The effects of Celecoxib on inflammation and synovial microcirculation in murine antigen-induced arthritis

H.H. Gebhard[1], S.P. Zysk[2], M. Schmitt-Sody[2], V. Jansson[2], K. Messmer[1], A. Veihelmann[2]

[1]Institute for Surgical Research, Ludwig-Maximilians University of Munich; [2]Department of Orthopaedics, Klinikum Grosshadern, Ludwig-Maximilians University of Munich, Germany.

Abstract

Objective

There is controversy about the effects of cyclooxygenase-2 (COX-2) on adhesion molecules and the microvasculature in inflamed tissue. Thus, the aim of this study was to assess COX-2-expression in Antigen-induced Arthritis (AiA) and to investigate the effects of selective COX-2 inhibition by Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide) (CXB), on synovial microcirculation and adhesion molecule expression in arthritic as well as healthy mice.

Methods

Balb/c mice were allocated to 4 groups; 2 control groups with saline or CXB and 2 groups with AiA which also received saline or CXB (30 mg/kg BW in 0.3 ml solution). The severity of arthritis was assessed by changes in the transverse joint diameter. On day 14 after AiA-induction, the patella tendon of the left knee joint was microsurgically resected and intravital fluorescence microscopy on synovial tissue was performed. Finally, the knee joint was removed for histology and immunohistochmistry.

Results

COX-2-expression in the inflamed synovium was demonstrated by immunohistochemistry. Application of Celecoxib resulted in a significant reduction in the rolling leukocyte fraction as well as in the number of leukocytes adherent to the endothelium (0.25 ± 0.1 and 96 ± 34 cells/mm^2 respectively) in comparison to the untreated animals with AiA (0.44 ± 0.03 and 206 ± 22 cells/mm^2 respectively). Additionally, CXB-treated arthritic animals showed significantly less knee joint swelling and reduced adhesion molecule expression.

Conclusion

In the present study, COX-2 expression in the synovial tissue of mice with AiA could be demonstrated. Selective COX-2 inhibition with CXB resulted in reduced leucocyte-endothelial cell interactions and decreased adhesion molecule expression. Evidence for a protective role of COX-2 in mouse AiA was not found.

Key words

Cyclooxygenase 2, antigen-induced arthritis, celecoxib, adhesion molecules, intravital microscopy.

Harry H. Gebhard, BS; Stefan P. Zysk*, MD; Markus Schmitt-Sody, MD; Volkmar Jansson, MD; Konrad Messmer, MD; Andreas Veihelmann, MD.*

**Both of these authors contributed equally to the study.*

This work was supported by a grant from Pfizer GmbH, Karlsruhe, Germany.

Please address correspondence and reprint requests to: Dr. med. Stefan P. Zysk, Department of Orthopaedics, Ludwig Maximilians University, Klinikum Großhadern, Marchioninistrasse 15, D-81377 Munich, Germany.
E-mail: szysk@med.uni-muenchen.de

Received on March 29, 2004; accepted in revised form on September 17, 2004.

Introduction

Non steroidal anti-inflammatory drugs (NSAIDs) exert their effects by blocking of prostaglandin synthesis due to inhibition of the enzyme cyclooxygenase (COX) (1). COX consists of two isoenzymes, COX-1 and COX-2 (2). Selective COX-2 inhibitors, such as Celecoxib (CXB) (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide), show equivalent analgesic and anti-inflammatory efficacy compared to classic NSAIDs combined with the advantage of fewer gastrointestinal side effects (3). Therefore, selective COX-2 inhibitors are widely used in the treatment of human osteoarthritis and rheumatoid arthritis (RA) (4). However, the initial hypothesis that the anti-inflammatory effects of NSAIDs result from COX-2 inhibition and that their side effects are due to the blockade of COX-1 turned out to be not entirely sufficient. COX-2 activity, for example, is known to play a key role in preserving gastric mucosal integrity (5) and therefore exerts protective functions as well.

A potential mechanism for the anti-inflammatory actions of selective COX-2-inhibitors may be the downregulation of adhesion molecule expression (6). Adhesion molecules are known to mediate leukocyte-endothelial cell interactions and, thereby, the influx of immune competent cells into the inflamed tissue (7). However, recent studies provided evidence of tissue specific differences in COX-2 regarding its role in adhesion molecule regulation. Cuzzocrea *et al.* reported a substantial reduction of ICAM-1 in the mesenteric microvasculature of rats with colitis after systemic COX-2 inhibition (6). In contrast, Bishop-Bailey *et al.* demonstrated the upregulation of adhesion molecule expression on smooth muscle cells by COX-2 inhibition *in vitro* (8).

COX-2 expression has previously been demonstrated in the synovial tissue of RA patients (9). However, COX-2 effects on synovial microvasculature and adhesion molecule expression in arthritis have not been determined yet.

