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COMPARISON OF IN VITRO-ACTIVITY OF COMMONLY USED TOPICAL GLUCOCORTICOIDS ON CYTOKINE- AND PHOSPHOLIPASE INHIBITION*

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Abstract

Background and Objectives: Topical glucocorticoids (GCs) are potent inhibitors of cellular inflammatory mediator production. Differences in receptor binding activities are believed to correlate with inhibition of mediator release and anti-inflammatory efficacy in vivo. To further assess this hypothesis we compared in cultured human monocytes the inhibitory activity of classic synthetic GCs on leukotriene B4 (LTB_4), prostaglandin E2 (PGE_2), interleukin 1 β (IL-1β) and c-phospholipase A 2 activity ($cPLA_2$).

Methods: Normal human monocytes (10^5/ml) were tested for 20hrs with increasing concentrations (range 10^{-12}-10^{-5}M) of triamcinolone acetonide (TAA) compared to beclomethasone dipropionate (BDP), budesonide (BUD), dexamethasone (DEX), or the ethanol diluent together with 10μg/ml of lipopolysaccharide (LPS). Mediator production and spontaneous $cPLA_2$-activity was determined by direct enzyme immunoassay methods.

Results: TAA at therapeutically relevant concentration (10^{-8} M) inhibited significantly ($p < 0.01$, $n = 9$) mediator production of TNF-α > IL-1β > TxB_2 > LTB_4 in a dose dependent manner by 75%, 65%, 41%, and 33%. IL-1β inhibition at 10^{-8} M by TAA (65%)> BDP (52%)> BUD (47%) was not different (ANOVA, $p>0.2$). Also spontaneous $cPLA_2$-activity at 10^{-8} M was inhibited to a similar degree (ANOVA, $p> 0.6$) by BUD (17.3%) > TAA (11.4%) > BDP (8.6%). In the same culture conditions spontaneous PGE_2-secretion was inhibited by BDP (28.8%) > BUD (24.2%) > TAA (11.4%) with no significant effect for TAA.

Conclusion: Clinically well established GCs have a similar inhibitory capacity on monocyte cytokine production and surprisingly only weak effects on AA-metabolism. Small receptor binding activity may account for the lack of cytokine inhibition by subtherapeutic ($<10^{-8}$ M) airway concentrations of TAA and BDP. Partial mediator inhibition by GCs at therapeutically known airway concentrations may be relevant to control bursts of airway inflammation during acute exacerbation but unfavourable to effectively delay progression of chronic airway inflammation.

Key words: asthma, rhinitis, beclomethasone dipropionate, budesonide, triamcinolone acetonide, cytokines; arachidonic acid metabolism

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways, and considerable progress regarding the underlying pathophysiological mechanisms was made during the last decade [3, 26] . Cell types that are representative for nasal and bronchial inflammation are activated eosinophils, mast cells, monocytes/macrophages and lymphocytes [5]. The invasion of these cells into airway tissue is stimulated by various adhesion molecules, which are regulated by a complex network of cytokines. Within the cells, the transcription of genes and thereby the production of proteins is regulated by transcription factors, of which NF-κB is particularly important for the regulation of inflammatory reactions. For example, the production of tumour necrosis factor alpha (TNF-α) and interleukin 1ß (IL-1β) is modified by NF-κB [5]. Interleukin-1β initiates the immune response by regulating the expression of many other genes, including cyclooxygenase, and the latter mechanism leads to increased concentrations of prostaglandins.

Glucocorticoids (GCs) are recommended for the control of chronic inflammatory reactions in patients with rhinitis and asthma [3, 4, 25, 26] . These drugs are able to reduce the transcription of genes coding for inflammatory mediators, particularly cytokines, and of inflammatory enzymes, such as inducible NO synthase or inducible cyclooxygenase [3]. The inhibitory effects of GCs on the immune response have been correlated with a down regulation of the transcription factors NF-κB and AP-1 via a conformational change in the glucocorticoid receptor (GR) that allows the glucocorticoid ligand bound GR to enter the nucleus and to bind to a glucocorticoid response element. The net result is a reduced production of pro-inflammatory mediators such as cytokines, histamine, LTE_4, or PGE_2

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by inflammatory cells. The last effects are believed to be mediated by depletion of arachidonic acid release from cell membrane phospolipids through the action of GCs on c-phospholipase activity ($cPLA_2$) [7]. Following $cPLA_2$-activation the archidonate substrate is further metabolized by the 5-lipoxygenase and the cyclooxygenase enzymes to generate leukotrienes, prostaglandins and thromboxan B2 [23].

