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RESEARCH PAPER

Similar Maximum Systemic but not Local Cyclooxygenase-2 Inhibition by 50 mg Lumiracoxib and 90 mg Etoricoxib: A Randomized Controlled Trial in Healthy Subjects

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ABSTRACT

Purpose Once daily doses of 100–400 mg lumiracoxib have been proposed to inhibit local prostaglandin synthesis longer than systemic prostaglandin synthesis due to local accumulation in inflamed, acidic tissue. Lower, less toxic doses, however, might still achieve the clinical goal and merit further contemplation.

Methods In a randomized, double-blind, placebo-controlled, three-way cross-over study, 18 healthy men received, with an interval of 24 h, two oral doses of 50 mg lumiracoxib or for comparison, 90 mg etoricoxib, for which local tissue accumulation has not been claimed as therapeutic component. Systemic and local drug concentrations, assessed by means of subcutaneous *in-vivo* microdialysis, were related to COX-2 inhibiting effects, quantified as inhibition of prostaglandin *ex-vivo* production in whole blood as well as local tissue prostaglandin (PG) concentrations.

Results Twenty-four hours after the first dose, only etoricoxib was detectable in plasma and inhibited PGE_2 production. In contrast, after the second dose, systemic PGE_2 concentrations were significantly reduced by both coxibs, indicating similar maximum systemic effects of the selected doses. The local COX-2 inhibition by etoricoxib was most pronounced for PGD_2. To the contrary, no indication was given of local inhibition of PG production by lumiracoxib at the dose tested.

Conclusions Doses of 50 mg lumiracoxib and 90 mg etoricoxib produced similar maximum inhibition of systemic COX-2 function whereas 50 mg lumiracoxib was ineffective in producing local COX-2 inhibition. At a 50 mg dosage, lumiracoxib does not provide peripheral effects that outlast its systemic actions in therapies of rheumatic diseases such as osteoarthritis.

KEY WORDS COX-2 · microdialysis · pharmacokinetcs · prostaglandins

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INTRODUCTION

Nonselective, traditional non-steroidal anti-inflammatory drugs (NSAIDs) have been the mainstay of the therapy of osteoarthritis, rheumatoid arthritis and acute pain. As their long term use may cause gastro-intestinal bleeding and ulcerations due to cyclooxygenase (COX) one inhibition, selective COX-2 inhibitors had been planned to replace the traditional NSAIDs. Unfortunately, most of the new substances have been withdrawn from the market (1,2) for differing reasons.

While rofecoxib and valdecoxib were associated with an inacceptable increase in the risk of cardiovascular events, the decision to withdraw lumiracoxib (Fig. 1) was based on cases of liver damage (2–5). The high dose of 400 mg lumiracoxib, initially recommended in Australia, might have facilitated toxicity. As the daily dosage of 100 mg recommended in Europe also led to severe liver damage in 111 cases, and the shortest intake duration with such a negative outcome was 16 days, a further dose reduction would seem likely to be advantageous. The patients with toxic effects were later identified as being carriers of a particular HLA (Human Leukocyte Antigen) haplotype (6) which triggered contemplations of its re-introduction.