The aim of this study was to investigate the expression of COX-2 in mouse AiA and to analyze the effects of selective COX-2 inhibition by Celecoxib on the synovial microcirculation using intravital microscopy as described previously (10), and on adhesion molecule expression. The data obtained were correlated with clinical and histological findings.

Methods

Animals

36 female inbred Balb/c mice (Charles River Wiga, Sulzbach, Germany) weighing 18-22 g were used. The animals were kept in an air-conditioned environment with 12h light/dark cycles, housed in single cages and fed laboratory chow (Ssniff, Soest, Germany) and water *ad libitum*. Before starting the experiment, they were randomly assigned to the following groups: control with saline (n = 8), control with Celecoxib (n = 7), AiAwith saline (n = 7) or AiA with Celecoxib (n = 8). The numbers given represent the number finally included after the exclusion of animals which experienced complications during the surgical preparation. All experiments were carried out according to German law regarding animal protection guidelines.

Antigen-induced arthritis

Arthritis was induced in mice using the protocol of Brackertz *et al.* (11). On days -21 and -14, mice were first immunized with a subcutaneous injection of 100 µg of methylated bovine serum albumin (mBSA) (Sigma, Deisenhofen, Germany) dissolved in 50 µL of saline. The mixture was supplemented with 50 µl complete Freund's adjuvant (CFA) (Sigma) and 2 mg/ml of heat-killed *Mycobacterium tuberculosis* strain H37RA (Difco, Augsburg, Germany). In accordance with the protocol, we gave additional intraperitoneal injections of 2 x 10^9 heat-killed *Bordetella pertussis* (Institute of Microbiology, Berlin, Germany) on days -21 and –14. Finally, arthritis was induced on day 0 by injecting of 100 µg of mBSA dissolved in 50 µL saline into the left knee joint. Control groups underwent the same procedure, but received equivalent volumes of saline only. The severity of antigen-induced arthritis was determined by means of the clinical and histological score described below.

Celecoxib administration
Celecoxib was generously provided by Pfizer GmbH (Karlsruhe, Germany). Stored at room temperature, the substance was diluted in sterile phosphate buffered saline solution (PBS) at a concentration of 2 g/ml for intragastral application, starting on day +7. 0.3ml of this solution (0.6 g; 30 mg/kg BW) were administered by gavage twice daily until the intravital microscopic measurements were performed on day +14. Control animals received equivalent volumes of vehicle (PBS).

Clinical assessment
Joint swelling was determined by measuring the transverse diameter of the knee joint using a caliper in units of 0.01 mm.

Surgical preparation
Intravital fluorescence microscopy was performed on the synovial microcirculation of the mouse knee joint. This model permits the intravital study of microhemodynamic parameters, including the segmented vessel diameter and red cell velocity in the microvascular network of the synovial microcirculation consisting of arterioles, capillaries, and postcapillary venules and quantitative assessment of the functional capillary density and leukocyte-endothelial cell interactions (12).
For the surgical preparation, anesthesia was induced by inhalation of isoflurane 1.2% and a combination of O_2/N_2O. For substance administration a catheter was placed into the tail vein. The mean arterial blood pressure was determined with an arterial catheter inserted into the tail artery and connected to a pressure transducer.
The microsurgical procedure was performed as described previously (12). Animals were kept on a heating pad to stabilize the body temperature, which was controlled by a rectal probe. The left hind limb was placed on a stage with the knee slightly flexed. Immobilization of the extremity was secured by fixation with silicon. Using a 1 cm incision the patellar tendon was carefully mobilized and partly resected. Then the intraarticular synovial tissue of the knee joint could be visualized. After superfusion with 2 ml of sterile saline, a coverslip was placed on the knee capsule and the intravital microscope was directed onto the synovium (12).
Special care was taken to avoid any constraints on the preparation. The animals were killed with 10 mg of pentobarbital intravenously after the end of the intravital microscopic observations.

Experimental protocol
The microscopic set-up has been described in detail elsewhere (13). A 20-fold water immersion objective was used to select 2-4 regions of interest in each animal. These 4 regions contained postcapillary venules and capillary areas for the measurement of the Functional Capillary Density (FCD) and vessel diameter. To measure the leukocyte-endothelial cell interactions, the fluorescent marker rhodamine 6G (Sigma) was injected intravenously in a single bolus of 0.15 mg/kg immediately before the measurement. Rhodamine epi-illumination was achieved with a 150 Watt variable HBO mercury lamp in conjunction with a Zeiss filter set 15 [band pass (BP) 546/12, Farbteiler (FT) 580, long pass (LP) 590]. The FITC measurements were made using a variable 12 V, 100 W halogen light source and the Zeiss filter set 09 (BP 450-490, FT 510, LP 520, Zeiss). Measurements of vessel diameter, venular RBC velocity and FCD were made after a bolus injection of the *in vivo* fluorescent plasma marker FITC-dextran (mol mass 150 kDa; 15 mg/kg body wt i.v.) (Sigma). The microscopic images were captured with a CLD camera and recorded on S-VHS videotape using both filter blocks consecutively. Data analysis was performed off-line using a computer-assisted microcirculation analysis system (14).

