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# Analgesic action of acetaminophen in symptomatic osteoarthritis of the knee

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***Objectives*. The study was designed to investigate the analgesic effects and mechanisms of acetaminophen (paracetamol) in symptomatic osteoarthritis (OA) of the knee.**

***Methods*. Twenty patients with symptomatic OA were randomly allocated to two groups treated with either acetaminophen or rofecoxib for 3 months. Visits and measurements were scheduled upon entry (T0), at month 1 (T1) and at month 3 (T3). The intensity of joint pain was evaluated with a 100-mm visual analogue scale (VAS). The physical function of the affected knee was evaluated with a questionnaire comparable to the Western Ontario McMaster Universities Osteoarthritis Index (WOMAC). Levels of serotonin, substance P (SP) and $\beta$-endorphin (BEND) were determined with commercial enzyme-linked immunoassay kits. The expression of $\kappa$ opioid receptor (KOR) in peripheral mononuclear blood cells (PBMCs) was quantified by real-time PCR.**

***Results*. Both acetaminophen and rofecoxib relieved pain considerably but with different kinetics, and affected different biomarkers. Rofecoxib appeared to be more efficient, reducing pain intensity by 56% at T1 ($P < 0.01$), whereas acetaminophen reduced it by only 29%. Physical function improved in both groups by T3. Correlated with the pain relief, acetaminophen significantly reduced plasma BEND levels, whereas rofecoxib did not do so. In both groups plasma SP levels were elevated compared with T0. A reduction in serum serotonin was detected in the rofecoxib group at T1 ($P = 0.004$) but had recovered at T3. No changes in KOR mRNA in PBMCs were observed in either group.**

***Conclusions*. There is a correlation between reduction in circulating BEND and OA pain relief in patients treated with acetaminophen.**

KEY WORDS: Acetaminophen, Beta-endorphin, Osteoarthritis, Pain, Rofecoxib.

Osteoarthritis (OA) is the most common form of arthritis and causes pain and loss of function. Its prevalence increases with age and leads to effects on health-related quality of life in older adults, and it is accompanied by a high economic burden [1–4]. As our population ages and lifespan increases, OA is expected to be the fourth leading cause of disability by the year 2020 [1].

There is no cure for OA. Therapeutic intervention is symptomatic; it aims to reduce pain and improve physical function. Based on efficacy, toxicity and overall costs, acetaminophen (paracetamol), an analgesic and antipyretic drug with weak anti-inflammatory properties and few gastric and platelet side-effects, is recommended by American College of Rheumatology Subcommittee on Osteoarthritis [5] and the European League Against Rheumatism [6] as the first-line pharmacological therapy. Although acetaminophen was introduced to medicine as early as 1893, its pharmacology remains a puzzle. It blocks prostaglandin (PG) production in a tissue-specific manner and has been suspected to be an inhibitor of the PGH synthase, cyclooxygenase (COX) [7]. Nevertheless, its activity cannot be fully explained by any of the COX variants that have been identified, i.e. COX-1, COX-2 or COX-3, a recently identified COX-1 variant in dogs [8]. This gives rise to the hypothesis that acetaminophen might function through other mechanisms dependent on or independent of the COX pathway.

$\beta$-Endorphin (BEND) is a subtype of endogenous opioid peptide with morphine-like analgesic effects. In addition to antinociception, it inhibits the production of the inflammatory cytokines tumour necrosis factor $\alpha$ and interleukin 1$\beta$, the production and enzymatic activity of matrix metalloproteinase 9 in the synovial cells of rheumatoid arthritis patients [9], and PG production in rats [10]; therefore, it is also involved in anti-inflammation and anti-arthritis processes.

Serotonin and substance P (SP) are two major inflammatory mediators. Both contribute to OA pain and joint destruction [11, 12]. SP is also involved in the production of $PGE_2$ from synoviocytes [13].

In the periphery, $\kappa$ opioid receptor (KOR) is found in fibroblast-like synoviocytes (FLS) [14] and immune cells [15]; its expression level is inversely related to the progress of arthritis [14, 16]. In the central nervous system, selective endogenous opioid receptor antagonists may attenuate the central antinociceptive effect of acetaminophen [17], implicating an interaction of acetaminophen and opioid receptors.