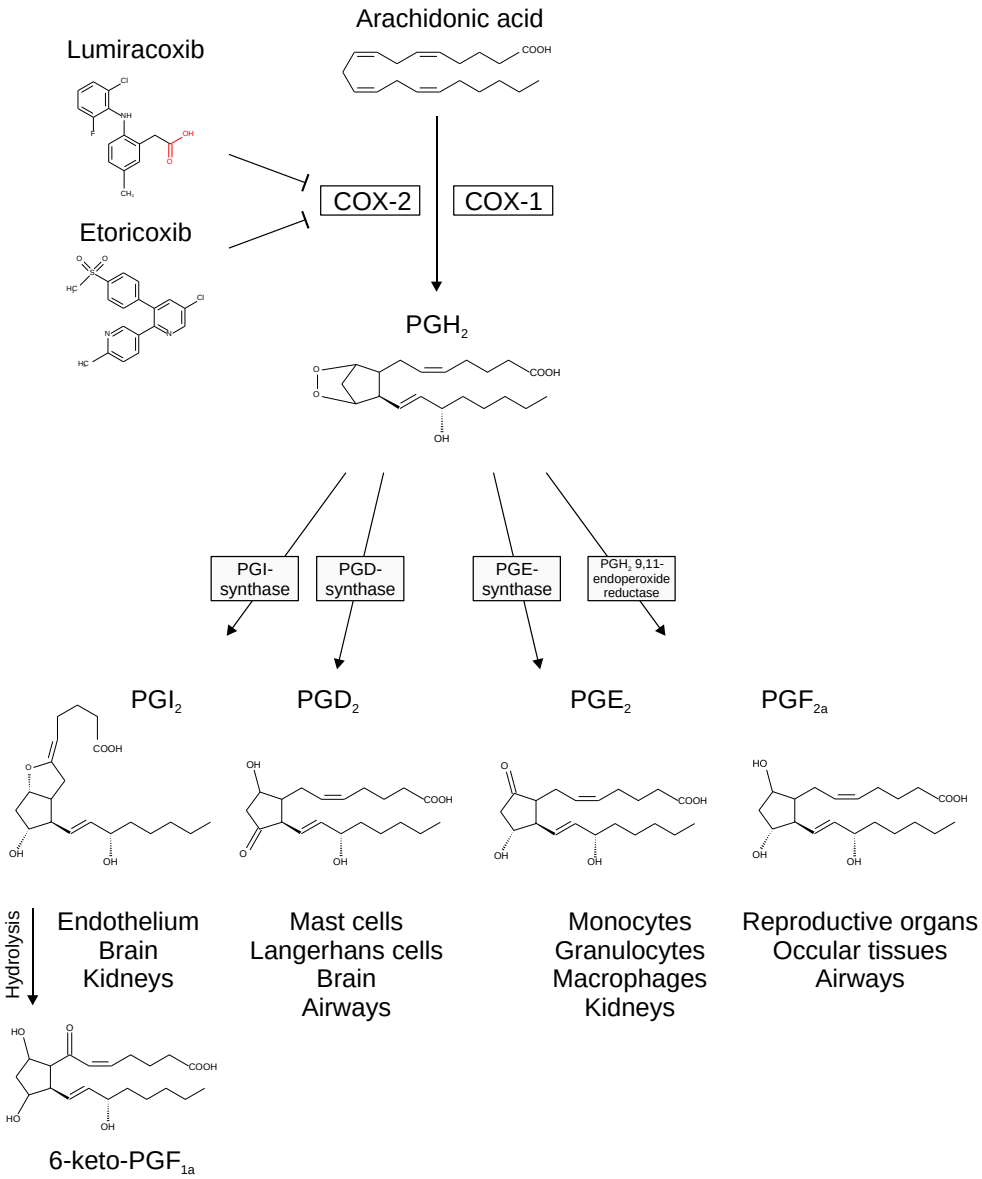

Fig. 1 Arachidonic acid metabolic pathway. Conversion of arachidonic acid to prostanoids is catalyzed by cyclooxygenases (existing as isoforms, COX-1 and COX-2) to the endoperoxide, prostaglandin H_2 (PGH_2). Depending on the presence of specific synthases, PGH_2 is subsequently metabolized to prostanoids with differing biological functions, including PGI_2 (prostacyclin, which is rapidly further hydrolyzed to the more stable 6-keto-$PGF_{1\alpha}$), PGD_2, PGE_2 and $PGF_{2\alpha}$. Lumiracoxib and etoricoxib selectively inhibit COX-2, thus, suppressing conversion of arachidonic acid with subsequent prostanoid synthesis. COX-2 inhibitory selectivity is achieved with molecules that fit to the binding site of COX-2 but are too large to block the active site of COX-1.

Lumiracoxib was recommended for a once daily dosing schedule. This was based on an observation that 24 h after administration its concentrations in synovial fluid were still high enough to maximally inhibit the production of prostaglandin (PG) E_2 (7), despite a plasma elimination half-life of only 6.5 ± 1.4 h (8). The long local persistence was explained by the physicochemical properties of lumiracoxib (Table I). It was claimed to be the only acidic selective COX-2 inhibitor and was thought to be trapped in inflamed and therefore, acidified tissue (8). If the hypothesis of local accumulation were true, a much lower dose might still achieve the clinical goal of COX inhibition in inflammation with fewer systemic side effects. Although the drug, in the meantime, seems to have been abandoned (9), the results are still important for future developments to judge whether an acidic COX-2 inhibitor may provide pharmacological advantages.

Table I Key Pharmacological Properties of Lumiracoxib and Etoricoxib

Parameter	Lumiracoxib	Etoricoxib
Physicochemistry		
Molecular weight [g/mol]	293.72 (8)	358.84 (39)
pK_a	4.11 (40)	19.69 (40)
Octanol water partition coefficient	3.89 (41)	3.14 (42)
Pharmacokinetics		
Protein binding [%]	99 (43)	92 (43)
Plasma elimination half-life, $t_{½}$ [h]	3–6 (44)	22 (12)
Volume of distribution, V_d [l]	13 (43)	120 (12)
Oral bioavailability (immediate-release formulation) [%]	74 (44)	100 (12)
Pharmacodynamics		
Selectivity (ratio COX-2:COX-1 inhibition)	433 (45)	344 (45)

MATERIALS AND METHODS

Subjects

The study (EudraCT-Nr. 2008-001880-12) followed the Declaration of Helsinki and was approved by the Ethics Committee of the Goethe-University Frankfurt am Main, Germany. Informed written consent was obtained from each participating subject. Between August 2008 and June 2009, healthy young men (n=18, age 25.1±3.6 years, mean±standard deviation, height 185±6.1 cm, weight 80.6±7 kg), not taking any medication or drugs, were enrolled after having provided informed written consent. The subjects' health was ascertained by medical questioning and basic clinical chemistry. The sample size was more than doubled from a previous study to increase statistical power (10).

Study Design and Medications

This was a randomized, placebo-controlled, double-blind, three-way cross-over study, with a washout period of at least 2 weeks. Randomization was obtained according to a Latin square (11) by means of a self-programmed software. As local coxib and prostaglandin concentrations were assessed by means of *in-vivo* microdialysis, placement of microdialysis probes in the subcutaneous tissue for more than 8 h, which would have raised ethical and technical problems, was circumvented by administering the drugs (50 mg lumiracoxib, Prexige®, Novartis, Nürnberg, Germany, and 90 mg etoricoxib, Arcoxia®, MSD, Haar, Germany) twice at 0 and 24 h and measuring concentrations from 4 h before to 4 h after the second administration. This provided information on variables at the end and at the beginning of the dosing period for both lumiracoxib and etoricoxib (half-life of 22 h (12)). With respect to the observation of acute effects, a 4-h sampling time following the second dosing can be regarded as sufficient based on reported onsets of analgesia of <30 min for etoricoxib (13) and 0.6–1.5 h for lumiracoxib (14). The dosage of 90 mg etoricoxib per day was chosen according to the approved medium dosage for adult patients, as specified in the product specification summary for this drug (15). Although the recommended dose of lumiracoxib was 100 mg per day (16), it was reduced by half because this had been reported to produce comparable COX-2 inhibition (17). All side effects spontaneously reported by the subjects were recorded.

