An exploratory microdialysis study investigating the effect of repeated application of a diclofenac epolamine medicated plaster on prostaglandin concentrations in skeletal muscle after standardized physical exercise

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Therapy with topical non-steroidal anti-inflammatory drugs (NSAIDs) relies on the ability of the active drug to penetrate the skin in sufficiently high amounts to exert a clinical effect.
- A significant cyclo-oxygenase dependent formation of inflammatory eicosanoids is associated with muscle injuries.

WHAT THIS STUDY ADDS

- This study hypothesizes the effect of repeated epicutaneous application of diclofenac on the local interstitial concentration of prostaglandins by describing PGE₂ and 8-iso-PGF_{2α} concentrations in the skeletal muscular tissue of the vastus lateralis under rest and dynamic exercise conditions.

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AIM

Muscle injuries and extensive exercise are associated with cyclo-oxygenase dependent formation of inflammatory prostaglandins. Since the effect of topical administration of non-steroidal anti-inflammatory drugs (NSAIDs) on local cyclo-oxygenase is unknown, the present exploratory, open label, non-randomized study set out to measure exercise induced release of prostaglandins before and after epicutaneous administration of diclofenac.

METHODS

Microdialysis was used to determine the local interstitial concentration of PGE₂ and 8-iso-PGF_{2α} as well as diclofenac concentrations in the vastus lateralis under rest, dynamic exercise and during recovery in 12 healthy subjects at baseline and after a treatment phase applying a total of seven plasters medicated with 180 mg of diclofenac epolamine over 4 days.

RESULTS

At baseline PGE₂ concentrations were 1169 ± 780 pg ml⁻¹ at rest and 1287 ± 459 pg ml⁻¹ during dynamic exercise and increased to 2005 ± 1126 pg ml⁻¹ during recovery. After treatment average PGE₂ concentrations were 997 ± 588 pg ml⁻¹ at rest and 1339 ± 892 pg ml⁻¹ during exercise. In contrast with the baseline phase no increase in PGE₂ concentrations was recorded during the recovery period after treatment (PGE₂ 1134 ± 874 pg ml⁻¹). 8-iso-PGF_{2α} was neither affected by exercise nor by treatment with diclofenac. Local and systemic concentrations of diclofenac were highly variable but comparable with previous clinical pharmacokinetic studies.

CONCLUSIONS

We can hypothesize an effect of topical diclofenac epolamine plaster on limiting the increase of local concentrations of the pro-inflammatory prostaglandin PGE₂ induced in the muscle of healthy human subjects following standardized physical exercise. No effect of diclofenac treatment on 8-iso-PGF_{2α} concentrations was observed, mainly since isoprostane is produced by a free radical-catalyzed lipid peroxidation mechanism independent of cyclo-oxygenases.

Introduction

Oral non-steroidal anti-inflammatory drugs (NSAIDs) act by reducing systemic prostaglandin production through direct inhibition of cyclo-oxygenase enzymes as well as modulation of arachidonic acid release and uptake. They are used for the treatment of acute and chronic pain of different aetiologies [1, 2]. However, systemically administered NSAIDs may cause dose-dependent adverse reactions in the upper gastrointestinal tract and in the renal, cardiovascular and respiratory system [1, 2]. Therefore, topical formulations for direct application at the site of pain have been developed [3]. Direct application results in continuous and localized drug delivery, while reducing systemic exposure to levels equalling 0.2% to 8% of the oral dose [3]. Topical formulations of diclofenac have been available in Europe for more than 20 years to relieve pain and inflammation due to rheumatic disease, osteoarthritis in muscles, tendons and joints or sports injuries [4, 5]. Previous clinical studies demonstrated the high tolerability and analgesic activity of diclofenac epolamine medicated plaster and detected a constant plasma concentration of diclofenac for up to 12 h after application [6–8]. However, after topical administration diclofenac concentrations in skeletal muscle tissue were found to be highly variable and below the lower limit of quantification of the analytical assay for the majority of the samples, despite significant diclofenac concentrations in the corresponding plasma samples [9–13].