There is increasing evidence that GR binding affinity and transactivation potency are important parameters to predict drug potency at the molecular level [37]. The relative binding affinities of topical glucocortioids (GCs) mometasone furoate (MF: 1235) > fluticasone propionate (FP: 813) > budenoside (BUD: 258) > triamcinolone acetonide (TAA: 164) were all higher relative to dexamethasone (DEX: 100). MF was the most potent GC with a binding affinity that was 12-fold more than that of DEX and this same order of receptor binding affinities (MF>FP> BUD> TAA> DEX) was also reported in other studies [12, 35]. Binding affinity of beclomethasone dipropionate (BDP) was inferior compared to TAA but its metabolite 17-BMP had a stronger binding affinity compared to BUD [9, 44]. Ratios of clinical efficacy of inhaled GCs with taking in account the effect of the drug delivery system were statistically determined by re-evaluation of 29 clinical studies including over 6000 patients. Effects of treatment compared for pairs of FP and other GCs were tested in matched groups suggested ratios of treatment success as compared to FP (1) for BUD (0.609) > BDP (0.561) > TAA (0.206) [29]. Since these previous reports suggested TAA the least effective GC further research on the anti-inflammatory activity of TAA seemed of further interest. TAA was developed as potent GC for the topical treatment of inflammatory disease, e.g. allergic rhinitis and asthma [43]. As a disease modifier TAA was superior to placebo and of similar efficacy compared BDP or BUD [5, 29].

The present experiments were designed to compare directly the effect of TAA with BDP and BUD at the cellular level on inflammatory mediator production at therapeutically relevant concentrations. The effect on $cPLA_2$ activity and the representative mediators of arachidonic acid metabolism, LTB_4, PGE_2, and the cytokines, IL-1β and TNF-α, was measured in monocytes from healthy volunteers. The hypothesis was that standard GCs, such as TAA, BDP and BUD control inflammatory mediator production as consequence of their receptor binding affinities by a steroid specific mode of action.

Subjects and Methods

Subjects

Five non-atopic, healthy non-smokers (3males, 2 females) volunteered in their invitation by donating blood. They were free from any respiratory tract infection within the 6 weeks prior to examination and had not received any medication. Fifty millilitres of venous EDTA blood was taken at 9 a.m., and monocytes were isolated from the samples as described below. The protocol was approved by the institutional review board of the University Hospital Bonn, and all subjects gave their written informed consent.

Methods

Cell Isolation

Monocytes were isolated as previously described [14]. Leukocyte-rich plasma was prepared from EDTA-blood by dextran sedimentation (6% dextran 500 for 30 minutes). Then monocytes were separated (Nyco-Prep, 1.068 g/ml, Nycomed, Norway) by density-gradient centrifugation (600g/15 min/22 °C). The interface cells were washed in 0.9% NaCl/10% autologous plasma and centrifuged through autologous EDTA-plasma at 50g for 10 minutes. Using light microscopy and a monoclonal platelet antibody (anti GPIIb-IIIa) it was assured that monocytes were free from platelets (<5%). The resulting platelet free monocytes were resuspended in HBSS. Cell viability (>95%) was assessed after 20hrs with trypan blue and lactate dehydrogenase (LDH) activity using the cytotoxicity assay (Boehringer Ingelheim, Germany) before and after incubation with TAA (Aventis Pharma, Germany), BDP, BUD and DEX bought from Sigma (Germany).

Cell Cultures

Aliquots of monocytes (10^5/ml) were incubated with the test substances at different concentrations (10^{-12}M to 10^{-6}M) in adherence to 48-well culture plates (Costar, Tecnorma, Fernwald, Germany) at 37 °C for 20 hours [15]. Cultures contained either 10μM LPS (Sigma) or 1μg/ml IL-1β (Cayman Chemical Corp.) for cell activation. Then, supernatants were harvested and kept frozen at -80 °C until assayed by direct enzyme immunoassay (EIA,Cayman Chemical Corp., Ann Arbor, MI, USA). In the same culture supernatants the representative monocyte AA-metabolites of each pathway, LTB_4, TxB_2/PGE_2 and the cytokine TNF-α and IL-1β were measured [15]. Cross reactivity between measured AA-metabolites and cytokine were below 0.0001%. In additional cultures monocytes (10^5/ml) were incubated with the GC for 20hrs. without the lipopolysaccharide (LPS) and the effect on spontaneous PGE_2 production was determined by EIA (Cayman Chemical Corp.).