In order to better understand the analgesic actions of acetaminophen, we investigated the changes in circulating BEND, SP, serum serotonin and KOR in OA patients treated with acetaminophen, along with clinical indexes. Our study revealed a correlation of joint pain relief and circulating BEND reduction in acetaminophen-treated patients.

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## Subjects and methods

### *Subjects*

*Patients and healthy subjects*. Twenty patients fulfilled the American College of Rheumatology criteria for knee OA [18] accompanied by joint pain [visual analogue scale (VAS) $\geq$60 mm] were recruited to the study. They had no secondary OA as a consequence of inflammation, gout, pseudogout or tumour. Kidney insufficiency was also an exclusion criterion. The patients had no OA-influencing concomitant diseases. Drug medication with opioids was not allowed. There was a washout period from non-steroidal anti-rheumatic agents of 14 days. After this period, patients were randomly allocated to two groups, which were treated with either acetaminophen up to 4 g/day ($n=10$, age 60–77 yr, 4 males, 6 females) or rofecoxib 25 mg/day ($n=10$, age 48–80 yr, 4 males, 6 females) for 3 months. Visits and measurements were scheduled upon entry (T0), at month 1 (T1) and at month 3 (T3). The functional status of the knee joint was assessed with a German questionnaire comparable to the Western Ontario McMaster Universities Osteoarthritis Questionnaire (WOMAC) [19]. The intensity of joint pain was evaluated with a 100-mm VAS at rest. All patients completed the study except two from the acetaminophen group after T1 (patients 3 and 6). Patient 6 withdrew from the study because of insufficient pain relief. No comorbidity was found in any patient during the study. As a control, blood from 20 age- and gender-matched healthy subjects was obtained from Blutspendedienst Zürich. The study was approved by the local ethical committee (Kantonale Ethikkommission, Spezialisierte Unterkommission für Spezialfächer; reference number 343). All patients signed informed consent. The trial is registered as ISRCTN14751911.

### *Methods*

*ELISA*. The serum serotonin level was determined with a commercial enzyme-linked immunosorbent assay (ELISA) kit (IBL Immuno-Biological Laboratories, Hamburg, Germany) according to the manufacturer's instructions. For plasma BEND and SP measurement, EDTA plasma was collected. Peptides were extracted with a C18 SEP-Column and subsequently analysed using BEND and SP ELISA kits (Morwell Diagnostics, Egg, Switzerland), respectively, as described by the manufacturer.

*RNA preparation and Taqman real-time RT-PCR*. Peripheral blood mononuclear cells (PBMCs) were isolated from heparin whole blood by Ficoll gradient centrifugation using Ficoll-Paque Plus reagent (Amersham Biosciences, Uppsala, Sweden). Total RNA was purified with an RNeasy Mini Kit (Qiagen, Basel, Switzerland) according to the manufacturer's instructions. Taqman real-time reverse transcription–PCR was performed as described elsewhere [14]. In brief, the first-strand cDNA was reversed transcribed by MultiScribe Reverse Transcriptase in the presence of random hexamer at 25°C for 10 min, 48°C for 40 min and 95°C for 5 min. Taqman real-time PCR was performed in duplicate with the KOR-specific primers 5′-CATCTGTTGG CATCTCTGCAA-3′, 5′-TGCAAGGAGCACTCAATGACA-3′ and FAM-labelled probe 5′-TCTTCCCTGACTTTGGTGCCTC CAAG-3′ (anti-sense), at 95°C for 15 s and 60°C for 1 min for 50 cycles. Relative quantities of KOR were determined with the standard curve method, using normal human cerebellum as calibrator and $\beta$-actin as endogenous reference to normalize RNA samples. For comparison, the relative quantity of KOR for each patient was determined as the percentage of the average quantity for healthy donors.

*C-reactive protein*. Serum C-reactive protein (CRP) was measured with an automated laboratory method (ELISA).

*Statistical analysis*. Results are presented as mean $\pm$ S.E.M. The Wilcoxon signed ranks test and the Mann–Whitney test were used to compare paired and non-paired variables, respectively. The relationship of two variables was measured using bivariate correlations. All analyses were performed with SPSS software 9.0 (SPSS, Chicago, IL, USA).