The anti-inflammatory effect of diclofenac is related to the inhibition of cyclo-oxygenase activity that results in the modulation of chemical mediators. Previous investigations gave evidence that dynamic exercise and high intensity static contraction induce the release of some eicosanoids, such as prostaglandins, from the skeletal muscle [14]. These mediators have several biological effects such as induction of inflammation and vasodilation [14].

The rationale of the present study was to verify the local pharmacodynamic effect of diclofenac released from the plaster and, in particular, if local exposure to diclofenac would alter prostaglandin concentrations, resulting from an over extension while exercising, in tissues such as skeletal muscle. The effect on prostaglandin production in muscle tissue was investigated using microdialysis for determination of exercise induced release of PGE_2 and 8-iso- $\text{PGF}_{2\alpha}$ before and after repeated epicutaneous applications of diclofenac epolamine to healthy volunteers. In addition, the diclofenac concentration in plasma and in the interstitial fluid was determined. Microdialysis is an established method based on sampling of analytes from the extracellular space by means of a semi-permeable membrane at the tip of a microdialysis probe. The principles of microdialysis have been described in detail previously [15, 16].

Methods

The study took place at the Department of Clinical Pharmacology, Medical University of Vienna, Austria. The study protocol was approved by the local Ethics Committee and Austrian Competent Authorities (AGES) and was registered at EudraCT (2009–015900-24). The study was performed in accordance with the Declaration of Helsinki 1964 (including current revisions), the Austrian Drug Law and the Good Clinical Practice Guidelines.

Study participants

Twelve healthy male Caucasian volunteers were enrolled in the study. Their ages were between 21 and 30 years; their mean bodyweight was 72.3 ± 8.8 kg and they had a mean body mass index of 21.9 ± 2.4 kg m⁻². Written informed consent was obtained from each volunteer prior to any study-related investigation or intervention. Within 14 days before the baseline period each volunteer underwent a screening examination, consisting of medical history, physical examination, body weight, vital signs, routine laboratory tests and a 12-lead electrocardiography. During the study the subjects were not allowed to take other medications, including intake of any medications within 2 weeks before the start of the study and medication containing diclofenac for 1 month before the study. Volunteers received standardized meals on study days and were instructed to avoid coffee, tea and any other food containing xanthines (i.e. coke, chocolate, etc.), alcohol and grapefruit juice during the entire study period.

Study design

The study was conducted as a single centre, open label, non-randomized, exploratory pharmacodynamic microdialysis study. The study included two periods, i.e. a baseline period (including study day 1) and a treatment period (including study day 2). After the first six subjects completed the study (group 1), a predefined interim analysis was performed. According to the results of the interim analysis, it was decided to modify microdialysate sample collection intervals slightly for the remaining six subjects (group 2). In detail, collection intervals were delayed by 10 min with respect to the beginning of the rest, dynamic exercise and recovery intervals, and the microdialysis flow rate was increased from 0.3 (group 1) to 0.5 $\mu\text{l min}^{-1}$ (group 2). These changes were introduced to permit an improvement of prostaglandin sample collection during each interval.

Baseline period

On microdialysis study day 1 the volunteers were admitted to the clinical research ward on the morning of the study day. Two microdialysis probes (CMA 63® microdialysis probes with cut-off 20 000, CMA, Solna, Sweden) were inserted into the skeletal muscle tissue of the vastus latera-

lis muscle for determination of prostaglandins. The deepness of the probe in the muscle tissue was of about 2–4 cm. Differences between each subjects are related to the individual anatomical characteristics. The microdialysis system was connected to a microinfusion pump and perfused with Ringer's solution at a flow rate of $0.3 \mu\text{l min}^{-1}$ (group 1) or $0.5 \mu\text{l min}^{-1}$ (group 2).

After a run-in period of 1 h (equilibration interval) to compensate for tissue trauma after probe insertion, collection intervals for PGE_2 and 8-iso-PGF $_{2\alpha}$ determination in microdialysates were selected based on previously published work [17]. After the run-in period microdialysed fluid was collected for 1 h (rest interval). Thereafter subjects performed dynamic exercise for 30 min on a bicycle ergometer with resistance calibrated at 70% of the calculated maximal resistance according to ergometer nomograms [18]. The second sampling period of 1 h started with the start of the dynamic exercise until 30 min after the end of the dynamic exercise (exercise interval). The final sampling interval of 1 h (recovery interval) was performed subsequently. Thereafter the microdialysis probes were removed.