Quantification of Cytosolic Phospholipase A2 ($cPLA_2$) Activity

In monocyte cell membrane fractures $cPLA_2$ activity was directly measured by enzyme immunoassay according to the instructions of the distributor (Cayman Chemical Corp.). Cell membrane fractures of monocytes were isolated as reported by Roshak et al [30]. Monocyte cultures (5×10^5/ml) were incubated with GCs (10^{-12}-10^{-5} M, n = 9-12) for 20 hrs in adherence to 48-well culture plates. Each experiment was set for n = 1 due to the limited amount of purified monocytes available. Following removal of culture supernatants wells were washed twice with phosphate puffer and the plates put on ice. The cells were treated with a

trypsin-EDTA solution (200µl/well) and incubated for 5 min. at 37 °C to remove adherent cells from culture plates. Cells and culture supernatants were collected in 1.5ml Eppendorf tubes and then sonificated on ice (3 x 5 sec.) in an ultrasound bath. Cell membrane fractions were then isolated by centrifugation (15min/10.000g/4 °C), culture supernatants shock frozen by N_2 exposure and stored frozen in 0.5ml Eppendorf tubes at -80 °C. Average supernatant volume of about 1300µl (1000ml supernatant +300ml EDTA-solution) was further concentrated (AMICON-Centricon, YM30 von Millipore, USA) by centrifugation (5000g/20min). The average resulting sample concentration was about 1:10. To achieve dose dependent effects of the test substances on $cPLA_2$-activity the amount of the smallest sample volume was adjusted to highest volume after sample concentration. In these purified and adjusted samples $cPLA_2$ activity was then measured directly by EIA. Addition of Ca^{++} (10^{-7} M) to monocytes cultures increased $cPLA_2$-activity by about 20%. Reproducibility of measured $cPLA_2$ activity was by ±10% in these conditions.

Statistical Analyses

For measurements of inflammatory mediators triplicate cultures were prepared from monocytes from each donor. Each culture was considered as independent random sample. All results are presented as means and standard errors of means for at least 9 random monocyte samples. They are expressed as the mean ± SEM as compared to the control without the GC. ANOVA analysis and the Mann & Whitney U nonparametric test were used for multiple statistical comparisons of steroid effects with the control and between effects of different steroids. Two-sided p values were considered significant if <0.05. All analyses were performed using the StatView 5.01 (SAS Institute Inc., North Carolina, USA) on a Apple-Macintosh computer.

Results

Inhibition of Arachidonic Acid Metabolites LTB_4 and TxB_2

The traditional topical steroid TAA, at therapeutically relevant airway concentrations of 10^{-10}-10^{-7} M, had a strong impact on the dominating mediators of AA-metabolism, LTB_4 and TxB_2, in response to stimulation with LPS (Table 1, Fig. 1). A dose-dependent increase of inhibition of the representative AA-metabolites of each pathway compared to baseline was observed with significant effects at 10^{-8} M TAA (LTB_4: -33.3%, TxB_2: -41%, $p<0.05$). Superior inhibition of TxB_2-production compared to LTB_4 only demonstrated a trend towards significance at higher TAA concentrations (10^{-7} M: LTB_4:- 38%, TxB_2:-50%, $p>0.05$, ANOVA).

After stimulation with IL-1β, monocytes responded in a similar manner and with the strongest inhibitory effect on TxB_2 –production (-24 ± 8%) at a therapeutic concentration of 10^{-8} M TAA (Fig. 2, Table 1). In contrast, stimulation with IL-1β had no influence on LTB_4 production and therefore no inhibitory effect of TAA could be demonstrated when monocytes were stimulated with IL-1β.

