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Original article

Effects of topically applied diclofenac and ketoprofen on prostaglandin E2 and Stat3 sera levels and body temperature in two different acute inflammation models in rats

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ABSTRACT

Introduction: Cytokines exert biological function through signal transducer and activator of transcription factors. Prostaglandins have function as promotors, where play a key role in generation of the inflammatory response and as ones that solve inflammatory process.

Non-steroidal anti-inflammatory drugs, inhibit prostaglandin synthesis but the existence of additional mechanisms is present. Thus, we aimed to explore effects of topically applied NSAIDs on the levels of PGE2 and Stat3 in the setting of two in vivo induced acute inflammation models.

Methods: Male Wistar rats were randomized into five equal groups: 4 treated and a control group. Diclofenac or ketoprofen patches were applied in two different doses, i.e. equivalent to human therapeutic dose, and three times higher dose. Three hours later either model of inflammation (with 20% yeast, or with 1% carrageenan) was induced.

Blood samples were taken 3 hours after and concentration levels of PGE 2 and Stat3 were determined using ELISA. Body temperature was measured at 0. 1st, 3rd and 5th hour after inflammation induction and presented in Celsius degrees. Shapiro-Wilk, Leven's, Welch's One-Way ANOVA, Kruskal-Wallis test and adjustment by Bonferroni correction were applied.

Results: In both inflammation models, no differences in the mean values of PGE 2 between control, low and high dose groups treated by either diclofenac or ketoprofen were found. In yeast inflammation, the mean value of Stat3 was significantly higher in both dose ketoprofen groups compared to control group. After ketoprofen application, no significant differences in body temperature between groups at hour 0 and 5 in either model of inflammation induced, while at 1st hour after carrageenan inflammation, significant differences were found with significantly higher values in low dose ketoprofen group compared to control group. In yeast application, significant differences in body temperature were found at hour 3 after inducing inflammation and post hoc pairwise comparison test revealed significant higher values in low dose ketoprofen group compared to control.

Conclusion: Elevated Stat3 values post ketoprofen application in yeast model of induced inflammation were detected. Further investigation of cytokine microenvironment as well as the mechanisms of ketoprofen influence on inflammation are needed.

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1. Introduction

In acute inflammation, the activation of phospholipase A2 is induced in the cell membrane and followed by release of arachidonic acid and inflammatory mediators such as cytokines and prostaglandins (PG). Multiple cytokines, including interleukins (ILs) and interferons (INFs), control immune and inflammatory systems by their biological function through Janus tyrosine kinases, signal transducer and activator of transcription (Stat) factors. PG biosynthesis, on the other hand, is blocked by nonsteroidal anti-inflammatory drugs (NSAIDs) and its topical use requires lower total systemic daily dose to achieve pain relief and reduce/resolve inflammation (Stanos and Galluzzi, 2013).

Diclofenac, an NSAID, in topical form, was investigated in various studies (Sugimoto et al. 2016, Takayama et al. 2011; Komatsu and Sakurada, 2012). Compared to its oral application, topically applied diclofenac achieves higher concentration in skeletal muscle than in plasma, while lower concentration than in plasma are achieved in synovial fluid (Moghaddami et al., 2005). Compared to diclofenac, ketoprofen, another NSAID, may exert more potential effect due to its smaller molecular weight (260 vs. 325 Da) (Stanos and Galluzzi, 2013) and consequent better permeability and absorption through the skin and soft tissues, thus suggesting its potential to be the first-choice topical treatment of inflammation (Stanos and Galluzzi, 2013).

Although the main mechanism of action of NSAIDs consist of inhibiting PG synthesis by blocking the enzyme cyclooxygenase (COX), clinical and experimental data strongly indicate the existence of additional mechanisms, including inhibition of NF-κB and Jak3/Stat3 signaling and down-regulation of pro-inflammatory cytokines to a level that inhibits inflammation and carcinogenesis (Vaish and Sanyal, 2011).

Therefore, we aimed to explore effects of topically applied NSAIDs (diclofenac and ketoprofen patches) on the levels of PGE_2, Stat3 and body temperature as pro-inflammatory signal in the setting of two in vivo induced acute inflammation models in rats.

2. Materials and methods

We conducted an experimental study in the setting of two in vivo induced acute inflammation models, i.e. yeast-induced and carrageenan-induced acute inflammation.

2.1. Reagents for inflammation induction

Yeast – 0.1 mL of an aqueous 10 w/v percentage suspension of dried brewer's yeast in physiological solution was injected subcutaneously (s.c) into the dorsal surface of right hind paw of the rat.

Carrageenan – λ-carrageenan (Sigma-Aldrich Co., Lot No. WA14463) – 1% solution in physiological solution was injected s. c. into the dorsal surface of right hind paw of the rat.

