac arms. Data are expressed as means  $\pm$  S.D.;  $N = 11$ .

cantly decreased pain intensity during hand immersion in cold water (cold pressor test). The effect was minor although, as the paracetamol–ketorolac combination did not affect the immersion time, in contrast to opioids [36–39]. Nociceptors of cutaneous veins appear to mediate cold pain via the activation of both  $A\delta$ - and C-fibres [23]. Ketorolac alone did not have an effect on the cold pressor test, which is

in agreement with its mechanism of action on prostaglandin synthesis and with a previous study [40]. Therefore, an enhancement of the effect of paracetamol is postulated, whose mechanism remains to be elucidated.

The results obtained confirm the potential benefit of combining paracetamol with NSAIDs as it is usually carried out clinically. This is in accordance with the evidence regarding a boosted analgesic effect of combining paracetamol and NSAIDs available from animal studies, experimental pain models in human being [28,41] and clinical studies of pain on osteoarthritis [42], dental surgery [43] or other selected surgical procedures [2,3]. However, the exact and quantitative nature of the interaction (additive or supra-additive) and its mechanisms are still under debate.

As proposed by a study in a rodent model of visceral pain for assessing the combination of paracetamol with several commonly used NSAIDs, using the isobolographic approach [5], the results obtained from the present study could suggest that the interaction is of a supra-additive nature. Indeed, neither of the two agents produced a significant effect on primary hyperalgesia to mechanical stimuli nor pain intensity to cold pain, whereas the combined treatments significantly increased these pain thresholds. Ketorolac alone had an effect on thermal hyperalgesia. On the other hand, the combination by itself increased the pain threshold to a greater extent than ketorolac alone. An isobolographic approach would have been necessary to determine the type of interaction (additive, infra-additive, supra-additive and antagonistic) [44]. Pharmacokinetic/pharmacodynamic (PK/PD) modelling combined with an isobolographic technique would have been interesting to provide such information. In our study, the effect of paracetamol alone on hyperalgesia was not significant, making it impossible to derive a dose–response curve. Therefore, the data were insufficient for PK/PD modelling as well as for an isobolographic approach. Although paracetamol has been used for decades, no clear relationship between analgesic effects and plasma concentrations has yet been determined [45].

Several mechanisms are thought to be involved in the mechanism of paracetamol-induced analgesia [7]. The proposed targets include the central serotonergic descending pain pathways [10,11] and the cannabinoid system [12,13,46,47]. The action of paracetamol on cyclooxygenase (COX) has been the subject of debate for several years. Furthermore, good evidence suggests that paracetamol has a weak inhibitory influence on peripheral prostaglandin synthesis, which would account for its lack of anti-inflammatory activity. However, it could be a potent inhibitor of prostaglandin synthesis in the CNS. Consequently, it would produce both analgesia and antipyresis [48]. Recent data have demonstrated that the analgesic effects of paracetamol were analogous to those of selective COX-2 inhibitors [9,49]. The enzyme commonly referred to as COX is in fact named PGHS. The PGHS catalyses the two-step oxygenation of arachidonic acid to prostaglandin  $H_2$  (PGH<sub>2</sub>), a prostaglandin precursor [9]. This reaction is catalysed by distinct active sites within the enzyme. The NSAIDs target the COX site,

Table 1.

Estimated pharmacokinetic parameters for paracetamol and ketorolac enantiomers. Values are mean  $\pm$  S.D.

Parameter	Paracetamol (combination versus paracetamol arm)	R-ketorolac (combination versus ketorolac arm)	S-ketorolac (combination versus ketorolac arm)
$C_{\max}$ ( $\mu\text{g/ml}$ )	17.52 $\pm$ 6.33 versus 18.53 $\pm$ 10.00	1.48 $\pm$ 0.44 versus 1.42 $\pm$ 0.25	0.68 $\pm$ 0.19 versus 0.68 $\pm$ 0.14
$t_{1/2}$ (h)	4.22 $\pm$ 0.79 versus 3.88 $\pm$ 0.66	5.51 $\pm$ 1.34 versus 5.98 $\pm$ 1.66	5.80 $\pm$ 0.97 versus 5.54 $\pm$ 1.09
CL (l/h)	25.4 $\pm$ 5.6 versus 25.8 $\pm$ 9.8	2.53 $\pm$ 6.98 versus 2.64 $\pm$ 1.22	8.79 $\pm$ 2.45 versus 8.99 $\pm$ 3.27
AUC ( $\mu\text{g/ml}$ per hour)	41.60 $\pm$ 11.52 versus 43.18 $\pm$ 13.77	4.25 $\pm$ 1.27 versus 4.50 $\pm$ 1.80	1.23 $\pm$ 0.39 versus 1.26 $\pm$ 0.46
$V_1$ (l)	56.6 $\pm$ 25.3 versus 58.6 $\pm$ 28.3	7.31 $\pm$ 2.12 versus 7.23 $\pm$ 1.12	15.4 $\pm$ 4.1 versus 15.4 $\pm$ 3.4