Inhibition of TNF-α and IL-1β by TAA

After monocytes had been stimulated with LPS, TAA inhibited the generation of TNF-α and IL-1β in a dose-dependent manner and stronger (ANOVA>0.05) compared to measured representative AA-metabolites (Fig. 1, Table 1). At 10^{-8} M, statistically significant effects were measured with an inhibition of TNF-α and IL-1β of 75% and 65%, respectively, as compared with the control. When IL-1β was used as stimulant (Fig. 2, Table 1), a similar inhibition of TNF-α (-65%) was observed as compared to baseline at a TAA concentration of 10^{-8}M.

Table 1. Profile of inhibition of inflammatory mediator production by TAA in LPS-stimulated human monocytes.

TAA	LTB_4 (pg/ml)		TxB_2 (pg/ml)		TNF-α (pg/ml)		IL-1β (pg/ml)
(M)	LPS	IL-1β	LPS	IL-1β	LPS	IL-1β	LPS
Control	80.7 ±6	8.5 ± 6	12663 ± 688	3584 ± 276	595.2 ± 69	530.5 ± 135	10897 ± 2092
10^{-10}	57.5 ± 6	-	*9793 ± 589	3269 ± 256	489.2 ± 97	310.5 ± 79	9676 ±2540
10^{-9}	68.4 ±5	-	11651 ± 789	3222 ± 264	499.6 ± 109	303.4 ± 81	9185 ± 2382
10^{-8}	*54.8 ± 5	-	*7473 ± 328	*2736 ± 230	*148.1 ± 33	156.0 ± 41	*3800 ± 1091
10^{-7}	*50.0 ± 5	-	*6286 ± 889	*2888 ± 240	*75. 9 ± 26	*116.5 ± 34	*2051 ± 722

Mean production (±SEM) of arachidonic acid metabolites (LTB_4 and TxB_2) and cytokines (TNF-α and IL-1β) in human monocytes (10^5/20hrs) stimulated with LPS (n = 9-11) or IL-1β (n = 12). LTB_4-production was not stimulated by IL-1β (1µg/20hrs) and could only be measured in supernatants of cultured monocytes (10^5/ml) at the detection limit of the enzyme immunoassay. Therefore, in these cultures no effect of TAA was determined.
* $p<0.05$ compared to control

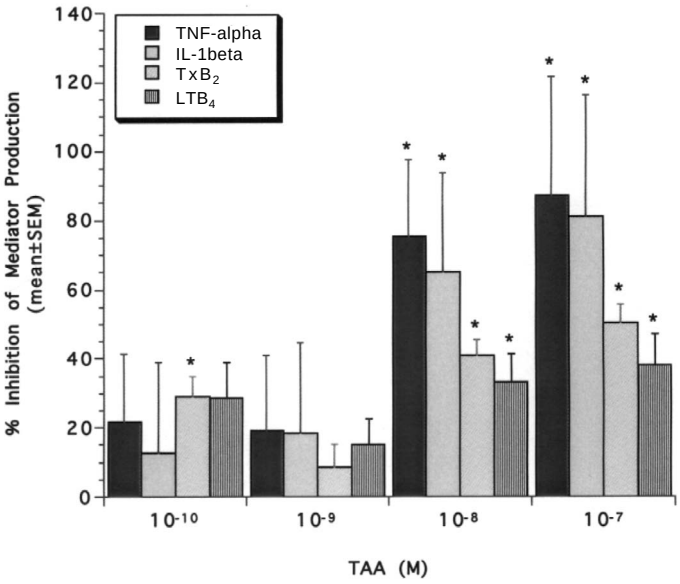

Fig.1. Inhibition of LPS-stimulated mediator production by TAA: Following incubation of monocytes with TAA LPS-stimulated mediator production was significantly (*$p<0.01$) inhibited at therapeutic airway concentrations of TAA (10^{-8}M) as compared to the controls.