Assessment of Concentrations of Lumiracoxib and Etoricoxib

Plasma Concentrations

Venous blood samples were collected into tubes containing potassium ethylenediaminetetraacetic acid before drug administration (baseline) and at 20, 21, 22, 23, 24, 24.25, 24.5, 24.75, 25, 25.5, 26, 26.5, 27, 28, 48, 72, 96, 120, and 144 h after the first administration of the medication. Plasma was separated within 15 min by centrifugation at 1,814 g for 10 min and stored with quality control samples at −80°C pending analysis.

Tissue Concentrations

A local cutaneous inflammation was induced 1 day prior to the study by applying a freeze lesion (18–20) on the surface of the right or left inner forearm with a copper cylinder 15 mm in diameter, 290 g weight and −30°C temperature. It was pressed perpendicularly onto the skin with a contact pressure provided solely by its own weight. For better thermal contact, a filter paper soaked with saline was placed between the skin and copper bar. Induction of the freeze lesion was slightly painful, evoking a perception of an electrical prickle, and rarely burning. Any spontaneous pain subsided within 2 h. The skin lesion remained slightly reddened and sensitive to mechanical stimulation for a few days (21).

Using *in-vivo* microdialysis (22), the concentrations of coxibs and prostaglandins were sampled for 8 h (−4 to +4 h relative to the second drug administration) from the subcutaneous tissue below experimental inflammation induced on the inner side of a forearm and from the non-inflamed contralateral control side. The catheter was constantly perfused with saline. Dialysate concentrations are proportional to tissue concentrations. The microdialysis catheter (CMA 62, membrane length 30 mm, membrane diameter 0.6 mm, molecular weight cut off 20 kDa, CMA, Stockholm, Sweden) was inserted into the subcutaneous space of the anterior forearm, as described previously (23), so that the membrane was localized underneath the freeze lesion or control tissue, respectively. The outlet tube was clipped at 12 cm to reduce dead space. The catheter was perfused with 0.9% sodium chloride using a microdialysis pump (CMA 100, CMA, Stockholm, Sweden) at a flow rate of 2 µL/min. Dialysates were collected at intervals of 30 min. The volume of dead space between microdialysis membrane and tube outlet was 5.8 µL, causing a lag time between diffusion of drug molecules through the membrane and sampling of the dialysate of 3 min. Time points were corrected correspondingly.

Dialysate concentrations were converted into tissue concentrations by employing the *in vitro* transfer rate assessed for lumiracoxib and etoricoxib. The dialysis probe was placed in glass test tubes that contained different concentrations of lumiracoxib or etoricoxib (1–10,000 ng/mL in five log concentration steps). The probe was perfused with sterile water at a flow rate of 2 µL/min and four dialysate samples at 30 min intervals were collected. Dialysate concentrations were plotted versus the respective concentration of the solution. Linear regression analysis was used to convert dialysate

concentrations into tissue concentrations, in which the recovery by microdialysis was approximated from the slope of the regression line (24).

Determination of Lumiracoxib and Etoricoxib

Concentrations of lumiracoxib and etoricoxib were measured by means of validated liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) assays (25) in human plasma and microdialysate as described in detail in the additional materials of this paper. A calibration curve, from 5 to 50,000 ng/mL for lumiracoxib and from 1 to 20,000 ng/mL for etoricoxib, was prepared by spiking 10 µL of the appropriate acetonitrile working solutions in the corresponding matrix. The lower limits of quantification (LLOQ) were 5 ng/mL for lumiracoxib and 1 ng/mL for etoricoxib.

Assessment of COX Inhibiting Effects of Lumiracoxib and Etoricoxib

Stimulation of Prostaglandin Production

COX-2 function was quantified using *ex-vivo* stimulation with LPS. Duplicate 1 ml aliquots of heparinized whole blood samples, obtained at baseline (time=0), 24 and 26 h after the first dosing, were incubated both in the absence and presence of 10 µL LPS (1 mg/mL; *Escherichia coli* serotype 026:B6) for 24 h at 37°C (26). The incubation was stopped by centrifugation (10 min, 32 g) and the separated plasma samples were immediately frozen at -80°C pending assay.

Analysis of Arachidonic Acid Metabolites

The content of PGE_2, PGD_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ in unstimulated and LPS-stimulated blood and dialysate was determined by LC-ESI-MS/MS as also described in the additional materials of this paper. Prostaglandins were separated with a Synergi Hydro-RP column (150×2 mm I.D., 4 µm particle size and 80 Å pore size from Phenomenex, Aschaffenburg, Germany) and determined in an API 4000 triple quadrupole mass spectrometer (AB Sciex, Darmstadt, Germany). The LLOQ was 10 pg/mL for each analyte. From the microdialysis samples, PGE_2, PGD_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and internal standards were extracted twice using liquid-liquid-extraction. The organic phases were removed at a temperature of 45°C under a gentle stream of nitrogen. The residues were reconstituted with 50 µL of acetonitrile/water/formic acid (20:80:0.0025, v/v, pH 4.0), centrifuged for 2 min at 10,000 g and then transferred to glass vials (Macherey-Nagel, Düren, Germany) prior to injection into the LC-MS/MS system.

Assessment of Antihyperalgesic Effects

Induction of freeze lesion was slightly painful, evoking a perception of electrical prickle and rarely burning, as reported previously. Any spontaneous pain subsided within 2 h. However, freezing produced marked inflammatory hyperalgesia within 20 h after application, being stable for approximately 8–10 h (18,27,28). The antihyperalgesic effects were estimated by measuring mechanical pain thresholds at the site of freeze injury and at the contralateral site on a homologous area of normal skin, which served as a control. The pain threshold to punctate mechanical pain stimuli was assessed by means of von Frey hairs of different strengths (0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 0.6, 1, 1.4, 2, 4, 6, 8, 10, 15, 26, 60, 100, 180, 300 g; North Coast Medical Inc., Morgan Hill, CA, USA). They were placed perpendicularly onto the left volar forearm and bent slightly to apply pressure. Von Frey hairs of different strengths were applied in a randomized order. The subjects had to indicate whether or not the stimulus was painful. During the experiments, the subjects had their eyes covered with a mask to avoid recognition of the strength of the von Frey hairs. The threshold to punctate mechanical pain was obtained by logistic regression of "yes/no" answers for the different von Frey hairs. Based on the logarithmic intervals between the von Frey hairs, the log-transformed thresholds were submitted to statistics.