Treatment period

The treatment period was initiated after a rest period of at least 3 days after day 1. The identical leg was always used for investigations in both study periods. During the treatment period healthy volunteers received multiple applications of diclofenac epolamine (DHEP) medicated plaster containing 180 mg diclofenac epolamine, which corresponds to 140 mg diclofenac sodium (IBSA Institut Biochimique S.A., Switzerland). On the first day of this period the subjects attended the clinical unit in the morning and the first plaster was applied by the study staff to the application area (anterior right or left thigh). Plasters were then applied at the clinical unit for 3 days in the morning and evening, i.e. each of the plasters remained *in situ* for 12 consecutive h. The plasters were fixed securely using a TegadermTM adhesive dressing during this time. After the sixth application on the evening of day 3 the subjects stayed in the clinic overnight. On the morning of day 4 (i.e. at least 1 week after microdialysis study day 1) microdialysis study day 2 was performed as described above. The seventh plaster was applied after the microdialysis probes were implanted and remained in place for at least 6 h. Probe equilibration, rest, exercise and recovery intervals were performed as described above for baseline.

In addition, microdialysis and blood samples for diclofenac analysis were collected for 3 consecutive h after the recovery period. Retrodialysis for probe calibration with regard to diclofenac was performed before removal of the microdialysis probes as previously described [19]. A final visit to check for the occurrence of adverse events was performed 7 days after microdialysis study day 2.

Chemical analysis

PGE_2 and 8-iso-PGF $_{2\alpha}$ were determined at BioProof AG, Germany, and analyzed using two distinct commercially available EIA kits from the Cayman Chemical Company (Ann Arbor, MI 48108, USA). The lower quantification limit (LQL) was 15.63 pg ml^{-1} for PGE_2 (RSD% 7.2, bias % 6.9) and 2.0 pg ml^{-1} for 8-iso-PGF $_{2\alpha}$ (RSD% 23.5, bias% 7.9). Diclofenac was determined in plasma (total diclofenac), microdialysate and retrodialysate (free diclofenac) samples at Pharmakin GmbH, Germany, using a validated LC-MS/MS method with a LQL of 150 pg ml^{-1} (CV% 13.19).

Pharmacodynamic and pharmacokinetic calculations and statistical analysis

The pharmacodynamic analysis consisted of the evaluation of potential effects of plaster application on the local interstitial concentrations of PGE_2 and 8-iso-PGF $_{2\alpha}$. Interstitial concentrations in muscle tissue determined during the baseline and treatment period were descriptively compared. Differences between the baseline and treatment period were analyzed at each sample time interval using either a paired *t*-test (or a Wilcoxon signed ranks test in case of lack of normality) and the evaluation of the 95% confidence intervals (CI) for the difference between collection intervals and treatment periods in terms of PGE_2 microdialysate content. Normality of data distribution was assessed using the Shapiro-Wilk test. All the statistical analyses were assessed using SAS[®] software version 9.1.3.

Descriptive pharmacodynamic and pharmacokinetic analyses were performed using the validated software WinNonLin (Version 5.2, Pharsight Corporation).

Results

Pharmacodynamic results

PGE_2 Mean PGE_2 concentrations for the first six subjects (group 1) and the further six subjects (group 2) are presented in Table 1. An expected, inter-individual variability in PGE_2 concentrations was observed at all collection intervals. The response was slightly different among the two groups, with group 2 showing a more pronounced effect of diclofenac on PGE_2 modulation than group 1. In group 2, a decrease in PGE_2 concentrations during the treatment period as compared with the baseline period was evident for all three microdialysate collection intervals, with reduction in PGE_2 being more pronounced during recovery (-51% , 2045.5 vs. $1010.9 \text{ pg ml}^{-1}$) and rest (-41% , 1227.2 vs. 726.5 pg ml^{-1}) than during dynamic exercise (-3% , 1162.6 vs. $1129.3 \text{ pg ml}^{-1}$). In group 1, the decrease was exclusively observed for the recovery interval (-37% , 1964.9 vs. $1236.5 \text{ pg ml}^{-1}$). However, no significant differences were observed for the analysis (95% CI) between the collection intervals in both periods for group 1 and group 2 separately (Table 2).