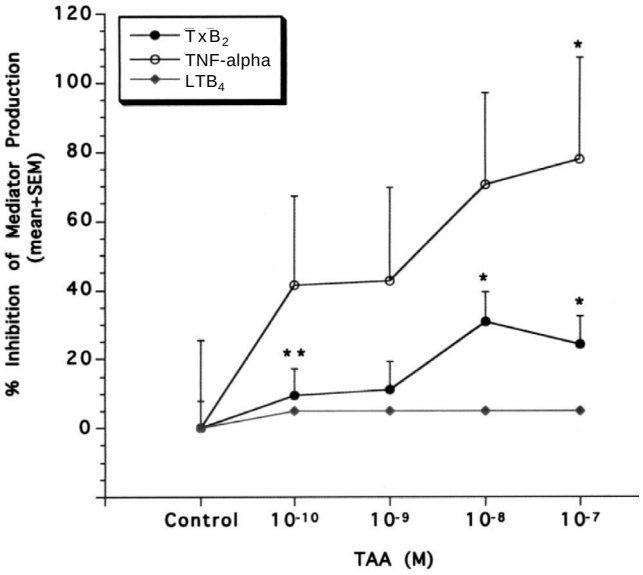

Fig. 2. Inhibition of IL-1β-stimulated mediator production by TAA: IL-1β-stimulated monocyte production of TNF-α and TxB_2 was significantly (*$p<0.01$, **$p=0.012$) inhibited by TAA. As compared to LPS-stimulation mediator production in the controls was less stimulated by IL-1β with no effect of IL-1β on LTB_4-production.

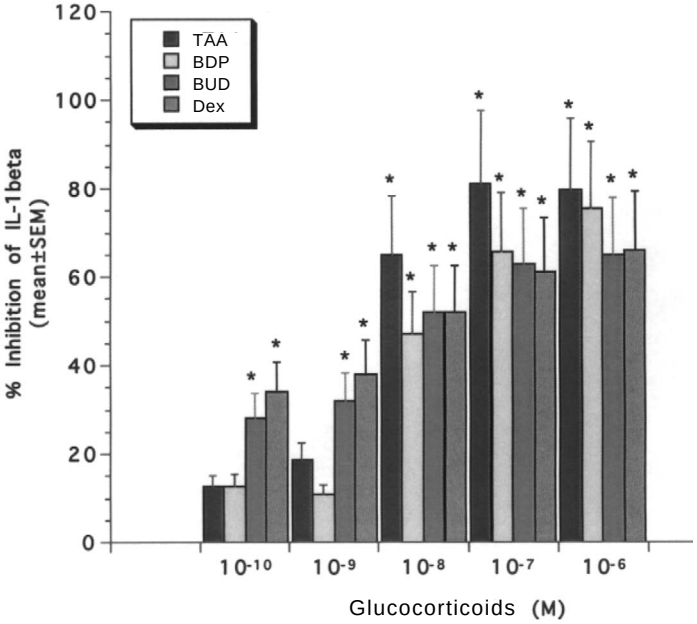

Fig. 3. Effect of topical glucocorticoids on LPS-stimulated IL-1β production: IL-1β was inhibited (*$p<0.02$, $n=11$-18) by topical glucocorticoids and dexamethasone in a dose dependent manner as compared to the control. Effects of topical steroids were not different at the therapeutic airway concentration (10^{-8} M) (ANOVA, $p>0.05$). IL-1β production was also inhibited to a similar degree (ANOVA, $p>0.2$) by dexamethasone and tested topical steroids.