Statistical Analysis

Basic pharmacokinetic parameters derived from lumiracoxib and etoricoxib plasma concentrations, i.e., the maximum concentration, C_{max}, and the time of its occurrence, t_{max}, were read from the data after the second drug administration, since after the first dosing no early measurements had been obtained. For statistical comparison, the values obtained at 24 h and at 26 h were used. The latter time point was the closest (nearest) value to the observed t_{max}. Values were submitted to analysis of variance for repeated measures (rm-ANOVA) against the within-subject factors "time" (24 or 26 h) and "medication" (etoricoxib, lumiracoxib or placebo). For tissue concentrations, the medians obtained over the periods, 4 h before (−4–0 h) and after (0–+4 h) the second dosing, were analyzed. Data were analyzed as for the plasma concentrations, with the additional within-subject factor "side" denoting the control versus freeze lesion side.

Pharmacodynamic parameters were assessed from the whole blood assays taken at the same time points, i.e., at 24 and 26 h after the first dosing, and from the pain thresholds to punctate mechanical stimuli. Specifically, arachidonic acid metabolite concentrations (PGE_2, 6-Keto-$PGF_{1\alpha}$, PGD_2 and $PGF_{2\alpha}$) were submitted to rm-ANOVA against the within-subject factors "time" (24 or 26 h) and "medication"

(etoricoxib, lumiracoxib or placebo), and in addition, "stimulation", denoting LPS stimulation versus non-stimulated blood concentrations. The potency of lumiracoxib and etoricoxib to inhibit COX-2 was calculated from the PGE_2 concentrations measured at 0 (pre-dose), 24 and 26 h in the *ex-vivo* blood. As described previously (17), the percentage change in plasma PGE_2 was determined relative to pre-dose plasma PGE_2 levels. For each value, basal PGE_2 concentrations measured in the absence of LPS were subtracted from PGE_2 concentrations determined in LPS-treated blood aliquots. The COX-2 inhibitory potencies were obtained (29) by fitting a sigmoid pharmacodynamic model to the percent inhibition of PGE_2 formation versus log coxib concentrations relationship, with baseline and maximum effect fixed at values of 0 and 100%, respectively. Data were fitted by nonlinear regression (GraphPad Prism, version 5.02; GraphPad Software, San Diego, CA). The pain thresholds were submitted to rm-ANOVA against the within-subject factors "time" (0, 20, 22, 24, 26 and 28 h), "medication" (etoricoxib, lumiracoxib or placebo) and "side" (control, versus freeze lesion side).

Post hoc comparisons were made, in the case of significant primary effects or interactions, by calculating t-tests. The α level was set at 0.05 and corrected for multiple comparisons according to Bonferroni. Statistical analyses were done using the SPSS software (version 21 for Linux, SPSS IBM Inc., Chicago, USA). Results are only reported for primary statistical effects and relevant interactions.

RESULTS

All subjects completed the study without side effects that required medical intervention. Due to technical problems, the tissue concentrations of arachidonic acid metabolites and medications in one subject (subject 4) and the data on inhibition of prostaglandin production in another subject (subject 2) had to be excluded from statistical analyses.

Concentrations of Etoricoxib and Lumiracoxib

Plasma Concentrations

While etoricoxib was still measurable in plasma 20–24 h after administration of the first dose (Fig. 2), lumiracoxib concentrations were below the LLOQ. Following the second administration of lumiracoxib or etoricoxib, plasma concentrations peaked within 2–3 h (27 ± 1.1 h and 26.4 ± 1.3 h, respectively, relative to the first administration). After the second dose, plasma concentrations of etoricoxib and lumiracoxib showed a similar increase, reaching a mean C_{max} of 1,612 ± 808 ng/mL and 1,881 ± 1,354 ng/mL, respectively. Plasma concentrations of etoricoxib (29 ± 38 ng/mL) were detectable up to 120 h after the second dose.