## Results

Evaluated by VAS, both acetaminophen and rofecoxib relieved pain considerably after 3 months of treatment ($VAS_{T3-T0}=-40.7$ mm, $P=0.018$; and $VAS_{T3-T0}=-42.5$, $P=0.005$, respectively). As shown in Fig. 1, one of 10 patients in each group (patients 8 and 110) had no pain at the end of the study. In a comparison of the two groups, rofecoxib appeared to be more efficient, taking effect on all patients in 1 month (mean $VAS_{T1-T0}=-41.5$ mm, $P=0.005$), whereas in the acetaminophen group only a moderate reduction was found at this time point in 7/10 patients (mean $VAS_{T1-T0}=-21.0$ mm, $P=0.020$); one patient (patient 9) experienced more pain; two others (patients 1 and 6) showed no improvement; and patient 6 quit the study after 1 month because of dissatisfaction with pain management.

We further evaluated functional improvement using a German questionnaire comparable to WOMAC [19]. Compared with T0, physical function of the affected knee increased significantly at T1 in the rofecoxib group ($P=0.001$) and T3 in both groups ($P<0.0001$ in the acetaminophen group and $P=0.009$ in the rofecoxib group); stiffness was alleviated only in the rofecoxib group compared with T0 (T1, $P=0.005$; T3, $P=0.033$) (Table 1).

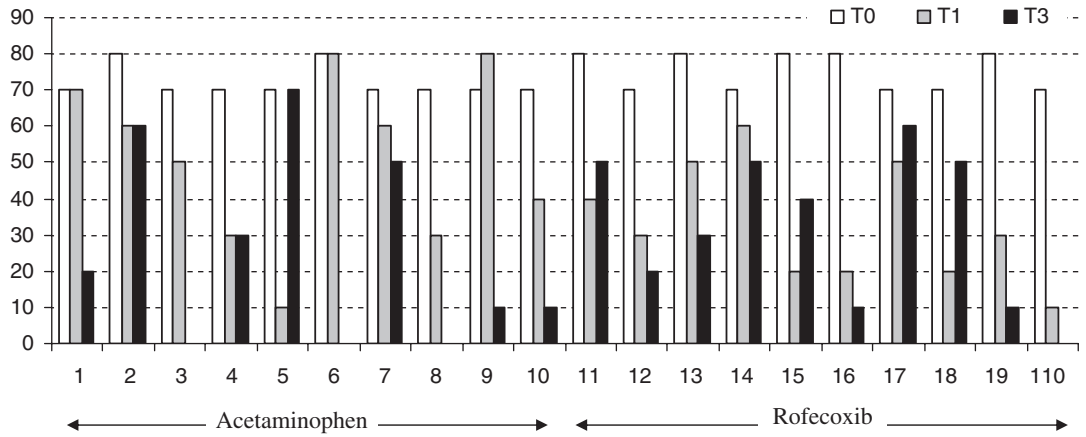


FIG. 1. Analgesic effects of acetaminophen and rofecoxib in symptomatic OA patients, using a 100-mm VAS.

There were no statistically significant differences between the two groups at any time point.

To explore the therapeutic mechanism of acetaminophen, we measured the plasma levels of BEND and SP, as well as serum serotonin and KOR in PBMCs in patients treated with acetaminophen in comparison with those treated with the COX-2 inhibitor rofecoxib.

TABLE 1. Changes in WOMAC index in OA patients treated with acetaminophen or rofecoxib in comparison with baseline

| | Acetaminophen | Rofecoxib |
|---|---|---|
| Pain | | |
| Baseline (T0) | 2.74 | 3.62 |
| Change at T1 | 0.26 | −1.6 |
| Change at T3 | −0.74 | −1.12 |
| Stiffness | | |
| Baseline (T0) | 2.85 | 4.2 |
| Change at T1 | 0.15 | −1.3 |
| Change at T3 | −0.54 | −0.8 |
| Activity | | |
| Baseline (T0) | 2.43 | 3.69 |
| Change at T1 | 0.05 | −1.19 |
| Change at T3 | −1.06 | −0.98 |

T1, 1 month after baseline; T3, 3 months after baseline.