Microcirculatory parameters
From video images after rhodamine-staining, the leukocyte endothelial cell interactions were quantified. Leukocytes interacting with the endothelium were classified as rolling or adherent cells. Rolling leukocytes were defined as cells that intermittently interact with the endothelial surface and pass through the vessel visibly slower than red blood cells in the centerline stream. To account for vessels of various diameters, flows and leukocyte fluxes, rolling leukocytes were divided by the total number of leukocytes passing through a plane on the vessel segment during the 30-second observation time (calculated as rolling cells/rolling + non-adherent cells to be independent from the total systemic leukocyte count). Adherent leukocytes are defined as those leukocytes that remain attached to the same location on the endothelial surface for the entire observation period of 30 seconds (expressed as cells/mm^2). The endothelial cell surface was calculated from the diameter and length. We determined the vessel diameter, RBC velocity and FCD from the video images obtained with FITC staining. RBC centerline velocity in postcapillary venules was assessed with Cap-Image and expressed as mm/second (14). FCD is defined as the length of RBC-perfused capillaries in the observation area (expressed as cm/cm^2).

Histology
After the animal was sacrificed, the knee joint was removed. After fixation in paraformaldehyde 8% at pH 7.2 over 12 hours, the joints were incubated in 20% EDTAat pH 7.2 for 3 days at room temperature to decalcify the bone. Samples were washed with phosphate buffer saline (PBS) and dehydrated with an automatic dehydrator. After embedding in paraffin, the joint was sliced into 3 µm thick sections that were stained with hematoxylin and eosin.
To evaluate the severity of the arthritis and leukocyte infiltration, we used the histological score introduced by Brackertz *et al*. (11): 0 = normal knee joint, 1 = normal synovium with occasional mononuclear cells, 2=two or more synovial lining cells and perivascular infiltrates of leukocytes, 3 = marked hyperplasia of synovium and dense infiltration of leucocytes (not only perivascular), 4 = synovitis, pannus formation, and cartilage/subchondral bone erosions. In each animal, two cuts were made, and in each section the number of infiltrated leukocytes was counted in 5 fields.

Immunohistochemistry

The E-selectin staining was performed according to the following protocol. The frozen sections of the synovium were fixed in acetone for 15 min and washed with PBS. To block the production of endogenous peroxidase, sections were quenched with 0.5% H_2O_2 methanol solution for 10 min. After washing with PBS, sections were incubated in goat serum 1.5% for 20 min to block any unspecific binding.

Afterwards, sections were incubated with a monoclonal rat anti-mouse CD-62E antibody 10E 6.9 directed against E-selectin (PharMingen, Hamburg, Germany) at a 1:100 dilution in PBS for 60 min. After washing, the sections were incubated with a biotinylated anti-rat IgG-antibody (Vectastatin, Vector Laboratories, Los Angeles, CA, USA) with mouse serum 5% for 30 min. Slides were rinsed with PBS and incubated with an avidin-biotin complex (Vectastatin) for 30 min. Following incubation with 3-amino-9-ethylcarbazol 0.01% as a substrate, sections were counterstained with haemalaun (Merck, Darmstadt, Germany), hydrated, and coverslipped. All incubations were performed at 24ºC in a humidity chamber, and all washes lasted for 10 min each. Each tissue block was stained with and without the primary antibody to monitor background staining.

The procedure for P-selectin staining by immunohistochemistry was identical to the above-described protocol, except using a polyclonal rabbit anti-mouse CD62P antibody (PharMingen) in place of the E-selectin antibody at a 1:200 dilution in PBS and a biotinylated anti-rabbit IgG-antibody (Vectastain) with rabbit serum 1.5%.

COX-2 was detected using the same protocol, but different antibodies: a monoclonal anti-mouse Ab as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a biotinylated anti-goat IgG-Ab as the secondary antibody were used.

For ICAM-1 and VCAM-1 staining, a similar procedure was performed with the following changes. Usage of either a biotinylated monoclonal rat anti-mouse CD54 antibody (PharMingen) at a 1:100 dilution in PBS for ICAM-1 or a rat anti-mouse CD106 antibody (PharMingen) for VCAM-1 detection. Adhesion molecule expression was assessed semiquantitatively by a blinded observer, who examined 3 tissue sections each (2 observation fields per section) from 3 individual animals, and scored the findings as follows: o = no staining, + = weak staining, and ++ = strong staining.

Statistical analysis

Data are expressed as the mean ± SEM. The histologic score is given as the median. For differences within the groups, we used the Kruskal-Wallis analysis followed by an all pairwise multiple comparison procedure. P values less than 0.05 were considered significant.