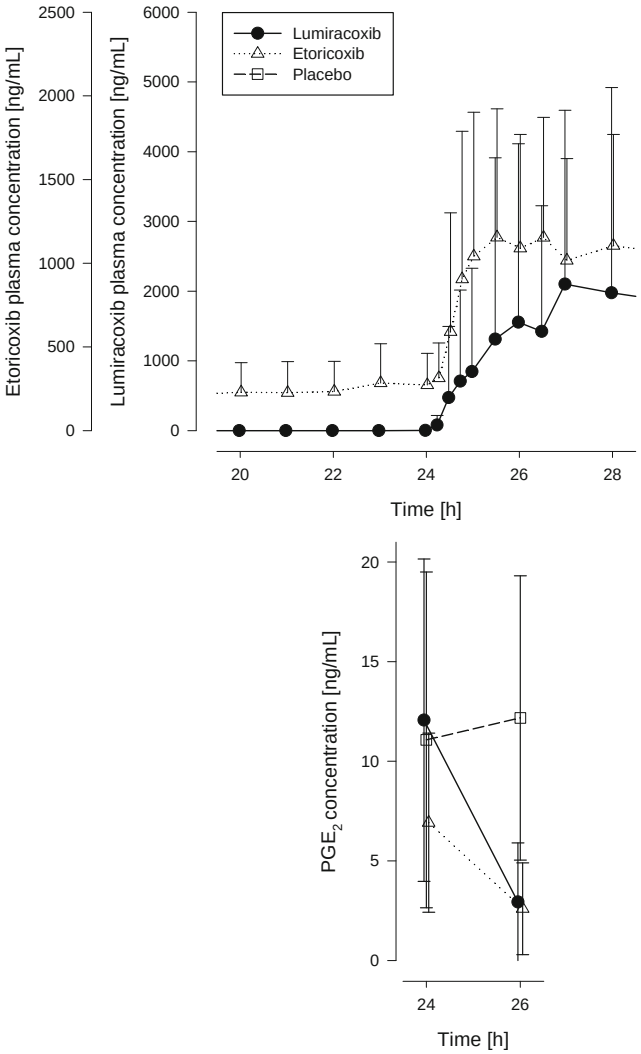

Fig. 2 Plasma concentrations of lumiracoxib and etoricoxib (top) during the period of primary pharmacodynamic assessments, ±4 h around the second administration of the study medication. The effects on PGE_2 production after *ex-vivo* stimulation with LPS (bottom) were consistent with the plasma concentrations and expectations, i.e., fell from a high level due to almost complete elimination of lumiracoxib before the second dose; fell from a moderate level because of incomplete elimination of etoricoxib; and remained at a high level during the placebo condition (rm-ANOVA effect "medication": $p = 0.000005$, interaction "time" by "medication": $p = 0.000009$).

Tissue Concentrations

The *in vitro* recovery of etoricoxib through microdialysis was approximately 23% and the calibration curve was linear over the concentration range of 1–10,000 ng/mL. The concentrations of etoricoxib in tissue (Fig. 3) changed as expected, i.e., they rose significantly from 27 ± 36 ng/mL and 28 ± 43 ng/mL at the control and freeze lesion sites, respectively, during the pre-2nd dose microdialysis sampling period (medians across the samples), to 162 ± 210 ng/mL and 154 ± 178 ng/mL during the post-2nd dose sampling period (rm-ANOVA effect "time": df = 1,17, $F = 12.66$, $p = 0.002$). Almost identical

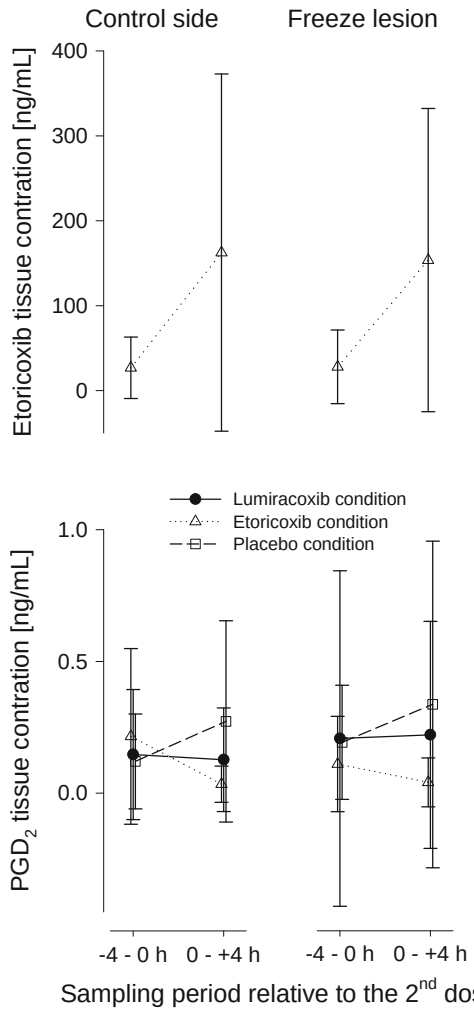

Fig. 3 Tissue concentrations sampled during the periods of the 4 h before or the 4 h after administration of the 2nd dose of the medication, via microdialysis tubes placed subcutaneously on the control side (left panels) or under the freeze lesion (right panels). Average and standard deviations of the median concentrations across the sampling periods are shown. In the top panels, the concentrations of etoricoxib are shown (rm-ANOVA effect "time": $p=0.002$, effect "side": $p=0.54$). Lumiracoxib was not detectable in the dialysate. In the bottom panels, the concentrations of PGD_2 are shown, revealing the only statistically significant effects of the medication on tissue concentrations of arachidonic acid products (rm-ANOVA interaction "time" by "medication": $p=0.007$).

concentrations were found at both sites (effect "side": df=1,17, $F=0.39$, $p=0.54$).

The *in vitro* recovery of lumiracoxib through microdialysis was approximately 14% and the calibration curve was linear over the range from 1 to 10,000 ng/mL. However, lumiracoxib was undetectable in the dialysate samples. Using the linear equation obtained during recovery analysis ($C_{dialysate}=0.1406 \cdot C_{tissue}$) and a LLOQ of 0.5 ng/mL, this means that tissue concentrations of free (i.e., not protein bound) lumiracoxib did not exceed 3.6 ng/mL during the 8 h microdialysis period.