As shown in Fig. 2, BEND was reduced in 8/10 patients in the acetaminophen group at T1, with an overall reduction of 8.62 pg/ml (T0, $30.00 \pm 10.16$ pg/ml; T1, $21.38 \pm 8.52$ pg/ml; $P = 0.017$). At T3 further reductions were found in 5/8 patients. Accordingly, the overall BEND level declined another 10.74 pg/ml, with a total reduction of 19.36 pg/ml since T0 ($P = 0.028$). The decrease in plasma BEND was significantly correlated with the corresponding VAS values (Pearson correlation = 0.998, $P = 0.037$; Spearman's $\rho$ correlation coefficient = 1.000, $P < 0.01$). No obvious changes were found in the rofecoxib group (Fig. 2) (T0, $18.56 \pm 2.09$ pg/ml; T1, $20.20 \pm 3.10$ pg/ml; T3, $18.47 \pm 2.30$ pg/ml). There was no statistically significant difference between the two groups at T0 ($P = 0.965$). Thus, in pain management acetaminophen may play a role through the BEND pathway, whereas rofecoxib does not [20].

At T1, serum serotonin was decreased in 7/10 patients in the rofecoxib group (Fig. 3), showing an average reduction of 13.25% from T0 ($132.15 \pm 14.86$ ng/ml) to T1 ($114.64 \pm 13.61$ ng/ml, $P = 0.037$), but had returned at T3 in five of the seven patients to an overall level comparable to that at T0 ($146.49 \pm 26.92$ ng/ml; T3 *vs* T1, $P = 0.059$; T3 *vs* T0, $P = 0.575$). With regard to the acetaminophen group, no significant changes were detected during the study (T0, $124.40 \pm 17.01$ ng/ml; T1, $124.12 \pm 17.04$ ng/ml; T3 = $136.25 \pm 16.40$ ng/ml).

We compared the plasma SP level in the two groups. As shown in the upper panel in Fig. 4, at T1 increases in plasma SP were

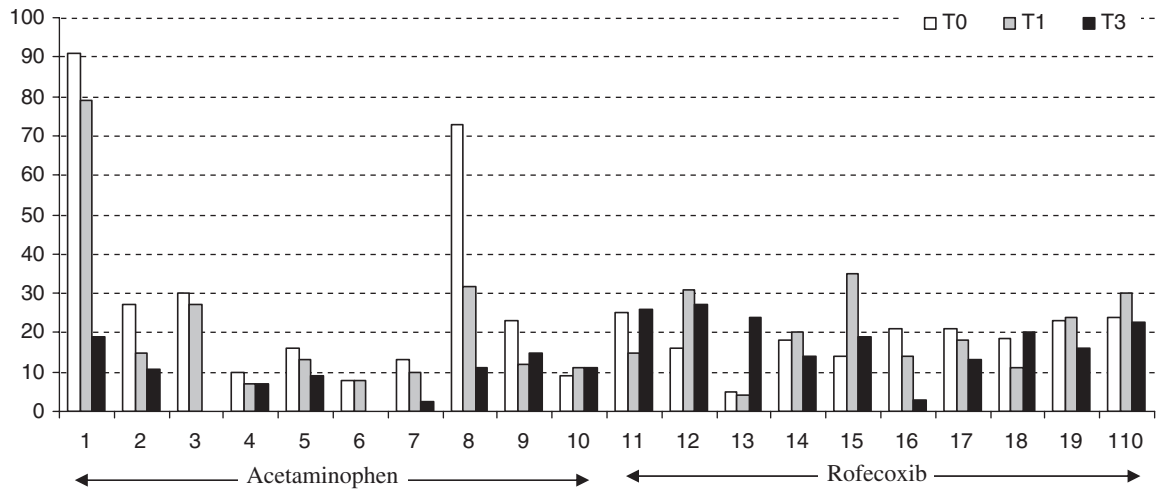


FIG. 2. Changes in plasma $\beta$-endorphin (BEND) in OA patients treated with acetaminophen or rofecoxib.