Results

Microcirculation and clinical analysis

The mean arterial blood pressure did not change in any of the 4 groups during the entire course of the experiments (TableI). Furthermore, the venular diameters were similar in all of the groups (Table I). CXB itself did not influence the leukocyte-endothelial cell interaction in healthy controls (Figs. 1 and 2). We found no significant changes in venular RBC velocity, compared to the control values and the values of the AiA group treated with Celecoxib (Table I). Regarding arthritic animals, the rolling fraction (Fig.1) and the number of leukocytes adherent to the endothelium (Fig.2) were significantly higher than in the control groups (Fig. 3). Drug application reduced significantly both leukocyte rolling and leu-

Table I. Macro- and microhemodynamic parameters and clinical analysis of mice from four experimental groups.

Parameter	Control (n 8)	Control + CXB (n 7)	AiA (n 7)	AiA+ CXB (n 8)
MAP(mmHg)	83 (3)	84 (3)	81 (5)	83 (2)
Venular diameter (µm)	25 (2)	22 (3)	24 (1)	23 (3)
RBC velocity (mm/s)	0.79 (0.12)	0.85 (0.14)	0.75 (0.18)	0.78 (0.09)
FCD (cm/cm²)	259 (18)	261 (29)	283 (12)	279 (18)
Δ Diameter LKJ (mm)	0.07 (0.01)	0.08 (0.02)	0.49 (0.07)[a]	0.14 (0.05)[b]

MAP: mean arterial pressure; RBC: red blood cell; FCD: functional capillary density; AiA: Antigen-induced Arthritis: CXB: Celecoxib; Δ Diameter LKJ: change in left knee joint diameter given as an increase (mm); data are given as the mean (SEM). [a]p < 0.05 vs control, [b]p < 0.05 vs. AiA.

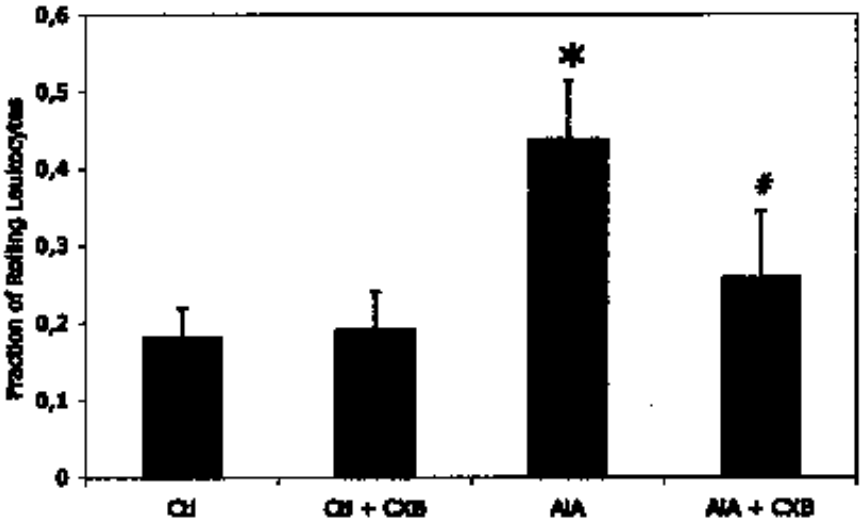

Fig. 1. Assessment of the fraction of rolling leukocytes using Rhodamine-staining and video microscopy. Calculation: rolling leukocytes/(rolling+non-adherent leukocytes). Ctl: Control; CXB: Celecoxib; AiA: Antigen-induced Arthritis. Data are given as mean ± SEM. *p < 0.05 vs control, #p < 0.05 vs AiA.

kocyte adherence to levels similar to those in healthy controls (Fig. 2).
FCD was slightly enhanced in both arthritic groups compared to controls. Celecoxib did not influence FCD either in AiA-mice or in healthy controls (Table I).
The clinical assessment revealed a significantly increased knee joint diameter (KJD) in the AiA group compared to controls, which was significantly diminished in CXB-treated arthritic animals.

Histology
We found dense infiltration of leukocytes in the synovium and synovial hyperplasia in the group with AiA. CXB reduced the extravasation of leukocytes in the synovium (only moderate infiltration and synovial lining cell proliferation was seen) compared to the AiA group. The difference in the quantified count of histological cuts was significant (Table II).

Immunohistochemistry
Immunohistochemistry revealed the strong expression of COX-2 in the synovial tissue of animals with AiA, while no expression could be detected in the sections of control animals (Fig. 4). ICAM-1 was strongly expressed in AiA, while expression was weak in controls. VCAM-1, E-selectin and P-selectin were expressed in AiA, but not in the synovia of healthy mice. Treatment with CXB decreased the staining of all of these molecules, to an extent comparable to that in healthy controls (Table III).