Effects of Etoricoxib and Lumiracoxib

Inhibition of Prostaglandin Production in Ex-Vivo Blood

Ex-vivo stimulation of whole blood with LPS significantly elevated the blood concentration of the pro-inflammatory PGE_2 (rm-ANOVA effect "stimulation": df=1,16, $F=48.06$, $p<0.001$; additional Table I). This was more pronounced at 24 h than at 26 h (effect "time": df=1,16, $F=13.35$, $p=0.001$; Fig. 2) and was significantly modulated by drug administration (effect "medication": df=2,32, $F=18.509$, $p<0.001$, interaction "time" by "medication": df=2,32, $F=17.09$, $p<0.001$). Specifically, after the second dose of lumiracoxib, PGE_2 concentrations decreased from 12.1±8.1 ng/mL (24 h) to 2.9±3.00 ng/mL (26 h). The PGE_2 production was comparatively lower after the first dose of etoricoxib [6.9±4.5 ng/mL (24 h)], indicating ongoing inhibition, and decreased to 2.6±2.3 ng/mL at 26 h, i.e., 2 h after the second dose. In contrast, PGE_2 production remained high (11.1 ±8.4 and 12.2±7.1 ng/mL at 24 and 26 h, respectively) during the placebo condition. Non-LPS stimulated blood concentrations of PGE_2 did not differ among time points and medications (0.08–0.17 ng/mL). Both coxibs inhibited the production of PGE_2 with similar potencies (Fig. 4). Specifically, the calculated values of EC_{50} for inhibition of PGE_2 production after LPS stimulation were 262.1 ng/mL for lumiracoxib (95% confidence interval, CI, of estimate: 125.4–547.9 ng/mL) and 198.5 ng/mL for etoricoxib (95% CI of estimate: 130–303 ng/mL). In contrast, $PGF_{2\alpha}$ did not show time or medication associated differences, concentration of 6-Keto-$PGF_{1\alpha}$ remained below the LLOQ after LPS stimulation and PGD_2 was found only at very low concentrations of approximately 0.4 ng/mL.

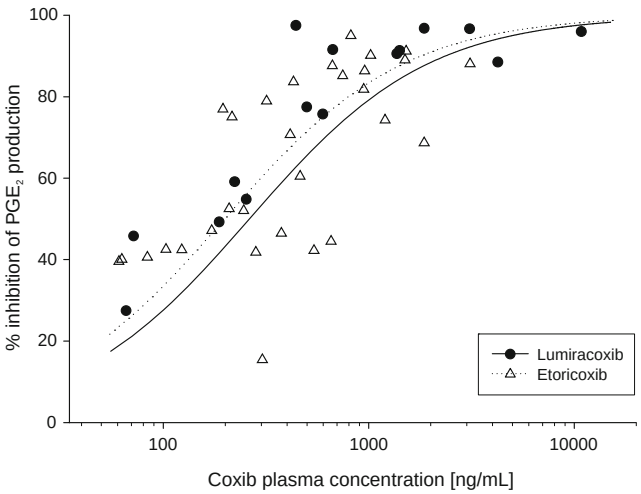

Fig. 4 Effect versus concentration relationships for lumiracoxib and etoricoxib, based on prostaglandin E_2 (PGE_2) production from peripheral blood monocytes stimulated *ex vivo* with bacterial LPS, which wass progressively more inhibited with increasing coxib plasma concentrations. A sigmoid pharmacodynamic model was applied. The values of EC_{50} for the inhibition of PGE_2 production were 262.1 ng/mL for lumiracoxib and 198.5 ng/mL for etoricoxib.

Modulation of Tissue Concentrations of Arachidonic Acid Metabolites

Changes in pre-dose, compared to post-dose concentrations of arachidonic acid metabolites in tissue were best observed with PGD_2 (Fig. 3). Its *in vitro* recovery was 47.7% and the calibration curve was linear over the range from 0.1 to 10 ng/mL. Under the placebo condition, its tissue concentrations tended to increase from the first half (pre-2nd dose) of the microdialysis sampling period (0.1 ± 0.2 ng/mL and 0.2 ± 0.2 ng/mL at the control and freeze lesion sides, respectively) to the second half (post-2nd dose: 0.3 ± 0.4 ng/mL and 0.3 ± 0.6 ng/mL, respectively). This rise was blocked by the active medications (rm-ANOVA interaction "time" by "medication": df=2,32, $F=5.76$, $p=0.007$, main effect "time": df=1,16, $F=0.022$, $p=0.885$), with a more pronounced tendency towards decreasing PGD_2 tissue concentrations after administration of etoricoxib than after lumiracoxib (Fig. 3 and additional Table II). Consistent with the latter observation, the tissue concentrations of PGD_2 correlated significantly with those in the LPS-stimulated *ex-vivo* blood at 2 h after administration of the second dose etoricoxib (control side: Spearman's $\rho=0.57$, $p=0.018$, freeze side: not significant), however, not at 24 h after administration of the first dose. Such a correlation was observed neither with lumiracoxib nor with placebo (p always >0.35). Finally, an effect of the sampling side on the PGD_2 tissue was not found (effect "side": df=1,16, $F=0.65$, $p=0.43$), neither was there a primary effect of the factor "medication" (df=2,32, $F=1.57$, $p=0.224$).

The recoveries of PGE_2, 6-Keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were 99.5, 100 and 100%, respectively. Their tissue concentrations did not differ between sides and did not differ significantly between medications (details not shown). The only exception to these negative findings was a side-differing change in tissue $PGF_{2\alpha}$ concentrations with time (rm-ANOVA main effect "time": df=1,16, $F=8.14$, $p=0.012$, interaction "time" by "side": df=1,16, $F=5.89$, $p=0.027$). While, at the control side, $PGF_{2\alpha}$ concentrations decreased from the sampling period before administration of the 2nd dose of the medications to the period after its administration, this was significantly less pronounced under the freeze lesion. However, the medication had no influence on this course (main effects and interaction including the factor "medication": $p>0.1$).