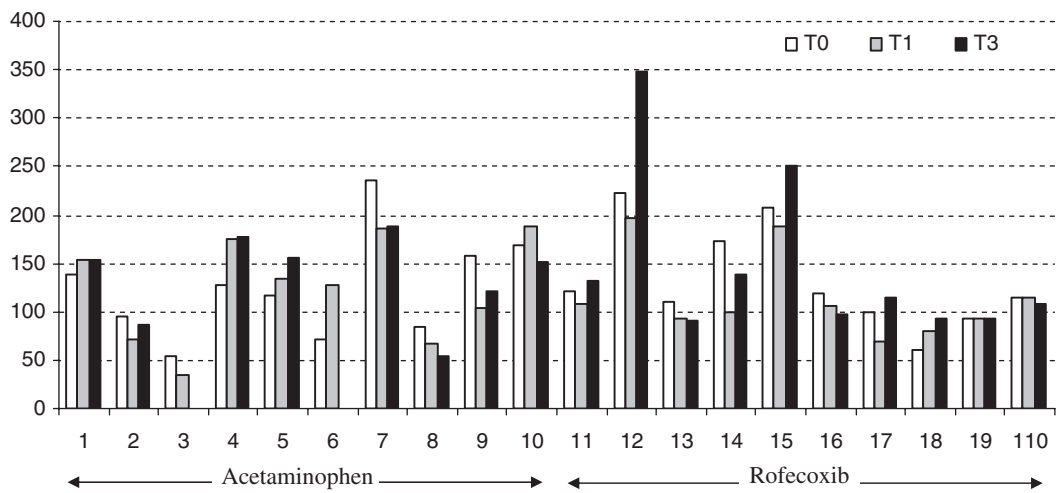


FIG. 3. Serum serotonin in OA patients treated with acetaminophen or rofecoxib.

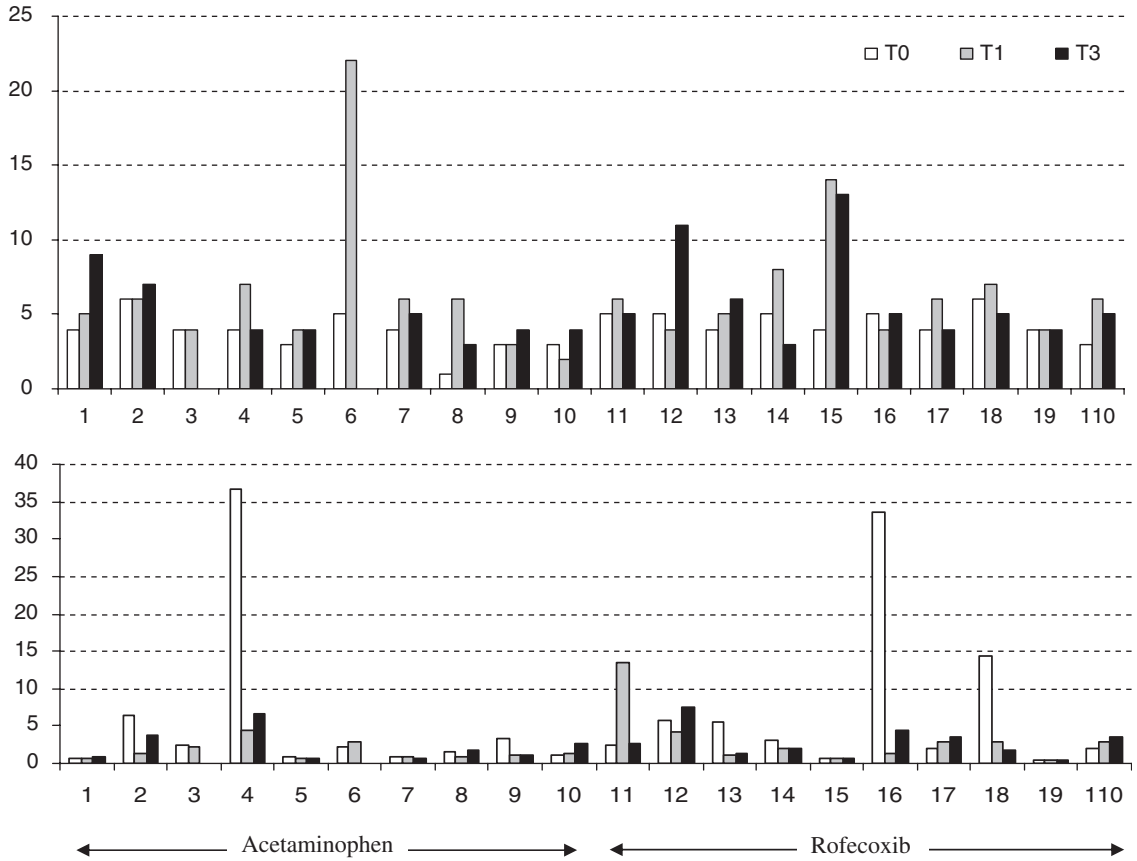


FIG. 4. Changes in plasma SP (upper panel) and CRP (lower panel) in OA patients treated with acetaminophen or rofecoxib.