2.2. Animals

Male Wistar rats weighing 220–290 g (breeding of Faculty of Medicine, University of Sarajevo) were used in all experiments. They were housed under controlled temperature (23±2 °C), humidity (55 ± 20 percentage) and 12 h/12 h light/dark cycle. The animals had access to the food and tap water *ad libitum.* All experiments were carried out in accordance with the *Guide for care and use of laboratory animals* (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals, 2011).

2.3. Investigated medicinal products

The following drug patches were used in this study: diclofenac patch (140 mg/140 cm^2; Voltadol® CerottiMedicati, GlaxoSmithKline Consumer Healthcare S.p.A., Milan, Italy) and ketoprofen patch (20 mg/70 cm^2; Keplat®, Hisamitsu UK Limited, London, United Kingdom). Patches were purchased as commercially available products in European Union.

2.4. Drug patch application

Animals were randomized into five equal groups (N = 6): two-groups (V1, V2) were treated with diclofenac bandage bend in two different doses; two groups (K1, K2) were treated with ketoprofen bandage bend in two different doses; one control group (C) received placebo bandage bend.

Of two different doses of diclofenac and ketoprofen, the first dose was calculated to be equivalent to therapeutic dose based on differences in body mass between humans and rats, and the second dose was calculated as the multiplication of the first dose (3 times higher dose) as follows: Voltadol® 1 cm × 0.6 cm (group V1) and 1 cm × 1.8 cm (group V2); Keplat® 1 cm × 0.3 cm (group K1) and 1 cm × 0.9 cm (group K2).Using the therapeutic or placebo bandage bend, patches were fixed in the center of the dorsal surface of right hind paw of the rat. Three hours later patches were removed to enable the initial measurements after which the model of inflammation (Model 1 with yeast or Model 2 with carrageenan) was applied. Inflammation was not induced in the control group

2.5. Induction of acute inflammation

Model 1: Induction of acute inflammation by yeast suspension

Acute inflammation was induced by s.c. injection of 0.1 mL 20% yeast suspension (1 g of brewer's yeast in 5 mL of physiological solution, equipping the suspension in a magnetic stirrer) into the dorsal surface of right hind paw of the rat according to the method by Randal Sellito (Randall and Selitto, 1957).

Model 2: Induction of acute inflammation by carrageenan solution

Acute inflammation was induced by s.c.injection of 0.1 mL 1% solution of carrageenan in sodium chloride solution on dorsal surface of right hind paw according to the method by Winter *et al.* (Winter et al., 1962).

2.6. Blood sampling

Blood samples for analysis of Immunological parameters were taken 3 h after s.c. yeast or carrageenan injections, when the most intense paw swelling was expected (Coura et al., 2015). Blood samples from *v. saphena magna* of opposite hind paw (in Model 1) and of treated paw (in Model 2) were obtained three hours after inflammation induction.

2.7. Determination of immunological parameters

Determination of immunological parameters was performed at the Center for Genetics, Faculty of Medicine, University of Sarajevo. Concentration of PGE_2 and Stat3 were determined by (pY705) (Human/Mouse/Rat) enzyme-linked immunosorbent assay (ELISA) Kits (Abnova, Taipei, Taiwan) on Hytec 288 device, The PGE_2 ELISA kit (Abnova, Taipei, Taiwan) a competitive immunoassay for the quantitative determination of PGE_2 in biological fluids, uses a monoclonal antibody to PGE_2 to bind, in a competitive manner, the PGE_2 in the sample.

The Sandwich ELISA kit (Abnova, Taipei, Taiwan) was used for the measurement of phospho-Stat3. An anti-pan Stat3 antibody has been coated onto wells. Stat3 present in a sample was bound to the wells by the immobilized antibody.

Body temperatures one of the signs of acute inflammation was measures at the hour 0, 1st, 3rd and 5th after inflammation induction, using Bioseb thermometer (www.bioseb.com) in a way that device was pressed against body back several seconds and temperature visualized on the screen as temperature in Celsius degrees (℃).

2.8. Statistical analysis

The descriptive and inferential statistical analysis were performed by using IBM SPSS version 20. Shapiro–Wilk test was employed to test continuous quantitative data distribution normality. Regarding to Leveńs test that showed statistical difference of homogeneity among groups, Welch's One-Way ANOVA was applied. When One-Way -ANOVA or Welch's One-Way ANOVA test showed significant findings, Tukeýs post-hoc test was used to identify which groups in the sample is significantly differ. For non-normally distributed quantitative data, non-parametric Kruskal–Wallis test was used to identify statistical difference between groups and for statistical findings of multiple pairwise comparison, test was adjusted by Bonferroni correction to identify the groups in the sample that significantly differ. Two sided tests with level of $p < 0.05$ were considered statistically significant.

Pearson or Spearman's tests were applied to identify a correlation between two normally distributed vs. non-normally distributed quantitative data. Two-sided tests with the level of $p < 0.05$ were considered statistically significant, respectively $p < 0.2$ for testing statistical significances of correlation (Bujang and Baharum, 2016).