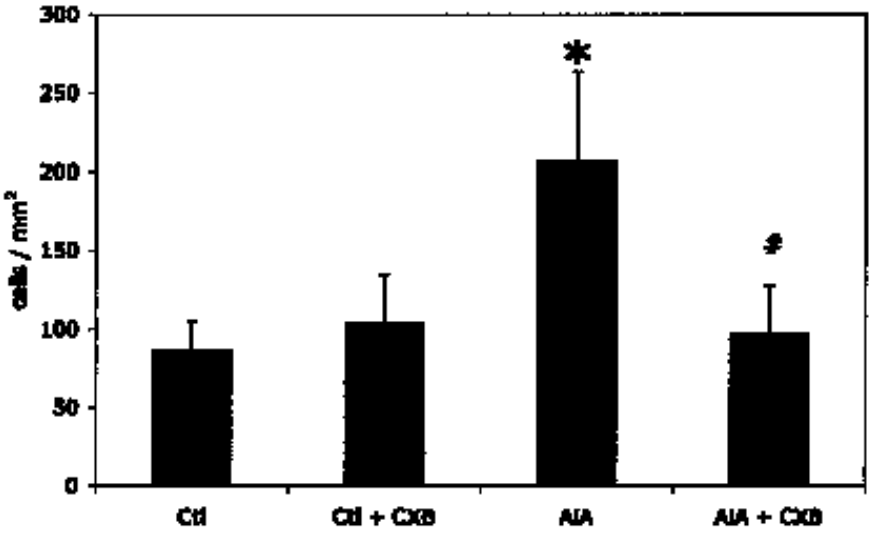

Fig. 2. Assessment of leukocytes adherent to the endothelium using Rhodamine-staining and video microscopy (expressed as cells/mm^2). Ctl: control; CXB: Celecoxib; AiA: Antigen-induced Arthritis. Data are given as mean ± SEM. *$p < 0.05$ vs control, # $p < 0.05$ vs AiA.

Discussion
The effects of COX-2 on adhesion molecule expression and therefore on leukocyte migration to inflamed tissue have been discussed and remain controversial. While selective COX-2-inhibition led to the upregulation of integrins in smooth muscle cells (8) and to the enhancement of leukocyte adhesion in mesenteric venules (15), other authors report less extravasation of leukocytes and, moreover, a decreased expression of selectins as well as of immunoglobulin-like proteins in mesenterial postcapillary venules (6).
The recruitment of leukocyte subsets to inflamed tissue is known to be a crucial factor in the pathogenesis of RA, but there are currently no data available regarding the effects of selective COX-2 inhibition on synovial leukocyte-endothelial cell interactions in an arthritis

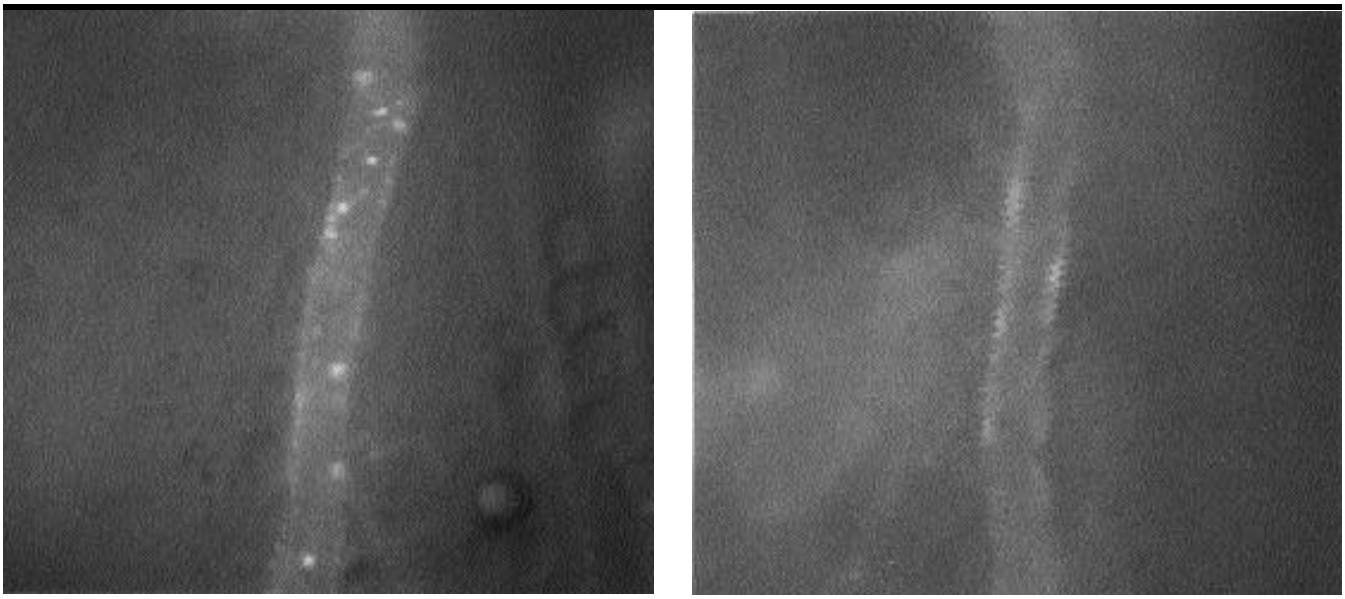

Fig. 3. Intravital microscopical view of postcapillary venules after staining of leukocytes with rhodamine 6G: (**a**) AiA: A large number of leukocytes can be seen (**b**) CTL: leukocytes can only occasionally be seen (magnification x432)