Table 1

Mean $\pm$ SD PGE₂ and 8-iso-PGF_{2 α} microdialysate concentrations (pg ml⁻¹) for group 1 (n = 6) and group 2 (n = 6) measured at rest, during dynamic exercise and during the recovery interval on day 0 (baseline) and on day 4 of treatment

	Group 1		Group 2	
	Baseline	Treatment	Baseline	Treatment
PGE ₂				
Rest	1111.4 $\pm$ 529.6	1266.7 $\pm$ 691.1	1227.2 $\pm$ 1023.9	726.5 $\pm$ 328.5
Exercise	1411.1 $\pm$ 358.6	1549.2 $\pm$ 756.5	1162.6 $\pm$ 546.6	1129.3 $\pm$ 1035.3
Recovery	1964.9 $\pm$ 797.6	1236.5 $\pm$ 1026.6	2045.5 $\pm$ 1466.5	1010.9 $\pm$ 745.5
8-iso-PGF _{2α}				
Rest	158.0 $\pm$ 63.9	134.0 $\pm$ 49.8	214.7 $\pm$ 161.4	348.6 $\pm$ 301.4
Exercise	118.0 $\pm$ 36.7	130.8 $\pm$ 76.2	280.8 $\pm$ 239.6	579.8 $\pm$ 662.3
Recovery	106.1 $\pm$ 34.3	110.5 $\pm$ 31.5	445.8 $\pm$ 327.7	467.4 $\pm$ 301.4

Table 2

PGE₂ results presented as 95% confidence intervals for the difference between microdialysis collection intervals at baseline (day 0) and after the treatment period (day 4) for group 1 (n = 6) and group 2 (n = 6)

Group	Day	Difference	n	Mean difference	SD	Lower 95%CI	Upper 95% CI
1	0	Exercise – Rest	6	299.68	427.50	-148.95	748.32
1	0	Recovery – Rest	6	853.53	856.37	-45.18	1752.24
1	0	Recovery – Exercise	6	553.85	810.15	-296.36	1404.05
1	4	Exercise – Rest	6	282.46	667.05	-417.57	982.48
1	4	Recovery – Rest	6	-30.20	979.96	-1058.61	998.20
1	4	Recovery – Exercise	6	-312.66	659.34	-1004.59	379.27
2	0	Exercise – Rest	6	-64.58	685.13	-783.58	654.41
2	0	Recovery – Rest	6	818.35	887.66	-113.19	1749.89
2	0	Recovery – Exercise	6	882.93	1098.71	-270.10	2035.96
2	4	Exercise – Rest	6	402.82	793.67	-430.08	1235.73
2	4	Recovery – Rest	5	375.02	577.96	-342.62	1092.66
2	4	Recovery – Exercise	5	278.93	361.93	-170.46	728.32

Mean PGE₂ concentrations for all 12 subjects, both study periods and all collection intervals are presented in Figure 1. PGE₂ was detectable in microdialysates at all three collection intervals, i.e. at rest, dynamic exercise and recovery. At baseline, the mean PGE₂ concentration was 1169.3 $\pm$ 779.6 pg ml⁻¹ at rest and 1286.8 $\pm$ 459.5 pg ml⁻¹ during dynamic exercise, increasing to 2005.2 $\pm$ 1126.3 pg ml⁻¹ only during the recovery interval. After the treatment period, the mean PGE₂ concentration was 996.6 $\pm$ 588.0 pg ml⁻¹ at rest, 1339.2 $\pm$ 891.8 pg ml⁻¹ during exercise and 1134.0 $\pm$ 873.6 pg ml⁻¹ during recovery. Thus, in contrast to the baseline period, no increase in PGE₂ concentrations was recorded during the recovery interval after repeated application of diclofenac medicated plasters, although the differences between the baseline and the treatment period observed did not reach statistical significance (as paired *t*-test analysis, data not shown) for any of the collection intervals (Figure 1). Indeed, the results presented as 95% CI for the difference between the different collection intervals, in both periods, showed significant differences between the recovery and the rest intervals and between the recovery and the exercise intervals, but not