3. Results

Comparison of concentration levels of $PGE_2$and Stat3 in control group vs. after application of low and high doses of NSAIDs in rats is showed in Tables 1 and 2.

In both models of acute inflammation (by 20% yeast or by carrageenan), no statistical differences in the mean values of both PGE_2 and Stat3 between control, low dose and high dose diclofenac groups were found.

In both models of acute inflammation, no statistical differences in the mean values of PGE_2between control, low dose and high dose ketoprofen groups were found. In the model of inflammation induced by carrageenan, no statistical differences in the mean values of Stat3 between control, low dose and high dose ketoprofen groups were found. In the model of inflammation induced by 20% yeast, the mean value of Stat3 was significantly higher in low dose ketoprofen group compared to control group, as well as in high dose ketoprofen group compared to control group.

Comparison of body temperature in control group vs. after application of low and high doses of NSAIDs was measured at 0, 1st, 3rdand 5th hour after induction of acute inflammation are presented in Tables 3 and 4.

The temperature in control group persisted at the approximately same level through all points of measurement and regardless of the inflammation model. After diclofenac application, in both models of inflammation, the lowest value of body temperature was detected at the 3rd hour in both treatment groups. There were no statistical differences in the mean values of body temperatures between control, low dose and high dose diclofenac groups at the 0, 1st, 3rd and 5th hour after inducing of inflammation by either model (20% yeast or carrageenan).

After ketoprofen application, no significant differences between groups were found at hour 0 and 5, in either model of inflammation induced by yeast ($p = 0.957$, $p = 0.094$, respectively) or carrageenan ($p = 0.395$, $p = 0.816$, respectively). At 1sthour after inducing inflammation by carrageenan significant differences were found ($p = 0.016$) and post hoc pairwise comparison test adjusted by Bonferroni correction revealed significantly higher values in low dose ketoprofen group (K1) compared to control group ($p = 0.021$). Also, after application of 20% yeast significant differences were found at hour 3 after inducing inflammation ($p = 0.035$), and post hoc pairwise comparison test adjusted by Bonferroni correction revealed significant higher values in low dose ketoprofen group (K1) compared to control ($p = 0.041$).

Correlation of Stat3 values and body temperature as measured at 0, 1, 3 and 5 h after induction of acute inflammation is shown in the Table 5.

Considering the small samples size by group in our research which is insufficient to identify statistically significant correlation between two quantitative variable we set level of statistical significance at $p < 0.2$ (Bujang and Baharum, 2016). In the model of inflammation induced by 20% yeast, negative significant correlation between Stat3 and body temperature was noticed in high dose diclofenac group ($p < 0.2$). In the model of inflammation induced by carrageenan, positive significant correlation between Stat3 and body temperature was noticed in high dose diclofenac group ($p = 0.111$) and high dose ketoprofen group ($p = 0.140$). Changes of Stat3 levels and body temperature in control group vs. after application of different doses of diclofenac or ketoprofen are presented in Figs. 1 and 2.

[a]Pearson correlation test, [b]Spearmańs correlation test, *statistical significance level $p < 0.2$.

4. Discussion

We found that topical administration of both low and high doses of diclofenac and ketoprofen patches resulted in no significant inhibition of PGE_2 production in either model of inflammation (Table 1), as well as of Stat3 production in carrageenan model (Table 2). However, in the model of inflammation induced by 20% yeast, the mean values of Stat3 in both (two different doses)

Table 1
Comparison of PGE_2 and Stat3 levels in control group vs. after application of low dose of diclofenac vs. after application of high dose of diclofenac in rats 3 h after inducing inflammation.

Measured Variables		Group			p-value
		Control (C, N = 6)	Diclofenac low dose (V1, N = 6)	Diclofenac high dose (V2, N = 6)	
PGE_2pg/ml	Y	506.67 ± 364.93	565.00 ± 315.18	360.00 ± 79.31	[a]0.294
mean ± SD or median (IQR)	C	615.00 (337.50–999.00)	70.00 (0.00–371.25)	505.00 (333.75–793.50)	[b]0.080
Stat3 µg/ml	Y	0.47 (0.25–1.20)	5.59 (0.68–13–53)	2.29 (0.89–5.84)	[b]0.091
median (IQR)	C	0.63 (0.47–4.12)	1.82 (0.62–17.24)	0.70 (0.46–10.10)	[b]0.320

Y- inflammation induced by yeast; C- inflammation induced by carrageenan; *mean ± SD* -arithmetic mean ± standard deviation, *median (IQR)*-median (interquartile range); [a] Welch's One-Way ANOVA; [b] Kruskal–Wallis test, (two-tailed test was applied), *the level of significance $p < 0.05$.