$C_{\max}$ , maximal concentration;  $t_{1/2}$ , terminal half-life; CL, clearance; AUC, area under the concentration–time curve;  $V_1$ , initial volume of distribution.

which catalyses the first step formation of prostaglandin  $G_2$  ( $\text{PGG}_2$ ) from the arachidonic acid. The peroxidase (POX) site catalyses the  $\text{PGH}_2$  formation from  $\text{PGG}_2$ . It is believed that paracetamol inhibits PGHS by its ability to serve as a reducing cosubstrate for the POX. Elevated peroxide levels evident in inflamed tissues decrease the inhibitory action of paracetamol on PGHS, which explains its lack of anti-inflammatory effect [49]. The inhibitory action of paracetamol on prostaglandin synthesis has also been demonstrated to exist in peripheral sites [8,49]. Thus, paracetamol could have a significant peripheral effect in addition to its main central effect only when it is combined with an NSAID. In the present study, the observed effect on primary hyperalgesia could result from an action of paracetamol on both the central and the peripheral PGHS, and our hypothesis being that ketorolac might enhance the action of paracetamol on PGHS by decreasing the peroxide levels through its anti-inflammatory effect, leading to a supra-additive antinociceptive effect. Therefore, the mechanism responsible for an increased analgesic effect of the combination could well be a result of the distinct mode and site of action.

A pharmacokinetic interaction between paracetamol and ketorolac was excluded by the pharmacokinetic analysis. Indeed, it was found that the pharmacokinetic data could be compared when each analgesic was given alone or in combination, and the obtained pharmacokinetic parameters were consistent with the previously published data [50–52].

The present study confirms the usefulness of the UVB model in investigating the primary antihyperalgesic effect of NSAIDs [30,53]. Additionally, it also confirms the remarkably stable conditions of primary hyperalgesia to thermal and mechanical stimuli lasting for more than 26 hr after UVB exposure, as reported earlier [26]. A different area was irradiated during each study session as tanned skin is less sensitive to UV. No drug effect was observed in the control contralateral skin zone, thereby confirming the prior observations that an inflammatory component is essential to assess NSAID efficacy in experimental pain models [30].

Two of the volunteers did not develop secondary hyperalgesia. Thus, they did not have allodynia to pinprick outside the irradiated site for one of the four study sessions. The potential reasons for this could be related to the fact that a lower dose of UVB was given, in comparison with Gustorff

*et al.* [26], and the minimal erythema dose was determined according to the skin type and not individually.

In the present study, no significant effect on secondary hyperalgesia between the treatments and placebo was observed. However, as expected, a trend towards an effect was observed when paracetamol was used. The limitation to the obtained results is probably related to the fact that the study is underpowered for this secondary end-point. Indeed, as our primary end-point was the heat pain threshold in the area of primary hyperalgesia, the present study was not designed to detect a difference in the area of secondary hyperalgesia to pinprick. According to the data obtained from Gustorff *et al.* [26], a 30% reduction in secondary hyperalgesia area can be detected with 11 volunteers in the cross-over design. However, as mentioned before, the dosage used for irradiation and the surface exposed was minimal. This explained the lack of power of the present study with the 11 volunteers. The inclusion of more volunteers in our study might probably have given more robust and significant results, on secondary end-points as well, and this could well have provided more information on the mechanisms of the interaction between paracetamol and ketorolac. However, a recent study with 16 healthy volunteers found no significant effect of paracetamol on hyperalgesic areas induced by intradermal electrical stimulation, although a trend was observed, which confirms, in agreement with several previous studies that used the same electrically evoked human pain model, that paracetamol displays a minor analgesic effect in this experimental setting [54].

In conclusion, the present clinical study in healthy volunteers showed that a combination of paracetamol and ketorolac was superior to either agent alone on an inflammatory pain model. The observed results suggest a supra-additive interaction between paracetamol and ketorolac that should be further investigated. An action on different steps of the prostaglandin synthesis could contribute to the observed increased effect of the combination on primary hyperalgesia. Further studies are warranted to evaluate an action on different steps of the prostaglandin synthesis of the combination.

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