Table II. Histological score (median) and number of extravasating leukocytes.

Groups	Histological score	Extravasated leukocytes
Controls, n = 8	0	12 (3)
Control + CXB, n = 7	0	15 (5)
AiA, n = 7	3 (1)	102 (14)[a]
AiA+ CXB, n = 8	2 (1)	43 (9)[b]

Histological score (11): 0 = normal knee joint; 1 = normal synovium with occasional mononuclear cells; 2 = two or more synovial cells and perivascular infiltrates of leukocytes; 3 = marked hyperplasia of synovium and dense infiltration of leukocytes (not only perivascular); 4 = synovitis, pannus formation, and cartilage/subchondral bone erosions. Data are given as the median.
Extravasated leukocytes: data are given as the mean (SEM).
CXB: Celecoxib; AiA: Antigen-induced Arthritis.
[a]$p < 0.05$ vs control , [b]$p < 0.05$ vs AiA.

model.

In this study, the model of antigen-induced arthritis originally described by Brackertz *et al.* (11) was used. It is a well accepted animal model for the study of human rheumatoid arthritis. Arthritic knee joints presented significant swelling during the entire observation period. The severity of arthritis was shown by the high histological score, leukocyte infiltration and pannus formation. Furthermore, using immunohistochemistry our investigation demonstrated that there is strong expression of the isoenzyme COX-2 in the synovial tissue of arthritic animals, in contrast to the tissue of healthy controls.

The present model – the intravital microscopy of synovial tissue (12) – preserves a stable preparation for at least 60 minutes. In accordance with this, the microhaemodynamic parameters assessed in the present investigation were found to be unchanged throughout the study period in controls as well as in the AiA groups without intergroup differences. Thus, differences in leukocyte rolling and adherence between the groups have to be mediated by changes in leukocytes or endothelial cells which affect the adhesive forces between the two (16). Arthritic animals showed a significantly increased fraction of rolling leukocytes in the postcapillary venules. The numbers of adherent cells as well as of subsequently extravasated leukocytes were significantly increased. There was strong staining of ICAM-1, VCAM-1, P-selectin and E-selectin in the vascular endothelium.

Systemic treatment with CXB led to a significant reduction of the fraction of rolling leukocytes, and the number of adherent as well as extravasated cells. We found less staining of ICAM-1, VCAM-1-, P-selectin and E-selectin in the immunohistochemical sections. It is likely that selective COX-2-inhibition leads to a downregulation of certain selectins and immunoglobulin-like proteins in synovial tissue, even though the exact mechanisms underlying these effects remain unclear. Proinflammatory cytokines such as TNF-α and IL-1 lead to stimulation of the nuclear factor κB (NF-κB), i.e. in endothelial cells, followed by an upregulation of ICAM-1, VCAM-1, P-selectin and E-selectin (7,17, 18). These cytokines are also known to regulate COX-2 expression (19). Furthermore, reactive oxygen species (ROS) might be involved in this process since ROS have been shown to play an active role in arthritic inflammation (20). Released in the presence of cytokines such as TNF-α, ROS are believed to induce the activation of NF-κB, and thus the upregulation of adhesion molecules such as ICAM-1, VCAM-1, E-selectin (18) and P-selectin (7). Recently Nakamura *et al.* (21) reported antioxidant effects of CXB *in vivo.* Thus, it can be suggested that these antioxidant effects might inhibit