Table 2
Comparison of PGE_2 and Stat 3 levels in control group vs. after application of low dose of ketoprofen vs. after application of high dose of ketoprofen in rats 3 h after inducing inflammation.

Measured Variables		Group			p-value	Post hoc test p-value
		Control (C, N = 6)	Ketoprofen low dose (K1, N = 6)	Ketoprofen high dose (K2, N = 6)		
PGE_2 pg/ml mean ± SD	Y	506.67 ± 364.93	174.17 ± 204.71	557.33 ± 281.25	[a]0.760	–
	C	613.00 ± 381.26	581.83 ± 353.2014	521.83 ± 393.74	[a]0.914	–
Stat3 µg/ml median (IQR)	Y	0.47 (0.25–1.20)	11.32 (3.07 – 33.94)	7.90 (1.26–29.82)	[b]0.015*	[c] C2 and K1, p = 0.028 C2 and K2, p = 0.045*
	C	0.63 (0.47–4.12)	2.82 (1.86–10.53)	1.00 (0.57–8.03)	[b]0.770	–

Y- inflammation induced by yeast; C- inflammation induced by carrageenan; *mean ± SD* -arithmetic mean ± standard deviation, *median (IQR)*-median (interquartile range); [a] Welch's One-Way ANOVA; [b] Kruskal–Wallis test, [c] pairwise comparison test adjusted by Bonferroni correction, (two-tailed test was applied), *the level of significance $p < 0.05$.

Table 3
Comparison of body temperatures in control group vs. after application of low dose of diclofenac vs. after application of high dose of diclofenac in rats at the 0, 1, 3 and 5 h after inducing inflammation by either 20% brewer's yeast or 1% carrageenan solution.

Group			Control (C, N = 6)	Low dose diclofenac ($D1_Y$,$D1_C$ N = 6)	High dose diclofenac ($D2_Y$,$D2_C$, N = 6)	p value
Body temperatures of rats at the time after induction of inflammation (^{o} C) *mean ± SD/median (IQR*	0 h	Y	36.5 (36.08–36.58)	36.30 ± 0.33	36.45 (36.00–36.50)	[a]0.76
		C	36.30 (36.23–36.33)	36.00 (35.58–36.30)	35.90 (35.5–36.18)	[a] 0.157
	1 h	Y	36.4 (35.78–36.55)	36.30 (36.20–36.35)	36.45 (36.28–36.5)	[a] 0.463
		C	36.28 ± 0.18	36.11 ± 0.42	36.50 ± 1.20	[b]0.436
	3 h	Y	36.37 ± 0.10	35.97 ± 0.67	35.85 ± 0.58	[c]0.117
		C	36.35 ± 0.16	36.0 (35.3–36.73)	36.26 ± 0.80	[a] 0.169
	5 h	Y	36.40 (35.63–36.5)	36.48 ± 0.16	35.7 ± 0.88	[a] 0.062
		C	36.35 ± 0.32	36.4 (35.93–36.5)	35.88 ± 0.43	[a]0.184

Y – 20% broweŕs yeast induced inflammation, C- carrageenan induced inflammation, , *mean ± SD* -arithmetic mean ± standard deviation, *median (IQR)*-median (interquartile range), [a] Kruskal Wallis, [b] Welch's One-Way ANOVA, [c] One-Way-ANOVA, (two-tailed test was applied), the level of significance $p < 0.05$.

Table 4
Comparison of body temperatures in control group vs. after application of low dose of ketoprofen vs. after application of high dose of ketoprofen in rats at the 0, 1, 3 and 5 h after inducing inflammation by either 20% brewer's yeast or 1% carrageenan solution.

Group			Control (C,N = 6)	Low dose ketoprofen ($K1_Y$, $K1_C$ N = 6)	High dose ketoprofen ($K2_Y$, $K2_C$, N = 6)	p value	Post hoc test p-value
Body temperatures of rats at the time after induction of inflammation (°C) *mean ± SD/median (IQR*	0 h	Y	36.50 (36.08–36.58)	36.50 (36.10–36.53)	36.47 ± 0.29	[a]0.957	–
		C	36.30 (36.23–36.33)	36.25 ± 0.46	36.50 (36.23–36.53)	[a]0.395	–
	1 h	Y	36.40 (35.78–36.55)	36.57 ± 0.43	36.50 (36.48–36.50)	[a]0.406	–
		C	36.28 ± 0.18	36.50 (36.50–36.60)	36.51 ± 0.16	[a]0.016*	[b] C and $K1_C$ p = 0.021*
	3 h	Y	36.37 ± 0.10	36.50 (36.50–36.60)	36.51 ± 0.16	[a]0.035*	[b] C and $K1_y$ p = 0.035*
		C	36.35 ± 0.16	36.37 ± 0.16	36.40 (36.33–36.43)	[a]0.937	–
	5 h	Y	36.40 (35.63–36.50)	36.58 ± 0.19	36.55 (36.30–36.60)	[a]0.094	–
		C	36.35 ± 0.32	36.42 ± 0.12	36.40 (36.38–36.68)	[a]0.816	–

