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Effects of steroidal and non-steroidal antiphlogistic drugs on eicosanoid synthesis in irritated skin: studies with the isolated perfused bovine udder

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Abstract

Using the isolated perfused bovine udder as an in-vitro model of skin inflammation, the effects of topically administered arachidonic acid on prostaglandin and leukotriene synthesis have been shown previously. In this study, the effects of indometacin (indomethacin) and clobetasol-17-propionate (administered topically) as well as flunixin meglumine and meloxicam (administered via the perfusion fluid) have been studied. Compared with controls, arachidonic acid caused a significant increase in the dermal prostaglandin E_2 (PGE_2) and peptidoleukotriene ($LTC_4/D_4/E_4$) concentration. Topical treatment with indometacin (1.6 mg cm^{-2}) and clobetasol-17-propionate (90 μg cm^{-2}), which were administered 60 min before arachidonic acid administration, inhibited the inflammatory reaction. Flunixin meglumine (1 μg mL^{-1} perfusion fluid) was administered 30 min after and meloxicam (3 μg mL^{-1} perfusion fluid) was administered 60 min before arachidonic acid application. Three hours after arachidonic acid administration, a significant inhibition of PGE_2 synthesis was induced by flunixin. In contrast, meloxicam showed only a slight effect. The effect of flunixin was comparable with in-vivo results. It is known from animal studies that anti-inflammatory effects of meloxicam are obvious within up to 6 h after treatment. Therefore, the incomplete effect of meloxicam may be explained pharmacokinetically. In conclusion, the described in-vitro model seems to be suitable for studies of pharmacological effects on eicosanoid synthesis in the skin.

Introduction

The isolated perfused bovine udder was introduced as an in-vitro model for studies of transdermal penetration and absorption (Kietzmann et al 1993). Additional experiments demonstrated the suitability of this udder model for studies of skin irritation (local tolerance) of topically administered compounds (Kietzmann et al 1996). Therefore we were interested to evaluate the potential of the isolated perfused udder for studies of inflammatory skin reactions. In similarity to the established mouse ear model (Opas et al 1985; Rao et al 1993; Lloret & Moreno 1995), arachidonic acid was administered topically onto the udder skin. As parameters, the dermal concentration of eicosanoids, which play a pivotal role in inflammatory processes, was measured in the skin (Bäumer & Kietzmann 2000).

We have examined the effects of topically and "systemically" administered antiphlogistic drugs on the eicosanoid synthesis in bovine udder skin irritated with arachidonic acid. Indometacin (indomethacin) and clobetasol-17-propionate were administered topically, meloxicam and flunixin meglumine were administered via the perfusion fluid.

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Materials and Methods

Isolated perfused bovine udder

Bovine udders were perfused according to former studies (Kietzmann et al 1993; Bäumer & Kietzmann 2000). In brief, the organs were collected immediately after the slaughter of healthy cows. The udders were infused immediately via the right and left external pudendal arteries to counteract clot formation. In the laboratory, the organs were perfused with oxygenated Tyrode solution as rapidly as possible by a peristaltic pump (Masterflex 7518-10; Cole-Parmer Instr., Chicago, IL). The temperature of the perfusion fluid was 38.5°C. The perfusate flux chosen for the experiments was 100–120 mL min^{-1}, which resulted in a sufficient skin vascularity and mean skin temperature of approximately 30°C. The viability of the perfused udder skin was demonstrated by almost unchanged glucose consumption, an initially decreasing and thereafter unchanged lactate production and an unchanged lactate dehydrogenase activity.

Topical administration of arachidonic acid

Arachidonic acid (3 mg cm^{-2}) was administered to the skin as an ingredient in an ointment (unguentum alcoholum lanae aquosum 0.45 g, arachidonic acid 0.03 g, dimethyl sulfoxide 0.025 g, glycerine 0.025 g), prepared immediately before each experiment. Skin areas of 10 cm^2 were treated with arachidonic acid. A second area (treated with the vehicle) was used as control. Skin biopsies (diam. 6 mm) were taken from treated and untreated areas 15, 60, 120 and 180 min after treatment. The skin was stored at −196°C. Using an enzyme immunoassay (Caymann Chemical Co., Ann Arbor, MI), prostaglandin E_2 (PGE_2) and peptidoleukotrienes ($LTC_4/D_4/E_4$) were measured after homogenization (30 s, 20000 rev min^{-1}, 4°C) as described by Bäumer (1999). Diluted supernatant was used for ELISA directly, because Sterl (1998) demonstrated that an additional extraction procedure was not necessary.