Effects on Experimental Hyperalgesia

Application of the freeze lesion clearly succeeded in inducing a significant hyperalgesia (Fig. 5). Pain thresholds to punctate mechanical stimuli dropped from 78.7 ± 67.9 g before freezing to 27.7 ± 30.2 g after freezing (grand average across medications) but remained stable at the control site. The effect of applying the freeze lesion was statistically significant (rm-ANOVA main effect "time": df=5,80, $F=11.49$, $p<10^{-6}$, main effect "side": df=1,16, $F=50.103$, $p=3 \cdot 10^{-5}$, interaction "time" by "side": df=5,80, $F=13.18$, $p<10^{-5}$). At the site of the freeze lesion, post hoc t-tests established both its significant effect on pain thresholds and its temporal stability ($p<0.05$ for the first threshold, taken before application of the freeze lesion, versus each of the thresholds measured after its application; control side: no such significant differences). However, the medication failed to produce any significant effects on pain thresholds on either side (effect "medication": df=2,32, $F=0.231$, $p=0.795$, interaction "medication" by "side": df=2,32, $F=1.125$, $p=0.337$, interaction "medication" by "time": df=10,160, $F=1.103$, $p=0.363$, interaction "medication" by "side" by "time": df=10,160, $F=0.496$, $p=0.891$). This implies that the two coxibs did not differ from placebo with respect to pain thresholds and the small rise in pain thresholds at the freeze lesion side following the administration of etoricoxib (Fig. 5 right) was not statistically significant.

DISCUSSION

The three main components of this study, plasma concentrations, tissue concentrations and experimentally induced hyperalgesia, were consistent throughout the study. Firstly, the plasma concentrations versus time profiles displayed the expected courses, with etoricoxib but not lumiracoxib still detectable at 24 h after the first dose, as anticipated by their half-lives, and with the expected rise in concentrations after the second dose. Similarly, the concentrations of PGE_2 in LPS-stimulated *ex-vivo* blood were in keeping with the concentration-dependent inhibition of their production by the coxibs but not by placebo. Secondly, tissue concentrations of etoricoxib were low, yet did not reach zero 24 h after the first dose and increased after administration of the second dose. Moreover, concentrations were almost identical on both sides, as expected, indicating that microdialysis had been performed successfully. Thirdly, the freeze lesion was effective in producing hyperalgesia, as indicated by the significant decrease in pain thresholds limited to the freeze side and the persistence of this unilateral hyperalgesia throughout the period of primary measurements. These indicators were taken to reflect the validity of the methods applied. A first finding of this study was that doses of 90 mg etoricoxib and 50 mg lumiracoxib were equipotent with respect to their systemic effects. This comparability resulted from the similar concentrations of PGE_2 in LPS-stimulated blood after the administration of the second dose of each of the medications. Both coxibs exerted inhibitory effects on the production of PGE_2 at similar potencies, as indicated by the values of EC_{50} in the sigmoid pharmacodynamic model, which were not statistically different when considering the confidence intervals of estimate. Moreover, the EC_{50} of COX-2 inhibition by etoricoxib

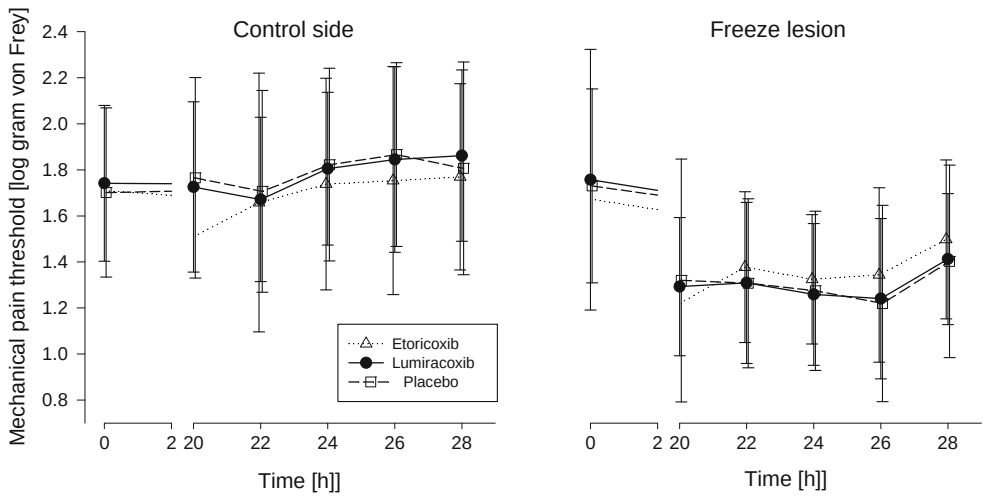

Fig. 5 Time courses of the thresholds to punctate mechanical pain (von Frey hairs), starting from the measurement at baseline (time = 0), taken before the freeze lesion was placed, and continuing for up to 20 h later when the measurements around the second dose of the medication (administered at 24 h immediately after the measurement) were started. The arithmetic means and standard deviations are shown. While the freeze lesion clearly and significantly decreased the pain thresholds, indicating hyperalgesia, the medications failed to influence the thresholds (rm-ANOVA main effect "time": $p < 10^{-6}$, main effect "side": $p = 3 \cdot 10^{-5}$, interaction "time" by "side": $p < 10^{-5}$, effect "medication": $p = 0.795$, interaction "medication" by "side": $p = 0.337$).

of 198.5 ng/mL, corresponding to 553.2 nM, agreed very well with the reported IC_{50} of 0.47 μM (30) and when considering the 95% CI of estimate of 362.3–844.4 nM, the values were similar to the previously reported potency. Similarly, the EC_{50} for lumiracoxib of 262.1 ng/mL, corresponding to 0.89 μM (95% CI of estimate 0.43–1.87 μM), was within the same order of magnitude as the reported potency of 0.13 μM (31). As expected, the systemic effects of etoricoxib persisted for 1 d while those of lumiracoxib disappeared, a finding that correlates with the different plasma half-lives and which was seen with the PGE_2 concentrations in 24 h LPS-stimulated blood. However, as 90 mg etoricoxib is the clinically recommended dose (32), a higher dose of lumiracoxib than the systemically equipotent dose of 50 mg raises the risk of overdosing (17) with respect to its systemic effects.