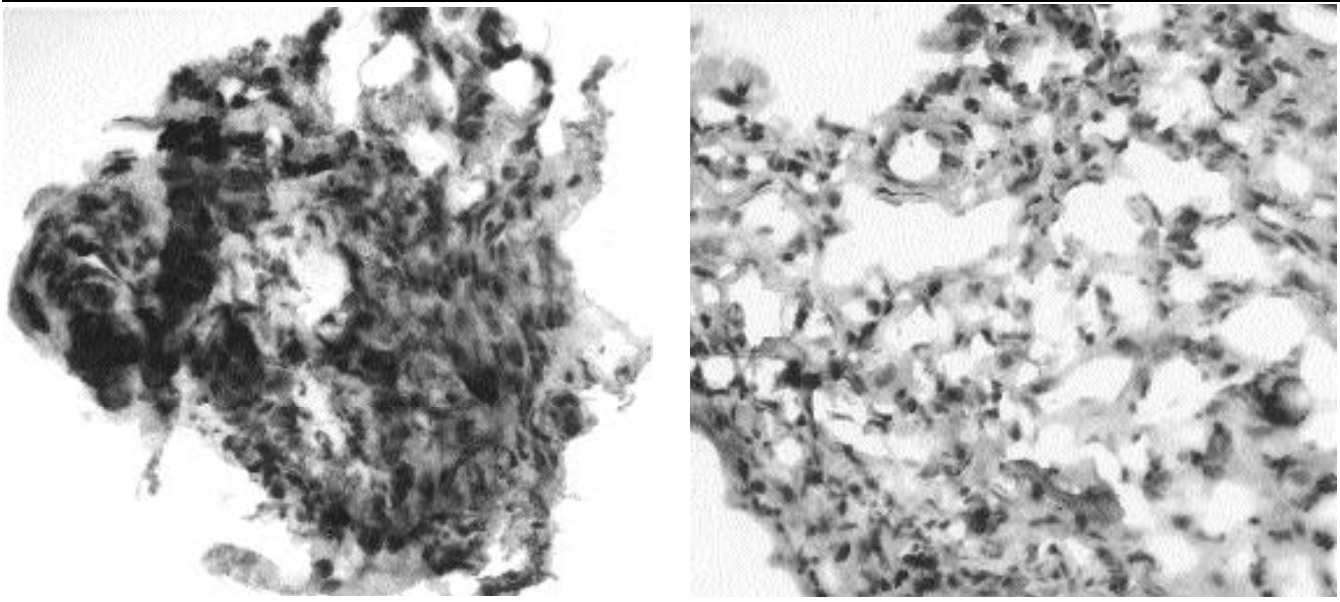

Fig. 4. Immunohistochemistry/COX-2 staining of synovial tissue from the mouse knee joint: (**a**) AiAvs. (**b**) CTL(magnification x160).

Table III. Expression of adhesion molecules in mouse knee joint synovial tissue*.

	Control		AiA	
	PBS	CXB	PBS	CXB
COX-2	o o o	o o o	++ ++ ++	+ o o
P-selectin	o o o	o o o	+ + +	o o o
E-selectin	o o o	o o o	o + +	o + o
ICAM-1	+ + +	++ + +	++ ++ ++	+ + +
VCAM-1	o o o	+ o o	+ + o	+ o o

*Scores are expressed as o (no staining), + (weak staining), or ++ (strong staining) in each of 3 tissue sections from 3 individual animals, assessed by immunohistochemical staining with monoclonal antibodies.
PBS: phosphate buffered aaline; CXB: xelecoxib; AiA: Antigen-induced Arthritis; COX-2: cyclooxygenase 2; ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion molecule 1.

cytokine-enhanced ROS generation and, therefore, adhesion molecule expression.

In the healthy controls CXB treatment did not lead to significant changes in leukocyte-endothelial cell interaction. Furthermore, there was no significant difference between controls and controls with CXB treatment regarding any of the paramenters assessed, including FCD, histology, extravasated cells or knee joint swelling. Consequently, there is no evidence that CXB itself leads to an activation in the synovial microvasculature. There was weak staining for constitutively expressed ICAM-1, but no staining for either VCAM-1 and P-selectin or for E-selectin in controls. This pattern did not change markedly in the controls with CXB treatment.

Bishop-Bailey *et al*. report an upregulation of ICAM-1 in human vascular smooth muscle cells after stimulation by IL-1β [8]. However, COX-2 inhibition by another selective COX-2 inhibitor, L-745,337, revealed a significant increase of ICAM-1 expression compared to IL-1β stimulated controls. Bishop-Bailey *et al.* found the same effect for VCAM-1 stimulated by IL-1β and IL-4, and inhibition with L-745, 337. Besides the different tissues and species, the different COX-2 inhibitors might give an explanation for these contrasting results. It has been proposed that the antioxidant properties of CXB contribute to the inhibition of adhesion molecule expression and there is evidence that not all COX-2 inhibitors exhibit antioxidant properties (21). Furthermore, Muscara *et al*. (15) used superfusion for drug administration, whereas CXB was applied orally in our study which corresponds to the clinical mode of application. Using CXB-superfusion Muscara *et al.* (15) found a significantly increased number of adherent leukocytes in rat mesenteric venules. In contrast, Cuzzocrea *et al*. (6) reported reduced adhesion molecule expression in the rat mesenteric microvasculature after oral CXB administration.