Topical administration of indometacin or clobetasol-17-propionate

Indometacin (1.6 mg cm^{-2}) or clobetasol-17-propionate (90 μg cm^{-2}) were administered occlusively to the skin 1 h before arachidonic acid treatment.

"Systemic" administration of flunixin or meloxicam

Flunixin (1 μg mL^{-1}) was administered via the Tyrode solution 30 min after topical treatment with arachidonic acid. Meloxicam (3 μg mL^{-1}) was added to the Tyrode solution 1 h before arachidonic acid treatment. Skin biopsies were sampled at identical time points as described above. The antiphlogistic drugs were added continuously in the same concentration until the last skin biopsy was taken.

Statistical evaluation

Results are given as median and quartiles (Tables 1 and 2) or median-boxes (Figures 1, 2, 3 and 4). The statistical analysis for the biopsies was performed with the Mann-Whitney test for independent samples (U-test).

Table 1 Leukotriene $C_4/D_4/E_4$ concentration (ng cm^{-2}) in the skin of isolated perfused bovine udders after topical administration of arachidonic acid. Influence of clobetasol-17-propionate or indometacin on leukotriene $C_4/D_4/E_4$ release in the skin.

Time		Vehicle control	Arachidonic acid 3 mg cm^{-2}	Clobetasol-17-propionate + arachidonic acid	Indometacin + arachidonic acid
15 min	Median	537	2571	2600	1880
	1./3. quartile	456/810	1111/3378	2243/2896	1309/2189
1 h	Median	960	2994	2191	2444
	1./3. quartile	223/1737	2533/3378	1489/3891	2137/4386
2 h	Median	987	4073	3198	2743
	1./3. quartile	718/1026	2664/7075	2876/3891	873/5497
3 h	Median	1024	4020	3960	2931
	1./3. quartile	1005/1737	3198/5140	2596/4977	2296/4524

Data are given as median, 1. and 3. quartile of six separated experiments; clobetasol-17-propionate or indometacin were administered 1 h before arachidonic acid application. There was no significant difference between arachidonic acid-treated and arachidonic acid combined with either indometacin- or clobetasol-17-propionate-treated areas.

Table 2 Leukotriene $C_4/D_4/E_4$ concentration (ng cm^{-2}) in the skin of isolated perfused bovine udders after topical administration of arachidonic acid. Influence of flunixin meglumine on leukotriene $C_4/D_4/E_4$ release in the skin.

Time		Vehicle control	Arachidonic acid 3 mg cm^{-2}
15 min	Median	801	1025.5
	1./3. quartile	569.5/941	939/1422
2 h	Median	868.5	1259
	1./3. quartile	775/1084	1132/2129
3 h	Median	988.5	2167
	1./3. quartile	645/1148	1397/2989

Data are given as median, 1. and 3. quartile of four separate experiments, flunixin meglumine was administered 1 h before arachidonic acid application. There was no inhibition of leukotriene $C_4/D_4/E_4$ release by flunixin meglumine.

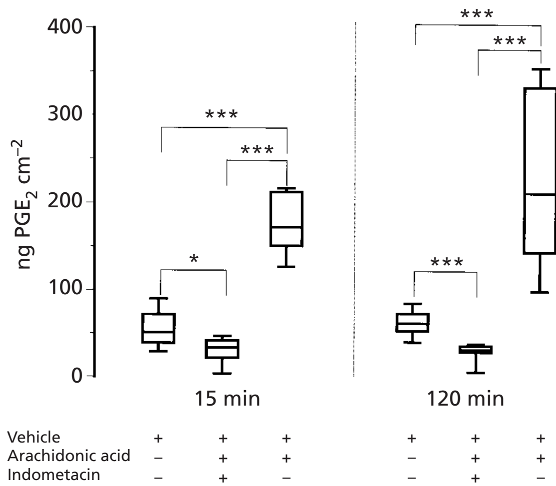

Figure 1 Prostaglandin E_2 concentration (ng cm^{-2}) in the skin of isolated perfused bovine udders after topical arachidonic acid administration. Influence of indometacin and arachidonic acid on PGE_2 release in the skin. Data are given as median boxes of six separate experiments. There was a significant difference between arachidonic acid-treated and the combination of arachidonic acid and indometacin-treated areas. Even the vehicle control generated more PGE_2 compared with the combination of arachidonic acid and indometacin-treated areas. $*P < 0.05$, $***P \leqslant 0.001$.