Y – 20% broweŕs yeast induced inflammation, C- carrageenan induced inflammation, *mean ± SD* -arithmetic mean ± standard deviation, *median (IQR)*-median (interquartile range), [a] Kruskal Wallis, [b] Post hoc pairwise comparison test adjusted by Bonferroni correction, (two-tailed test was applied), *the level of significance $p < 0.05$.

ketoprofen groups were significantly higher compared to control group. In addition, we have used the measure of body temperature as pro-inflammatory signal in both models of artificially induced inflammation. After setting level of statistical significance at $p < 0.2$ (due to small sample size), correlation analysis indicated that elevation of Stat3 concentrations is accompanied by slightly elevated body temperature (rho 0.676; p = 0.140) in inflammation induced by carrageenan and applying high dose ketoprofen patches. Positive significant correlation between body temperatures and Stat3, was also present in high dose diclofenac group (rho 0.714; p = 0.111), while in the model of inflammation induced by 20% yeast, negative significant correlation between Stat3 and body temperature was noticed in high dose diclofenac group (rho-0.725; p = 0.103). Interestingly, significant correlation was not noticed in low and high ketoprofen groups after inflammation induced by 20% yeast (p = 0.414, p = 0.249, respectively), in which the Stat3 was significantly higher compared to control (p = 0.028; p = 0.045, respectively). It is questionable if ketoprofen exert a weaker effect in acute inflammation in general or its weaker effect compared to diclofenac is addressed only to its effect on temperature as the inflammation parameter.

Stat3 was originally known as the acute-phase response element with ubiquitous distribution. It is closely related to Stat1 and is activated by IL-6-type cytokine family (Elkasmi et al. 2006), but also with many unrelated agonists such as oncogenes and IFNs. IL-6 and number of other cytokines and growth factors can cause the induction of Stat3 through their respective receptors. IL-10R produces a strong anti-inflammatory response via Stat3, which serves as an antagonist to proinflammatory signals that are activated during the innate immune response. However, anti-inflammatory response is not unique to the IL-10R-dependant signal transduction but can be produced by a number of cytokines that activate Stat3. In addition, many forms of cancers exhibit an enhanced Stat3 activity (Levy and Lee, 2003).

Table 5
Correlation of Stat3 and body temperature in control group vs. after application of low dose of diclofenac or ketoprofen vs. after application of high dose of diclofenac or ketoprofen following induction of inflammation by either 20% yeast or 1% carrageenan solutions.

	STAT 3 mcg/ml						
		Diclofenac groups	Rho	p-value	Ketoprofen groups	rho	p-value
Body temperatures (°C)	20% yeast induced inflammation	Control (N = 6)	−0.96a	0.856	Control (N = 6)	−0.960a	0.856
		Low dose (N = 6)	−0.478[a]	0.338	Low dose (N = 6)	0.384[b]	0.414
		High dose (N = 6)	**−0.725[b]**	**0.103***	High dose (N = 6)	0.888[b]	0.249
	carrageenan induced inflammation	Control (N = 6)	0.118[b]	0.824	Control (N = 6)	0.118[b]	0.824
		Low dose (N = 6)	0.058[b]	0.913	Low dose (N = 6)	0.551[b]	0.257
		High dose (N = 6)	**0.714[b]**	**0.111***	High dose (N = 6)	**0.676[b]**	**0.140***

[a] Pearson correlation test, [b] Spearmańs correlation test, *the level of significance p < 0.2.