Figure 1

Mean $\pm$ SD PGE₂ microdialysate concentrations (pg ml⁻¹) measured on day 0 (baseline) and on day 4 of treatment at rest, during dynamic exercise and during the recovery interval (n = 12). ▨, baseline; ■, treatment

Table 3

PGE₂ results presented as 95% confidence intervals for the difference between microdialysis collection intervals at baseline (day 0) and after the treatment period (day 4)

Day	Difference	N	Mean Difference	SD	Lower 95% CI	Upper 95% CI
0	Exercise – Rest	12	117.55	576.73	–248.89	483.99
0	Recovery – Rest*	12	835.94	831.77	307.46	1364.42
0	Recovery – Exercise*	12	718.39	936.26	123.52	1313.26
4	Exercise – Rest	12	342.64	701.80	–103.26	788.54
4	Recovery – Rest	11	153.99	811.52	–391.20	699.18
4	Recovery – Exercise	11	–43.76	604.32	–449.75	362.24

*The 95% confidence interval for the mean difference between intervals does not contain the value 0, implying that there is a statistically significant difference between the considered intervals.

Figure 2

Mean $\pm$ SD 8-iso-PGF_{2α} microdialysate concentrations (pg ml⁻¹) measured on day 0 (baseline) and on day 4 of treatment at rest, during dynamic exercise and during the recovery interval ($n = 12$). ▨, baseline; ▤, treatment

between the rest and the exercise intervals at baseline. These significant differences didn't recur after treatment (Table 3), supporting the hypothesis that the diclofenac released by the plaster inhibits the PGE₂ production locally.

8-iso-PGF_{2α} 8-iso-PGF_{2α} was detectable in microdialysates of all 12 subjects at rest, during dynamic exercise and during the recovery interval for both study periods (Figure 2). At baseline the mean 8-iso-PGF_{2α} concentration of all 12 subjects was 186.4 $\pm$ 120.7 pg ml⁻¹ at rest, 199.4 $\pm$ 184.2 pg ml⁻¹ during exercise and 276.0 $\pm$ 284.3 pg ml⁻¹ during the recovery interval. On the last day of diclofenac plaster application, the mean 8-iso-PGF_{2α} concentration was 241.3 $\pm$ 234.5 pg ml⁻¹ at rest, 355.3 $\pm$ 507.0 pg ml⁻¹ during exercise and 289.0 $\pm$ 276.6 during recovery. Therefore 8-iso-PGF_{2α} tended to increase due to exercise. However, because of the wide inter-individual variability in microdialysate concentrations no clear effect on 8-iso-PGF_{2α} concentrations was observed between rest and

dynamic exercise or recovery, even when considering results from group 1 and group 2 separately (Table 1). No statistically significant differences in 8-iso-PGF_{2α} concentrations were observed for any of the time intervals between baseline and treatment period (paired *t*-test analysis, data not shown).

Pharmacokinetic results

Tables 4 and 5 summarize the pharmacokinetic data for diclofenac in plasma and in muscle tissue of all 12 subjects. In plasma samples, diclofenac (total concentration) could be detected in all subjects at all time points with the mean concentration of 4199.6 $\pm$ 1707.1 pg ml⁻¹ (Table 4). Free diclofenac concentrations obtained by microdialysis in skeletal muscle were highly variable and below the lower limit of quantification (BLQL) for the majority of subjects (Table 5). Overall, free diclofenac was detectable in 7 out of 12 samples. After recovery, corrected mean concentration, i.e. considering 'zero' as value corresponding to BLQL, of all samples was 164.9 $\pm$ 250.5 pg ml⁻¹.

Safety results

No serious adverse events occurred during the study. Overall 11 adverse events were observed in five (41.7%) subjects during the study. Proteinuria, pyuria and application site erythema were experienced by two (16.7%) subjects each, followed by back pain, sunburn, headache and skin irritation, reported by one subject (8.3%) each. Erythema and skin irritation events were judged to be caused by the TegadermTM adhesive tape used to keep the plaster in place and not by the DHEP medicated plaster itself. No systemic NSAIDs were used throughout the study.