found in 6/10 acetaminophen- and 7/10 rofecoxib-treated patients and these resulted in overall increases of 75.68 and 40.66% in the two groups (acetaminophen, T0, $3.70 \pm 0.42$ pg/ml, T1, $6.50 \pm 1.79$ pg/ml, $P = 0.041$; rofecoxib, T0, $4.55 \pm 0.25$ pg/ml, T1, $6.40 \pm 0.95$ pg/ml, $P = 0.046$). From T1 to T3, reductions in SP were found in three of the six patients treated with acetaminophen, but in two of them SP remained higher than at baseline, whereas moderate increases were detected in two other patients in the group. With regard to the rofecoxib group, SP was decreased in six of the total of 10 patients, but remained higher or equal to SP at T0 in 8/10 patients. Therefore, despite moderate overall reductions compared with T1, at T3 there were still 35.14 and 34.07% increases in SP in the acetaminophen and rofecoxib groups, respectively, compared with T0 (acetaminophen, T3, $5.00 \pm 0.71$ pg/ml; rofecoxib, T3, $6.10 \pm 1.03$ pg/ml). No statistical differences were found between T1 and T3 in either group or, at any time point, between the two groups. Because the concentrations of peripheral SP could be affected by various inflammatory diseases [21, 22], we further evaluated the CRP level in these patients; CRP generally reflects the presence and intensity of an inflammatory process. In contrast to SP, the average levels of CRP at T0, T1 and T3 were normal in both groups (acetaminophen, T0, $5.61 \pm 3.50$ mg/ml, T1, $1.64 \pm 0.39$ mg/ml, T3, $2.24 \pm 0.72$ mg/ml; rofecoxib, T0, $6.43 \pm 2.98$ mg/ml, T1, $3.19 \pm 1.20$ mg/ml, T3, $2.81 \pm 0.66$ mg/ml). Abnormal elevation of CRP was detected in only one patient in each group at the start of the study (patients 4 and 16); levels returned to normal after 1 month's medication, coinciding with pain relief but not with the increase in SP (Fig. 4, lower panel).

KOR mRNA was detected in PBMCs of 85% OA patients enrolled in the study (8/10 in the acetaminophen group, 9/10 in the rofecoxib group) but at significantly low levels ($P < 0.001$): only $16.46 \pm 2.77\%$ (acetaminophen) and $13.48 \pm 2.40\%$ (rofecoxib) of the average for healthy individuals ($n = 20$) at T0. No obvious modulation effect was found after either acetaminophen or rofecoxib medication at T1 (acetaminophen, $14.18 \pm 4.44\%$; rofecoxib, $15.82 \pm 3.91\%$) and T3 (acetaminophen, $15.83 \pm 4.29\%$; rofecoxib, $13.44 \pm 3.04\%$), and no difference was observed between the two groups at any time point investigated.

## Discussion

To explore the therapeutic mechanism of acetaminophen in symptomatic OA management, we compared its effects on joint pain and function with those of the COX-2 inhibitor rofecoxib. Although both drugs relieved pain and improved the physical function of OA patients significantly in 3 months, they exhibited different kinetics, indicating different pharmacological mechanisms.

In the blood, BEND is released not only from pituitary gland [23] but also from immune cells [24]. BEND can bind to its receptors locally through the circulation. Endings of primary sensory neurons, synovial tissues [25], osteoarthritic cartilage and chondrocytes [26] contain BEND binding sites. Activation of BEND receptors in the joints can inhibit local pain directly [27, 28]. At the start of the study, the average level of BEND in the acetaminophen-treated group was slightly higher than that in the rofecoxib group as well as in age- and gender-matched healthy subjects ($19.5 \pm 1.48$ pg/ml, $n = 20$); however, there was no statistically significant difference (acetaminophen *vs* rofecoxib, $P = 0.965$; acetaminophen *vs* healthy subjects, $P = 0.764$). Also, the BEND level in patients was not related to the severity of pain in either group. Surprisingly, after acetaminophen administration, plasma BEND was reduced in correlation with the pain relief.