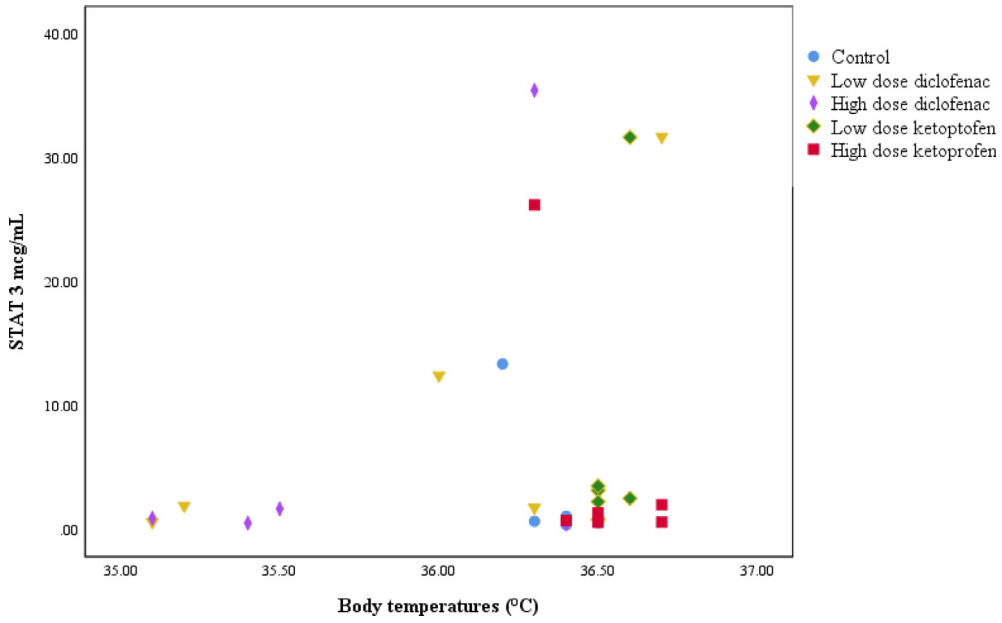

Fig. 1. Scatter plot of body temperatures and Stat3 mcg/ml in control group vs. after application of low dose of diclofenac vs. after application of high dose of diclofenac vs. after application low dose ketoprofen vs after application in rats 3 h after inducing inflammation by 20% brewer's yeast.

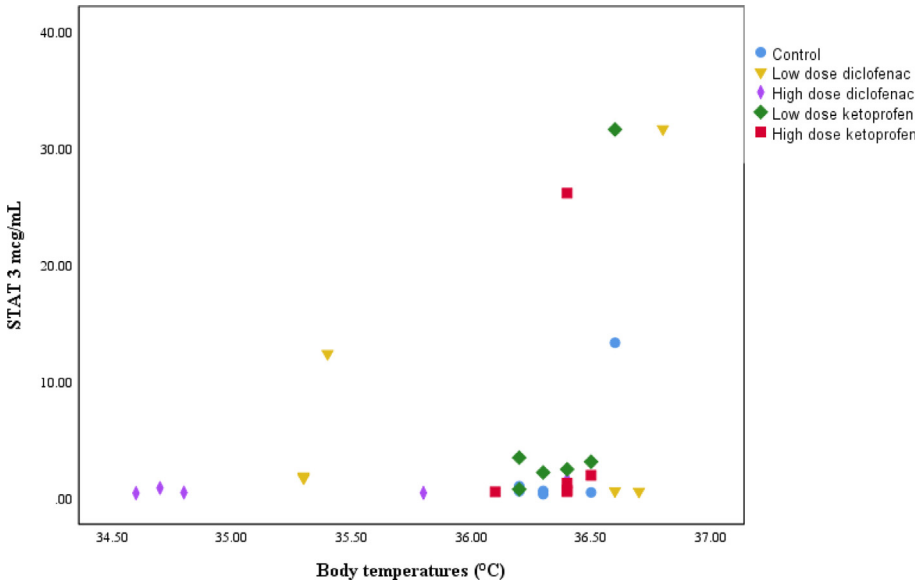

Fig. 2. Scatter plot of body temperatures and Stat3 mcg/ml in control group vs. after application of low dose of diclofenac vs. after application of high dose of diclofenac vs. after application low dose ketoprofen vs after application in rats 3 h after inducing inflammation by 1% carrageenan solution.

The signaling from receptors using the same Jak-Stat pathway is different. Jak1-Stat3 pathway is activated in macrophages when they bind to either IL-6 or IL-10, but the downstream signaling pathway in macrophages for IL-6 and IL-10 are different irrespective of the fact that both utilize Jak1-Stat3 pathway. IL-10, a negative regulator of inflammation, utilizes Stat3 and indirectly targets a few Stat3-regulated genes. However, IL-6, despite using Jak1-Stat3 pathway, does not induce the anti-inflammatory response (Elkasmi et al. 2006).

The cytokine signaling is suppressed by multiple mechanisms, which include competition with Stats for the sites of receptor phosphorylation, inhibition of Jak activity and/or proteosomal degradation after binding to signaling proteins. Suppressors of cytokine signaling proteins are depicted as key negative regulators of cytokine signaling, most important of which is inhibition of the Jak-Stat pathway. Their synthesis is induced by various signals including IL-6, TNF, TGF-β and LPS (Krebs and Hilton, 2001).

The question is what is indicated by the activation of Stat3 and its significantly elevated values during ketoprofen application, in either model of induced inflammation, since Stat3 shows dual, anti-inflammatory or pro-inflammatory role (O'Sullivan et al., 2007, Kubo et al., 2003). Named authors suggest that cytokine receptors aggregate after binding to their respective agonist, resulting in the activation of Janus kinases. Induction of Janus kinases causes' receptor tyrosine phosphorylation and activation of other proteins involved including the Stats (O'Sullivan et al. 2007). Suppressor of Cytokine signaling 1 (SOCS1) directly binds to the Jak and inhibits its catalytic activity (Kubo et al., 2003). According to Levy and Lee from 2003, the role of Stat3 is dependent of cytokine environment; if it is in IL-6 rich microenvironment – it has pro-inflammatory effect, likewise if it is in IL-10 microenvironment.