The putative systemic overdose of lumiracoxib, however, conflicts with its reduced local effects on the metabolism of arachidonic acid. The present results provided no indication of a local effect of lumiracoxib at the administered, systemically equipotent dose. The inhibition of the rise in tissue PGD_2 concentrations was less pronounced after administration of lumiracoxib than after administration of etoricoxib. Moreover, the absence of local effects of lumiracoxib is further indicated by the lack of a correlation between PGD_2 concentrations in subcutaneous tissue or in *ex-vivo* whole blood, even at its peak plasma concentrations 2–3 h after administration. In contrast, a respective local vs. systemic correlation between PGD_2 concentrations was seen during etoricoxib treatment, although it was weak, only observed unilaterally and therefore has to be interpreted with caution. It thus appears that for a stable local effect, the lumiracoxib dose needs to be increased above the recommended dose, which contrasts with its systemic effects which already were equivalent to those of etoricoxib at the doses administered.

A second finding of this study was that the results did not support the accumulation of the acidic lumiracoxib in the injured tissue. The local production of PGD_2 in subcutaneous tissue was significantly inhibited by both active coxibs. However, this was only observed after administration of the second dose. An on-going pharmacological effect of lumiracoxib, as a consequence of ion trapping in acidic tissue, would have required this effect to be present also at the beginning of the microdialysis sampling period, 20 h after the first dose. This would have been seen even when lumiracoxib concentrations were low, but high enough to inhibit prostaglandin production. Unfortunately, the low recovery and the high protein binding of lumiracoxib prevented its microdialysis from subcutaneous tissue at measurable dialysate concentrations, and therefore, the results are solely based on indirect evidence on effects and should therefore be regarded with caution. This contrasts with the observation of three-fold higher lumiracoxib concentrations found in synovial fluid of rheumatoid arthritis patients compared to plasma.(8) However, these values were obtained after 7 days of treatment with lumiracoxib 400 mg once daily, which substantially exceeds the present dosage.

The patterns of inhibition of arachidonic acid metabolites in tissue, with coxibs acting mainly upon PGD_2 production, emphasise the different cellular composition of the subcutaneous space in comparison to that of the blood, where the main effects of coxibs were seen on PGE_2 production. This agrees with the observation that blood macrophages produce mainly PGE_2, whereas mast cells predominantly generate PGD_2 (33). Specifically, PGD_2 derived from mast cells and

epidermal dendritic cells makes an important contribution to skin inflammation (34) and plays a major role in niacin-induced flushing, as it binds to D prostanoid 1 (DP1) receptors located on epidermal Langerhans cells and keratinocytes (35), causing vasodilatation that can be blocked by the DP1 receptor antagonist, laropiprant (36).

A third finding of this study is the apparent insensitivity of the freeze lesion pain model to the effects of etoricoxib and lumiracoxib. The freeze lesion has been shown to be sensitive to the effects of NSAIDs such as ibuprofen (19) and diclofenac (37) and of opioids such as remifentanil (21), morphine and morphine-6-glucuronide (28). The response to freezing involves strong up-regulation of COX-2 and activation of nuclear factor kappa B (NF-κB), whereas COX-1 expression is slightly reduced (10). The model is sensitive to selective COX-2 inhibitors considering the reported effectiveness of 40 mg parecoxib in a similar setting (10). Therefore, since the freeze lesion obviously produced the expected hyperalgesia, an alternative explanation for the lack of antihyperalgesic effects of coxibs might relate to the dosing regimen. Etoricoxib was administered at the clinically approved dose of 90 mg. This dose corresponds to the median dose reported in the context of an extensive analysis of adverse effects of COX-2 inhibitors (38). In the same analysis, the median dose of valdecoxib was 23 mg/d (38). This corresponds to 27.1 mg of its prodrug, parecoxib, when calculated on the basis of molecular weights. Since in the analysis of 114 randomized double-blind clinical trials, doses of coxibs were equipotent, i.e., 27.1 mg of parecoxib corresponded to 90 mg of etoricoxib, the dose of 40 mg parecoxib that produced antihyperalgesic effects in the freeze lesion (10), in potency terms, was larger than the presently used dose of 90 mg etoricoxib. The observed, non-significant increase in pain thresholds on the freeze lesion side, following the administration of etoricoxib, also points towards a dosage-related influence. When further considering the similar systemic effects of etoricoxib and lumiracoxib in the present study, the dose of 50 mg lumiracoxib was also too low to produce antihyperalgesic effects, when compared to 40 mg parecoxib, which is in keeping with the lack of antihyperalgesia in the freeze lesion model. However, lumiracoxib produces analgesic effects slower than etoricoxib (13,14) and the onset time of 0.6–1.5 h was reported following administration of 400 mg (14). At a low dose of 50 mg, the threshold of effective tissue levels may have been crossed later, if at all, providing a further possible explanation of the lack of analgesic effects of lumiracoxib.

The once-daily dosing recommendation for lumiracoxib was based on tissue ion trapping in inflamed tissues, which was thought to outlast its presence at effective concentrations in plasma. In contrast, the study showed that at the systemic level, a dose of 50 mg lumiracoxib is equipotent to a clinically effective dose of 90 mg etoricoxib. This implies that higher doses once a day possibly represent an overdose. However, in the local inflamed tissue, the doses were not equivalent, which suggests the need for a higher dose of lumiracoxib, which would then be associated with increased systemic effects. Therefore, the results of the present study do not support a revival of lumiracoxib under the previously recommended dose regimen for the chronic therapy of rheumatic diseases such as osteoarthritis. However, the study did not accommodate steady-state conditions following multiple dosing, which remains to be addressed. With shorter dosing intervals, however, the claimed advantage of lumiracoxib over other coxibs consisting of local effects outlasting systemic effects disappears. Still, it may be used as an alternative highly selective COX-2 inhibitor provided strong patient selection including intact liver and HLA genotyping.

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