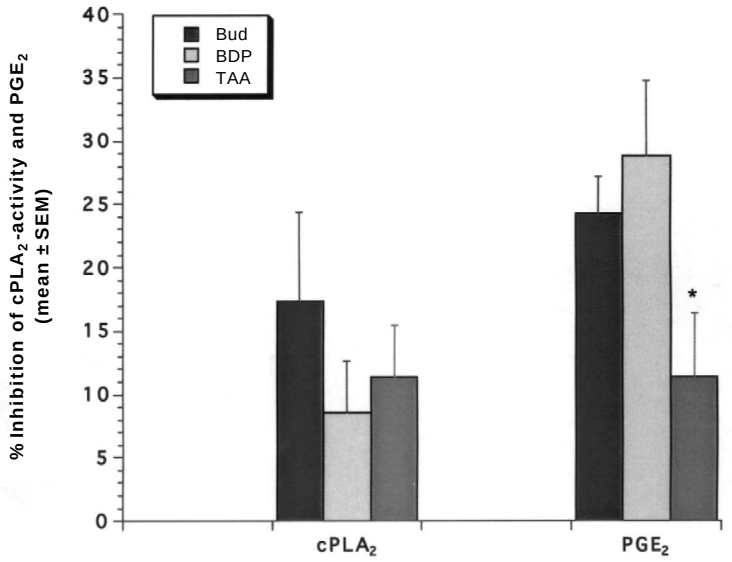

Fig. 4. Effect of topical glucocorticoids on spontaneous $cPLA_2$ activity and PGE_2 secretion: Spontaneous $cPLA_2$ activity and PGE_2 secretion was determined in supernatants of cultured human monocytes (10^5/20hrs). Topical steroids (10^{-7} M) only demonstrated weak inhibitory effects on $cPLA_2$ activity ($p \leq 0.05$). Spontaneous PGE_2 production was inhibited to stronger degree by BDP and BUD as compared to the control with no significant effect on PGE_2 (*p>0.08) only for TAA. However, inhibition of PGE_2 production by BDP was not significantly different compared to measured suppression of $cPLA_2$-activity (ANOVA, p = 0.39).

Comparison of Topical Glucocorticoids

The effects of TAA, BDP and BUD on IL-1β production were compared to DEX in LPS-stimulated monocytes. Following a dose-dependent inhibition of IL-1β by all tested steroids, a similar and statistically not different degree of IL-1β inhibition at 10^{-8} M (ANOVA >0.05) in a range of about 45-65% was detectable (Fig. 3). Topical steroids had the same capacity compared to DEX to suppress LPS-stimulated IL-1β production in monocytes.

Glucocorticoid Effects on c-Phospholipase Activity and the COX-pathway

The effect of BUD, BDP and TAA to inhibit spontaneous c-PLA_2 activity and non-stimulated PGE_2 production was analysed by direct EIA-assay in the same culture systems of long-term incubated human monocytes. Surprisingly, only a relative small degree of inhibition of spontaneous c-PLA_2-activity by BUD (-17.3%) > TAA (-11.4%) > BDP (-8.6%) was detectable at 10^{-7}M (Table 2). These effects were significant as compared to the controls at 10^{-7}M with no significant difference in between all three steroids tested (ANOVA >0.6). Spontaneous PGE_2-secretion (n = 12) from a baseline of 169.7 ± 4pg/ml was inhibited (10^{-7}M) by BDP (-28.8%) > BUD (-24.2%) > TAA (-11.4%) with no significant effect for TAA using the same culture conditions (Fig. 4). The inhibitory effect of BDP on spontaneous PGE_2 (28.8 ± 9%, n = 12) was not significantly stronger compared to its effect to suppress $cPLA_2$-activity (-8.5 ± 4%; ANOVA, p = 0.39). These data do not clearly suggest additional inhibition of COX-2 activity by the tested topical steroids.

Discussion

There is still no certainty that all topical GCs will behave similarly, either in vivo or in different in vitro cellular systems. In particular, since the availability of highly sophisticated topical GCs clinicians used to raise the questions if the commonly used topical GCs are still to be recommended and weather these traditional GCs could have different anti-inflammatory activities. So far, this question was not addressed in any trial. For this reason we have compared the potency of budenoside (BUD), beclometason dipropionate (BDP) and triamcinolone acetonide (TAA) as compared to dexamethasone (DEX) on inflammatory mediator production in human monocytes following stimulation with LPS.

Table 2. Effects of topical glucocorticoids on spontaneous $cPLA_2$-activity in human monocytes.

mol/min/ml x 10^{-3}	n	Control	10^{-8} M	p-value	10^{-7}M	p-value	10^{-6}M	p-value
TAA	9	3.769 ± 0.841	3.622 ± 0.213	0.612	3.341 ± 0.123	0.017	3.404 ± 0.115	0.018
BDP	12	3.515 ± 0.127	3.251 ± 0.220	0.39	3.215 ± 0.129	0.05	3.144 ± 0.130	0.038
BUD	11	3.425 ± 0.179	2.992 ± 0.168	0.056	2.833 ± 0.212	0.04	2.717 ± 0.168	0.012

Spontaneous $cPLA_2$-activity was determined by direct enzyme immunoassay in cultures monocytes (5 x 10^5/ml) following 20hrs incubation with steroids. P-values < 0.05 as compared to the control were estimated significant using the Mann & Whitney-test.

The present experiments suggest that TAA, despite its low receptor binding activity, is able to suppress the whole range of measured production of both arachidonic acid metabolites and cytokines in stimulated human monocytes in vitro. This effect could be demonstrated in LPS-and IL-1β-stimulated monocytes at the expected therapeutically relevant concentration of 10^{-8} M. This concentration is recognised as the average airway disposition achieved following use of different inhaler systems as determined for BUD [39, 42].