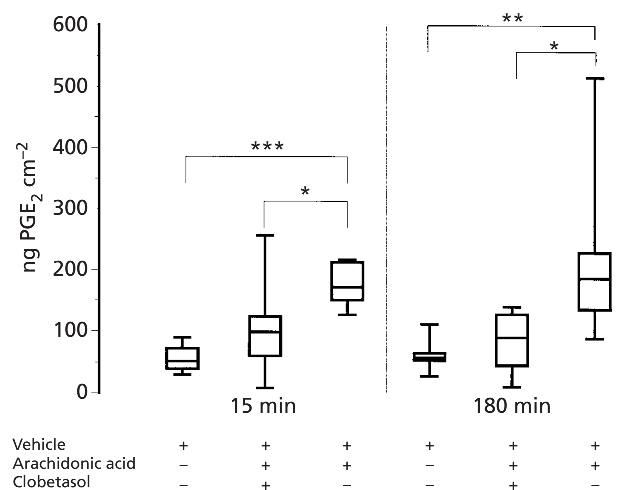

Figure 2 Prostaglandin E_2 concentration (ng cm^{-2}) in the skin of isolated perfused bovine udders after topical administration of arachidonic acid. Influence of clobetasol-17-propionate on PGE_2 release in the skin. Data are given as median boxes of six separate experiments. Clobetasol-17-propionate was administered 1 h before arachidonic acid application. There was a significant difference between arachidonic acid-treated and the combination of arachidonic acid and clobetasol-17-propionate-treated areas 15 and 180 min after arachidonic acid irritation. $*P < 0.05$, $**P < 0.01$, $***P \leqslant 0.001$.

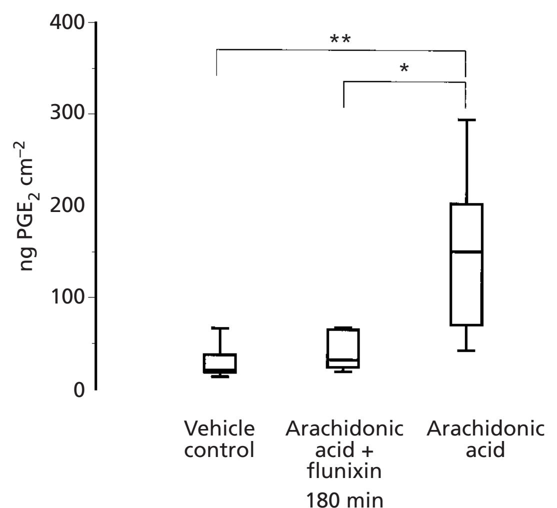

Figure 3 Prostaglandin E_2 concentration after administration of flunixin in the skin of isolated perfused bovine udders. Data are given as median boxes of six separate experiments. Flunixin (1 μg mL^{-1}) was administered 30 min after arachidonic acid application, and 3 h after that arachidonic acid administration the PGE_2 concentration was significantly reduced compared with non-antiphlogistic-treated udders. $*P < 0.05$, $**P < 0.01$.

Results

Topical administration of indometacin or clobetasol-17-propionate

Indometacin significantly inhibited the arachidonic acid-induced increase of PGE_2 over 3 h. The PGE_2 levels were below that of controls (Figure 1).

After treatment with clobetasol-17-propionate, the prostaglandin concentration was also reduced. The inhibitory effect was not complete (Figure 2).