We have not detected statistical differences in values of PGE_2 for both models of acute inflammation, in both ketoprofen and diclofenac groups. Sugimoto et al. 2016, evaluated analgesic effects of S-flurbiprofen (SFPP), diclofenac, ketoprofen, loxoprofen SRS, felbinac, indomethacin and flurbiprofen patches in adjuvant-induced arthritis (AIA) model of chronic pain in rats and all investigated NSAIDs exerted analgesic effects compared with the control group. However, only SFPP, diclofenac and flurbiprofen had consistent analgesics effect during the whole period of chronic inflammation pain in AIA model (day 1–5). Ketoprofen exerted analgesic effect on days 4 and 5, which was not persistent through the whole period. Nevertheless, the rates of inhibition of pain on 5^{th} day were similar both for ketoprofen and for diclofenac (44% vs. 45%, respectively). Unlike our results, these authors referred to a previously reported single topical application of SFPP (Sugimoto et al. 2016) that led to rapid decrease of both PGE_2 level in paw exudate and of inflammatory pain, suggesting that SFPP exerts a potent analgesic effect through the suppression of PGE_2 production. In addition, while discussing the role of PGE_2-mediated plasmatic extravasation in inflammatory edema, authors suggested that reduction of edema via NSAID mediated inhibition of PG production could be expected in several days (Sugimoto et al. 2016).

Komatsu et al. 2012, measured PGE_2 levels in the hind paw of rats with carrageenan-induced edema and yeast-induced hyperalgesia when topically applied nine different NSAIDs including ketoprofen. Authors found that NSAIDs inhibited PGE_2 production in accordance with their anti-inflammatory and analgesic effects, and ketoprofen reduced level of PGE_2 to almost level achieved in control animals.

COX-1 and COX-2 isoforms at site of inflammation has been implied as prerequisite to reduce production of total PGE_2 (Komatsu and Sakurada, 2012), and NSAIDs inhibitory potency and selectivity towards COX-1 and COX-2 varies. Ketoprofen is considered to exert greater COX-1 inhibitory activity, while diclofenac has more pronounced COX-2 inhibitory activity (Hunter et al., 2015, Bruno et al., 2014). In our study, PGE_2 values were not significantly different when either ketoprofen or diclofenac were applied to animals and compared to control group. (Tables 1 and 2).

Previous researches of NSAIDs were mainly focused on their influence on COXs with focus on COX2.A contribution of COX-2 to both, early and late phase of inflammation was reported (Ricciotti and FitzGerald, 2011). Zhang et al. (1999), reported that COX-2 expression and proinflammatory PGE_2 levels increased transiently early in the course of carrageenan-induced pleurisy in rats. Later during the response, COX-2 was induced again to even greater levels and generated anti-inflammatory prostaglandins, such as PGD_2 and 15-deoxy-Δ^{12-14}-PGJ_2 but only low levels of the proinflammatory PGE_2, suggesting that besides its pro-inflammatory, COX-2 also has its anti-inflammatory role. Administration of a COX-2 inhibitor during the resolution phase exacerbated inflammation in a carrageenan-induced pleurisy model (Zhang et al., 1999). Studies with COX-2 inhibitors suggest that products of this enzyme may play a role in resolution in several models of inflammation (Ricciotti and FitzGerald, 2011). However, the identity of such products, whether formed directly by COX-2 or due to substrate diversion consequent to COX-2 inhibition, remains, in many cases, to be established.

The main limitations of our study are small sample size, which was chosen respecting 3Rs principle in animal research, and the change of blood sampling place. Namely, as inducing acute inflammation by s.c.injection of yeast suspension into the dorsal surface of right hind paw of the rat caused such *hyperalgesia*, we adjusted blood sampling and used *v. saphena* from the opposite side leg for sampling. The large edema disabled additional blood sampling. Another possible limitation was non-existence of control with orally applied non-steroid anti-inflammatory drugs.

5. Conclusion

In conclusion, we have not detected statistical differences in values of PGE_2 for both models of acute inflammation, after application of ketoprofen or diclofenac. Significantly elevated Stat3 values post ketoprofen application in yeast model of induced inflammation needs further exploration, especially considering the dual role of Stat3 in inflammation, as anti-inflammatory or pro-inflammatory factor. In addition, we have used the measure of body temperature as pro-inflammatory signal and correlation analysis indicated that elevation of Stat3 concentrations is accompanied by slightly elevated body temperature in inflammation induced by carrageenan for high dose ketoprofen and diclofenac groups. On the contrary, negative significant correlation accompanied application of high dose diclofenac in inflammation induced by yeast. Stat3 was significantly higher compared to control in low and high ketoprofen groups after inflammation induced by 20% yeast, while interestingly significant correlation was not noticed between Stat3 and body temperature. As this Stat3 dual role is still blurred, there is a need for further investigation of cytokine microenvironment as well as the mechanisms of ketoprofen effects on inflammation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Stanos, S.P., Galluzzi, K.E., 2013. Topical therapies in the management of chronic pain. Postgrad. Med. 125, 25–33.