The TAA concentration of 10^{-8} M, which produced significant effects in the present experiments, is comparable to therapeutic levels achieved with inhaled GCs in the airways. A study on the absolute bioavailability of TAA revealed that after inhalation of 800μg TAA, 10% of the labelled dose (or 80μg) was absorbed into the systemic circulation via the lungs [2]. Mapping of aerosol distribution using sophisticated PET and SPECT technology showed that approximately two thirds of the inhaled TAA dose was deposited in the conducting airways, particularly in the 3rd to 5th airway generation, and one third reached the 15th to 23rd generations of acinar airways [19].

The effect of TAA to inhibit monocyte AA-metabolism was investigated by measuring the key metabolites LTB_4 and TxB_2, which are both known as representative metabolites of the 5-lipoygenase [11] and the cyclooxygenase (COX) pathway [13] in LPS-stimulated monocytes. Since both AA-metabolites were measured in the same culture supernatants our data characterise TAA as an inhibitor of AA-metabolism with stronger inhibitory effects on AA-metabolism via the cyclooxygenase pathways and weaker effects concerning the 5-lipoxygenase pathway. To further determine weather the underlying mode of action of TAA may either involve direct c-PLA_2- inhibition or additional and differential inhibitory effects on both pathways of AA-metabolism cPLA_2-actvitiy was directly determined. Surprisingly all tested topical GCs with low receptor binding activity only demonstrated small effects on cPLA_2-activity in a range of 9-17% with the least effect for TAA. Since these effects could only be shown at relatively high concentrations (10^{-7} M), we cannot expect that the topical steroids tested in our experiments will be active inhibitors of cPLA_2-activity in the airways. But GCs were reported to control COX-1 and –2 expression in rheumatoid synovial tissues [10].

Similar effects of TAA on COX-pathway were reported by Lewis and co-workers who described the inhibition of prostaglandin synthesis by GCs in human endothelial cells [20]. Dexamethasone (DEX), hydrocortisone, and TAA inhibited PGI_2 production by approximately 50-60%. The suppression was concentration-dependent, and the highest effect of TAA was observed at 10^{-7} M. Aksoy and co-workers measured the inflammatory-mediator induced eicosanoid release in cultured (BEAS-2B) airway epithelial cells [1]. After exposure to TAA, BUD, and fluticasone (FP), markedly reduced COX-2 activities were found, whereas GCs had little effect on COX-1, phospholipase 2, or inducible phospholipase 2 immunoreactivity. The authors concluded that as consequence of COX-2 suppression, PGE_2 production was reduced to 54% compared to baseline at TAA concentrations of 10^{-8} M. In the present study, production of a different COX metabolite (TxB_2) was determined and we found a similar suppression of 41% compared to the control (10^{-8} M). Since we were not able in our experiments to stimulate cPLA_2-activity using various stimuli and to determine this effect by direct measurements of cPLA_2 the effects of GCs were tested also on spontaneous PGE_2 production. Comparison of spontaneous PGE_2 and cPLA_2 production revealed for BDP (+20%) and BUD (+7%) a significant inhibition of the representative COX-2 metabolite in monocytes and no additional effect of TAA. These effects, however, were not statistically different from cPLA_2 inhibition. These results further suggest that commonly used GCs may control prostaglandin production al least in part by COX-2 inhibition and support a previous study [42]. The underlying mechanism of GC inhibited COX-activity is known as indirectly mediated by inhibition of NF-kappa B enhanced expression of COX-2 [18, 46]. Additionally there is also evidence for non-genomic effects of GCs, i.e. properties of GCs not requiring gene expression that suggest direct effects on the COX-enzymes.

In previous investigations PGE_2 production was inhibited by 52% and 44% with BUD and FP, respectively, at GC concentrations of 10^{-8} M [16]. In these studies FP-concentrations $<10^{-8}$ M had no inhibitory effect on COX-activity despite its higher receptor binding activity. Other ex vivo experiments revealed that BUD also reduced TxB_2 production by zymosan-stimulated peripheral monocytes [38]. Taken these results together, there is evidence that topical GCs influence the AA-metabolism as inhibitors of prostaglandin and thromboxane synthesis possibly via suppression of cyclooxygenase gene expression or direct inhibition of the two COX-enzymes and these effects seem not directly dependent on GC-receptor binding activity. Particularly in rhinitis inhibition of AA-metabolism seems to be important for acute symptom relief [21, 22].