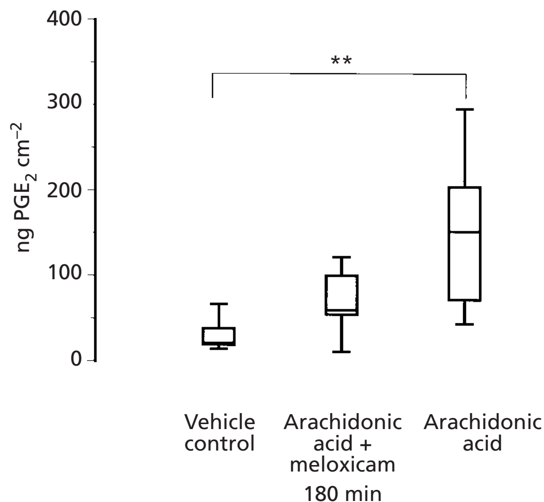

Figure 4 Prostaglandin E_2 concentration after administration of meloxicam in the skin of isolated perfused bovine udders. Data are given as median boxes of seven separate experiments. Meloxicam (3 μg mL^{-1}) was administered 60 min before arachidonic acid application, and the PGE_2 concentration 3 h after that arachidonic acid administration was slightly reduced compared with non-antiphlogistic-treated udders. $^{**}P < 0.01$.

In contrast to PGE_2, the concentration of peptidoleukotrienes in the clobetasol-17-propionate-or indometacin-treated areas did not differ significantly from that of skin areas treated with arachidonic acid alone (Table 1).

"Systemic" administration of flunixin or meloxicam

The perfusion with flunixin resulted in a significant inhibition of the PGE_2 synthesis 3 h after arachidonic acid administration (Figure 3), but there was no inhibition of the $LTC_4/D_4/E_4$ synthesis (Table 2).

Three hours after arachidonic acid administration, meloxicam caused only a slight reduction of PGE_2 synthesis (Figure 4).

Discussion

The arachidonic acid-induced skin inflammation in mice is a model frequently used in experimental pharmacology (Opas et al 1985; Rao et al 1993; Lloret & Moreno 1995). Therefore, we have established a comparable in-vitro model of arachidonic acid-induced skin inflammation. Former studies demonstrated the increase of eicosanoids in arachidonic acid-irritated skin of isolated perfused bovine udders (Bäumer & Kietzmann 2000). In this study we have examined the effect of topically and "systemically" administered antiphlogistic drugs on the eicosanoid synthesis in the irritated bovine udder skin.

Indometacin, a potent inhibitor of cyclooxygenase (COX), shows a highly significant reduction of PGE_2 synthesis without any effect on 5-lipoxygenase. The inhibition of PGE_2 synthesis below that of untreated controls indicates a strong affinity to the "housekeeping enzyme" COX 1 (Laufer et al 1999). This strong inhibition is comparable with in-vivo results on inflamed mouse ears (Lloret & Moreno 1995).

In that model of inflammation the substrate (arachidonic acid) was added, and so the inhibition of the phospholipase A_2 as one mechanism of action of the glucocorticoids was ineffective (Flower & Blackwell 1979). The partial reduction of PGE_2 synthesis by clobetasol-17-propionate may be due to the inhibition of COX-2 expression caused by the irritation with arachidonic acid (Masferrer et al 1992; Barry et al 1999).

The inhibitory effect of flunixin meglumine was significant only 3 h after topical administration of arachidonic acid. After 1 h, a slightly enhanced PGE_2 concentration was obvious. Compared with the in-vivo results of Espinasse et al (1994), where the inhibition set in 30 min after administration of flunixin, our model took a longer time. The reason for this is because in contrast to the in-vivo experiments where the eicosanoids were measured subcutaneously, in our in-vitro model flunixin had to diffuse into the epidermis and dermis as the loci of inflammation.

As expected, flunixin meglumine had no influence on the lipoxygenase pathway. This confirmed the in-vivo results of Espinasse et al (1994).

The incomplete inhibition of PGE_2 concentration by meloxicam 3 h after arachidonic acid administration can be explained pharmacokinetically or possibly by its preferential COX-2 inhibition. It was obvious that the arachidonic acid inflammation model was mainly mediated via the COX-1 pathway (Puigneró & Queralt 1997). Therefore it might be that meloxicam, which showed a 3- to 10-fold inhibition of COX 2 compared with COX 1 (Engelhardt et al 1996a, b), was not as potent in this inflammation model as a non-selective antiphlogistic drug such as flunixin. The perfusion with the meloxicam-containing solution was performed over 4 h. This may have been too short a time for inducing a significant inhibition of PGE_2.

In conclusion, the described in-vitro model seems suitable for studying effects of topically and "systemically" administered antiphlogistic drugs on eicosanoid synthesis in the skin. Further studies should demonstrate a dose-dependent response of the prostaglandin inhibition by antiphlogistic drugs. Due to the large skin area obtained by the isolated perfused bovine udder, different substances and formulations can be tested at the same time.

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