Sugimoto, M., Toda, Y., Hori, M., Mitani, A., Ichihara, T., Sekine, S., Hirose, T., Endo, H., Futaki, N., Kaku, S., Otsuka, N., Matsumoto, H., 2016. Analgesic effect of the newly developed S (1)-flurbiprofen plaster (SFPP) on inflammatory pain in a rat adjuvant induced arthritis model. Drug Dev. Res. 77, 20–28.

Takayama, K., Hirose, A., Suda, I., Miyazaki, A., Oguchi, M., Onotogi, M., Fotopoulos, G., 2011. Comparison of the antiinflammatory and analgesic effects in rats of diclofenac sodium, felbinac and indomethacin patches. Int. J. Biomed. Sci. 7 (3), 222–229.

Komatsu, T., Sakurada, T., 2012. Comparison of the efficacy and skin permeability of topical NSAID preparations used in Europe. Eur. J. Pharm. Sci. 47, 890–895.

Moghaddami, M., Mayrhofer, G., Cleland, L.G., 2005. MHC class II compartment, endocytosis and phagocytic activity of macrophages and putative dendritic cells isolated from normal tissues rich in synovium. Int. Immunol. 17 (8), 1117–1130.

Vaish, V., Sanyal, S.N., 2011. Non-steroidal anti-inflammatory drugs modulate the physicochemical properties of plasma membrane in experimental colorectal cancer: a fluorescence spectroscopic study. Mol. Cell Biochem. 358 (1–2), 161–171.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals, 2011. 8th edition. Washington (DC): National Academies Press (US), https://www.ncbi.nlm.nih.gov/books/NBK54050/ doi: 10.17226/12910.

Randall, L.O., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissue. Arch. Int. Pharmacodyn. Ther. 111 (4), 409–419.

Winter, C.A., Risley, E.A., Nuss, G.V., 1962. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547.

Coura C.O., Souza R.B., Rodrigues J.A., Vanderlei Ede S., de Araújo I.W., Ribeiro N.A., Frota A.F., Ribeiro K.A., Chaves H.V., Pereira K.M., da Cunha R.M., Bezerra M.M., Benevides N.M., 2015. Mechanisms involved in the anti-inflammatory action of a polysulfated fraction from Gracilaria cornea in rats. PLoSOne. 10, 3, e0119319. http://doi.org/10.1371/journal.pone.0119319. eCollection 2015.

Bujang, M.A., Baharum, N., 2016. Sample Size Guideline for Correlation Analysis. World J. Soc. Sci. Res. 3 (1), 37–46.

Elkasmi, K.C., Holst, J., Coffre, M., Mielke, C., et al., 2006. General nature of the STAT3-activated anti-inflammatory response. J. Immunol. 177, 7880–7888.

Levy, D.E., Lee, C-k, 2003. What does Stat3 do?. J. Clin Invest. 109 (9), 1143–1148.

Krebs, D.L., Hilton, D.J., 2001. SOCS proteins: Negative regulators of cytokine signaling. Stem Cells. 19, 378–387.

O'Sullivan, L.A., Liongue, C., Lewis, R.S., Stephenson, S.E., et al., 2007. Cytokine receptor signaling through the JAK-stat-socs pathway in disease. Mol. Immunol. 44, 2497–2506.

Kubo, M., Hanada, T., Yoshimura, A., 2003. Suppressors of cytokine signaling and immunity. Nat. Immunol. 4, 1169–1176.

Hunter, T.S., Robinson, C., Gerbino, P.P., 2015. Emerging evidence in NSAID pharmacology: important consideration for product selection. Am. J. Manag. Care. 21 (7), S139–S147.

Bruno A., Tacconelli S., Patrignani P., 2014. Variability in the response to non-steroidal anti-inflammatory drugs: mechanisms and perspectives. 114, 1, 56–63. http://doi.org/10.1111/bcpt.12117.

Ricciotti, E., FitzGerald, G.A., 2011. Prostaglandins and Inflammation. Arterioscler. Thromb. Vasc. Biol. 31 (5), 986–1000. https://doi.org/10.1161/ATVBAHA.110.207449.

Zhang, X., Morham, S.G., Langenbach, R., Young, D.A., 1999. Malignant transformation and antineoplastic actions of nonsteroidal anti-inflammatory drugs (NSAIDs) on cyclooxygenase-null embryo fibroblasts. J. Exp. Med. 190, 451–459.