The inhibition of LTB_4 production in the present study by about 40% at TAA 10^{-6} M was comparable to that obtained with FP and BUD in previous experiments [16]. However, FP significantly inhibited LTB_4 production at a concentration of only 10^{-10} M (27%) with a maximum effect of 36% at 10^{-7} M, whereas a concentration of BUD 10^{-9} M was required to achieve a significant 18% suppression of LTB_4. These results suggested that FP has a stronger affinity to block production of leukotrienes as compared to prostaglandins and it is still not clear weather this effect might be related only to its higher GC-receptor binding affinity. No significant effects of TAA, however, were notable in our study at a concentration of $\leq 10^{-9}$ M on the representative mediators of the 5-lipoxygenase or the cyclooxygenase pathways and on cytokine production. A study in asthmatic patients showed that LTC_4 concentrations in bronchoalveolar fluid could be decreased by 44% compared to baseline after 12 weeks of treatment with FP [27]. These results suggest that inhaled GCs are capable of inhibiting leukotriene formation in vivo and that these findings may translate into clinically relevant effects. It seems evident, that in local therapy, such as for allergic rhinitis, about 100-fold (10^{-6} M) higher local concentrations of commonly used GCs

would probably induce the same effects on inflammatory mediators with no respect to GC-receptor binding activity.

Compared to the inhibition of AA-metabolites the effects of TAA in relevant upper and lower airway concentrations on LPS-or IL-1β-stimulated monocyte production of TNF-α and IL-1β were about 2-fold stronger (80±10%). Comparison of the inhibitory effects of tested topical GCs on IL-1β production revealed similar inhibitory effects at concentrations ≥ 10^{-8} M. Therefore, our results characterise commonly used GCs as strong inhibitors of cytokine production. This is a group-specific mode of action of GCs in inflammatory processes [17] that is known be mediated by inhibition of cell-transcription. However, at concentrations ≤ 10^{-9} M, inhibition of IL-1β was only demonstrable for BUD with no effect of BDP or TAA and these results are in line with the range order of receptor binding activities of GCs. These effects of commonly used topical GCs on IL-1β were not statistically significant as compared to dexamethasone (DEX). Since BDP is hydrolized in the lung to 17-BMP, which displays a GC-receptor affinity 25-times higher than that of BDP, our data with BDP may be still underestimated [44]. There is also increasing evidence, that lung tissue may be active in metabolising GCs by the activity of 11β-hydroxysteroid dehydrogenase in epithelial cells [47].

Clinically, the efficacy of high concentrations of inhaled BDP (2 x 1000μg/day) was equivalent with daily prednisolone of 10mg in patients with asthma [8]. After systemic treatment with prednisolone in moderate asthmatics a reduction in the number of cells expressing mRNA for interleukin-4 and interleukin-5 was observed in bronchoalveolar lavage fluids [30]. Inhaled FP caused a significant decrease of IL-1β, IL-8, and LTB_4 in sputum of patients with bronchiectasis [40]. A 50% inhibition of IgE-dependent IL-4 generation by human basophils after TAA was observed and in these experiments TAA was more potent than dexamethasone or hydrocortisone [33]. Our data are in concert with recent studies with GCs in various cellular systems and suggest for commonly used inhaled GCs stronger effects of BUD and equal effects of BDP and TAA that were all reported inferior as compared to FP or MF [9, 35, 36, 37].

In summary, our data add to the knowledge that commonly used topical GCs can be classified as strong inhibitors of cytokine production in LPS -and IL-1β-stimulated monocytes with smaller effects on arachidonic acid metabolism. As consequence of this mode of action up-regulated COX-2 activity in lung parenchyma of chronic obstructive pulmonary disease may not sufficiently controlled by topical GCs and this may have an impact on the progression of inflammatory responses in lung diseases [45]. Besides receptor binding affinity variation of lipid solubility, binding to lung tissue at sites other than GC-receptors and differences in the activation of GC metabolising enzymes seem of importance as these factors can dramatically influence the potency and duration of action of GCs in the airways. For this reason receptor binding activity may only be partially useful to effectively characterise the potency of topical GCs.

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