Discussion

Prostaglandins have been shown to modulate protein metabolism within skeletal muscle as well as inflammation and pain [20]. PGE₂ is formed in a variety of cells from PGH₂, which itself is synthesized from arachidonic acid by the

Table 4Mean $\pm$ SD diclofenac plasma concentrations (pg ml⁻¹) on day 8 as pre-dose and at 3, 4 and 5 h post-dose ($n = 12$)

Plasma diclofenac (pg ml ⁻¹)	Pre-dose	3 h	4 h	5 h
Mean $\pm$ SD	BLQL	4365.37 $\pm$ 1771.46	4508.59 $\pm$ 1856.77	3724.83 $\pm$ 1589.31
Min – Max	BLQL	626.90–8009.90	1275.30–4404.60	670.90–6404.50
CV%	NC	40.58	41.18	42.67

Table 5Mean $\pm$ SD diclofenac microdialysate concentrations (pg ml⁻¹) on day 8 at different time intervals after the drug administration ($n = 12$). Diclofenac was detected in microdialysis samples of 7 (58.3%) of the 12 subjects. Corrected mean concentration, i.e. considering 'zero' as value corresponding to BLQL, of all samples was calculated

Microdialysate diclofenac (pg ml ⁻¹)	2–3 h	3–4 h	4–5 h
Mean $\pm$ SD	92.17 $\pm$ 144.43	231.03 $\pm$ 664.53	171.55 $\pm$ 289.13
Min – Max	BLQL – 359.90	BLQL – 2295.90	BLQL – 808.50
CV%	156.71	287.65	168.54

enzyme prostaglandin synthetase [20]. PGE₂ has a number of biological actions, including vasodilation, both anti- and pro-inflammatory action, modulation of sleep/wake cycles and facilitation of human immunodeficiency virus replication [20]. In contrast 8-iso-PGF_{2 α} is an isoprostane which is formed *in vivo* by a non-enzymatic free radical catalyzed oxidation of arachidonic acid without the direct action of cyclo-oxygenase (COX) enzyme [17, 21]. Isoprostanes are non-classical eicosanoids and possess potent biological activity as inflammatory mediators that augment the perception of pain [17, 21].

PGE₂ and 8-iso-PGF_{2 α} were detectable in microdialysates at all collection intervals. At baseline, PGE₂ basal concentrations were comparable with those in previous published papers [22, 23] and they were similar during rest and exercise and increased during the recovery interval. These data indicate that the experimental procedures used in the present study may have delayed the detection of the prostaglandins produced during exercise, an effect attributable to the low microdialysis perfusion rates used in the present study, i.e. 0.3 μ l min⁻¹ and 0.5 μ l min⁻¹. The low perfusion rate was chosen to achieve maximum recovery levels of prostaglandins, since neither PGE₂ nor 8-iso-PGF_{2 α} can be purchased in a quality suitable for administration to humans and therefore no calibration of probes by the retrodialysis procedure was possible. While they compared with previous studies lower perfusion rates may have increased the recovery of prostaglandins in microdialysates and also may have delayed the detection of an increase in prostaglandins produced during dynamic

exercise [22]. This pattern was clearly and comparably observed in both groups of subjects, supporting the assumption that impact of the slight changes introduced, in terms of both perfusion rate (flow rate increased from 0.3 μ l min⁻¹ to 0.5 μ l min⁻¹ in group 2) and shifting of the collection intervals (collection intervals delayed by 10 min at the beginning of each 1 h interval in group 2), can be considered as irrelevant to the scope of our research. The dose of diclofenac administered and the study design were the same for the two groups. As compared with the baseline period, a reduction in PGE₂ concentrations was detected analyzing all 12 subjects, especially during the post-exercise recovery interval (–43%), after 4 days of treatment with topical diclofenac administered via the DHEP medicated plaster.