This effect was acetaminophen-specific and independent of the rofecoxib-affected COX-2 pathway, as no changes were found at any time point in the rofecoxib-treated group. Accumulation and binding of BEND with its receptors in local tissue may lead not only to pain relief but also to a reduction in free BEND in the circulation. There is increasing evidence that immune-derived BEND is critical for peripheral analgesia [29]. In a painful inflammation model, circulating leucocytes underwent site-directed migration and released BEND locally [30]. Acetaminophen may play a direct or indirect role in promoting local accumulation of BEND. In line with this, Stein's group reported increased transport of immune-derived BEND after local injury [31]. Further investigation is to be undertaken to test the hypothesis.

In both OA groups, the expression level of KOR mRNA in PBMCs is dramatically lower that that in healthy subjects. This is in line with our observations in FLS [14]. Nevertheless, neither acetaminophen nor rofecoxib had any modulating effects on KOR mRNA in these patients. Thus, unlike in the central nervous system [17], circulating KOR may not be related to the analgesic action of acetaminophen. However, this does not exclude the possibility that acetaminophen may function through other subtypes of peripheral opioid receptors.

Rofecoxib (Vioxx®) is one of the selective COX-2 inhibitors, which were originally developed to reduce the gastrointestinal side-effects of other non-steroidal anti-inflammatory drugs (NSAIDs), possibly due to the inhibition of COX-1. It was voluntarily withdrawn from the market at the end of September 2004 because of the risk of heart attack and stroke after long-term medication (longer than 18 months). We included rofecoxib in our study as a COX-2 inhibitor control. This study was performed and accomplished before the withdrawal of rofecoxib. No severe adverse effects were found during the course of the study. We observed a temporary reduction in serotonin in the rofecoxib group, indicating that rofecoxib might act on the serotonin pathway and secondarily control the release of inflammatory mediators besides its known anti-inflammatory action as a selective COX-2 inhibitor.

Despite effective pain management and functional improvement, plasma SP was increased significantly in the patients treated with either acetaminophen or rofecoxib. Peripheral SP is released from nerve endings, inflammatory cells and also FLS [21, 22]. Its concentration in plasma could be affected by various inflammatory diseases [21]. Using CRP as a marker of inflammation, we did not observe any correlation between SP and CRP levels, suggesting that the increase in SP is less likely to be a result of inflammatory conditions in the patients we investigated. BEND is known to be able to suppress SP release [32]. The reduction in peripheral BEND could facilitate SP release. Nevertheless, such a correlation was only found in two acetaminophen-treated patients. In fact, the increase in SP was found not only in acetaminophen-treated patients with lower plasma BEND but also in rofecoxib-treated patients with a normal level of BEND. Thus, the increase in SP may not result from a decrease in BEND. Acetaminophen and rofecoxib may share some general machinery in the induction of SP; this needs to be investigated further with a control group without NSAIDs.

## Conclusions

We investigated the actions of acetaminophen in the symptomatic management of OA. This investigation lacked an NSAID-free control group, which is a weakness of the study. However, comparison with such a group could cause ethical problems because pain medication (at least a rescue medication) for symptomatic OA with a pain intensity of VAS $\geq$60 mm has to be provided. NSAIDs and opioids were not allowed in our study design. Acetaminophen (the rescue medication in most clinical trials) is disadvantageous because of the study aim. Our data showed that acetaminophen medication improved joint function and relieved pain and that this was accompanied by a decrease in BEND in the circulation. This suggests that acetaminophen, in addition to being a COX inhibitor, might function through the BEND pathway via a novel mechanism, to be further characterized.

| *Rheumatology* | Key messages |
|---|---|
| | • Acetaminophen facilitated pain relief in patients with symptomatic knee osteoarthritis.<br>• Circulating $\beta$-endorphin is reduced in acetaminophen-treated osteoarthritis patients.<br>• $\beta$-Endorphin may be involved in acetaminophen-mediated analgesia. |

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