Also, different factors in microdialysis procedure could affect the results. The distance of the probe from the drug application site could result in a different level of inhibition as reported by Mikkelsen *et al.* [24]. Although the study used a different design, the inhibition of PGE₂ production in samples collected after 30 min of exercise, with the probe placed at 4 cm from the indomethacin application site, was about 40% compared with the baseline concentration [24]. This value is very near to the mean value observed in the present study, with an inter-individual variability that could be the result of inter-individual differences in the probe deepness and distance from the epidermis (on average, around 2–4 cm) comparing baseline and exercise phase.

In contrast, no effect of diclofenac treatment on the cyclo-oxygenase independent production of 8-iso-PGF_{2 α} was observed [21]. Although the very wide inter-individual variation in interstitial skeletal muscle concentrations of 8-iso-PGF_{2 α} hampers the interpretation of data, the lack of difference between the three sampling intervals, both for the rest and treatment period, indicate that production of 8-iso-PGF_{2 α} was not significantly altered by the dynamic exercise during the observation period.

The diclofenac concentrations determined for plasma and tissue were in good agreement with previous findings [25]. As observed in many previous studies, local diclofenac concentrations in skeletal muscle tissue were highly variable and below the lower limit of quantification of the analytical assay for the majority of the samples, despite significant diclofenac concentrations in the corresponding

plasma samples [9–13]. The main reason for this observation is that in plasma total diclofenac concentrations are determined, whereas in microdialysates only the fraction of diclofenac which is extracellular and not protein-bound can be detected [25]. Since the protein binding of diclofenac is more than 99%, the expected concentrations in microdialysates are about 100-fold lower than in plasma [26]. In the present study, based on the pooled data of total diclofenac concentrations measured in plasma ($4199.6 \pm 1707.1 \text{ pg ml}^{-1}$) the calculated free concentrations in plasma would be around 40 pg ml^{-1} . Compared with the pooled diclofenac concentrations in tissue of $164.9 \pm 250.5 \text{ pg ml}^{-1}$, this would indicate that local free, i.e. pharmacodynamically active, concentrations of diclofenac are approximately four times higher than systemic concentrations, supporting the concept of topical delivery of diclofenac to the muscle tissue below the plaster application area.

Some limitations of the present study were the relatively short sampling period for determination of prostaglandins and the fact that only two prostaglandins were investigated. However, the low flow rate resulted in sampling volumes between 20 and $30 \mu\text{l h}^{-1}$ which restricted analysis to only two substances. In addition, the high variability of both diclofenac and prostaglandins has to be noted. Possible explanations include differences in skin penetration of diclofenac [10–13, 25] as well as inter-individual differences between the studied healthy volunteers, especially with regard to physical fitness. Moreover, the study was limited to only 12 subjects and was performed in an open label, non-randomized fashion. However, while blinding could have been achieved by use of placebo applications, the physical exercise (30 min on a bicycle ergometer with resistance calibrated at the 70% interval of the calculated maximal resistance) was considered highly reproducible between the two periods and the outcome parameter, i.e. concentrations of prostaglandin as measured by an independent laboratory was considered objective.

In summary, our results confirm that there is an increase in PGE_2 production in muscle of healthy human subjects following standardized physical exercise which, under the experimental conditions used, is particularly evident during the recovery period, when compared with the rest and the exercise period at baseline. Despite high inter-subject variability this increase during the recovery period was reduced (and no more statistically significant) when compared with the rest and the exercise period, after seven consecutive 12 h applications of topical diclofenac epolamine-containing medicated plaster. However, the difference between the baseline and treatment period in terms of PGE_2 production during the recovery interval was not found to be statistically significant due to the high variability of the results and the limited number of subjects. The present exploratory findings are fully in line with previous clinical observations of the efficacy of diclofenac

epolamine plasters for treatment of soft tissues injury of different origin.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). Research funding for this study was sponsored by IBSA Institut Biochimique SA. VF, SR and GM are employees of IBSA Institut Biochimique SA. CL and AV are employees of CROSS SA, which has been contracted by IBSA Institut Biochimique SA as CRO for the conduction of this study and has received financial support for its services. AB, RC, MK, BB, MB and MZ are employees of the Medical University of Vienna, which, as an institution, received financial compensation for performing the study. The authors declare that they have no other relationships or activities that could appear to have influenced the submitted work.

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