In the present study, swelling of the mouse knee joint was significantly decreased in the AiAgroup treated with CXB, which is consistent with the data obtained from intravital microscopy. This is in accordance with the results in the treatment of human RA using CXB (22). PGE_2 generated by COX-2 amplifies vascular permeability changes induced by complement fragments, histamine and leukotrienes. It is obvious that CXB helps to keep the endothelial barrier intact, resulting in decreased swelling and less leukocyte infiltration of inflamed tissue (23). Furthermore, Anderson *et al.* (24) report that selective inhibition of COX-2 reverses inflammation in rat adjuvant arthritis. Thus, suppression of COX-2 derived prostaglandins prevents mediation from a variety of proinflammatory effects, including enhancement of local plasma, cellular exsudation and upregulation of IL-6 and COX-2 itself in this animal model of arthritis (24). Ochi *et al*. (25) clearly demonstrated that COX-2, but not COX-1 contributes to inflammation in a mouse Collagen Induced Arthritis (CIA) model. They reported that there is a strong correlation between COX-2 mediated PGE_2- and TBX_2-formation and paw edema as well as histological lesions in mouse CIA(25).

In conclusion, our study provides evidence for COX-2 expression in the synovial tissue of mice with antigen-induced arthritis. CXB treatment of arthritic animals was associated with a decreased leukocyte-endothelial cell interaction and decreased adhesion molecule expression. We propose that CXB, probably due to its antioxidant properties, decreases the inflammatory reaction in acute arthritis. CXB itself did not enhance leukocyte-endothelial cell interaction in healthy animals. There were no signs of a protective role of COX-2 in arthritis in the present investigation.

References

1. VANE JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 1971; 231: 232-5.
2. FLETCHER BS, KUJUBU DA, PERRIN DM, HERRSCHMANN HR: Structure of the mitogen-inducible TIS 10 gene and demonstration that the TIS 10-encoded protein is a functional prostaglandin G/H synthase. *J Biol Chem* 1992; 267: 4338-44.
3. MCKENNA F, ARGUELLES L, BURKE T, LEFKOWITH J, GEIS GS: Upper gastrointestinal tolerability of celecoxib compared with diclofenac in the treatment of osteoarthritis and rheumatoid arthritis. *Clin Exp Rheumatol* 2002; 20: 35-43.
4. SIMON LS, WEAVER AL, GRAHAM DY *et al.*: Anti-inflammatory and upper gastrointestinal effects of celecoxib in rheumatoid arthritis: a randomized controlled trial. *Jama* 1999; 282: 1921-8.
5. MARICIC N, EHRLICH K, GRETZER B, SCHULIGOI R, RESPONDEK M, PESKAR BM: Selective cyclo-oxygenase-2 inhibitors aggravate ischaemia-reperfusion injury in the rat stomach. *Br J Pharmacol* 1999; 128: 1659-66.
6. CUZZOCREA S, MAZZON E, SERRAINO I *et al.*: Celecoxib, a selective cyclo-oxygenase-2 inhibitor reduces the severity of experimental colitis induced by dinitrobenzene sulfonic acid in rats. *Eur J Pharmacol* 2001; 431: 91-102.
7. KROMBACH F: Molekulare Mechanismen der Leukozyten-Endothelzell Interaktion. *Intensivmed* 1996; 33: 375-85.
8. BISHOP-BAILEY D, BURKE-GAFFNEY A, HELLEWELL PG, PEPPER JR, MITCHELL JA: Cyclo-oxygenase-2 regulates inducible ICAM-1 and VCAM-1 expression in human vascular smooth muscle cells. *Biochem Biophys Res Commun* 1998; 249: 44-7.

9. SANO H, HLA T, MAIER JA *et al.*: In vivo cyclooxygenase expression in synovial tissues of patients with rheumatoid arthritis and osteoarthritis and rats with adjuvant and streptococcal cell wall arthritis. *J Clin Invest* 1992; 89: 97-108.
10. VEIHELMANN A, HARRIS AG, KROMBACH F, SCHUTZE E, REFIOR HJ, MESSMER K: In vivo assessment of synovial microcirculation and leukocyte-endothelial cell interaction in mouse antigen-induced arthritis. *Microcircu - lation* 1999; 6: 281-90.
11. BRACKERTZ D, MITCHELL GF, MACKAY IR: Antigen-induced arthritis in mice. I. Induction of arthritis in various strains of mice. *Arthritis Rheum* 1977; 20: 841-50.
12. VEIHELMANN A, SZCZESNY G, NOLTE D, KROMBACH F, REFIOR HJ, MESSMER K: A novel model for the study of synovial microcirculation in the mouse knee joint in vivo. *Res Exp Med* (Berl) 1998; 198: 43-54.
13. HARRIS AG, HECHT R, PEER F, NOLTE D, MESSMER K: An improved intravital microscopy system. *Int J Microcirc Clin Exp* 1997; 17: 322-7.
14. ZEINTLH, SACK FU, INTAGLIETTA M, MESSMER K: Computer assisted leukocyte adhesion measurement in intravital microscopy. *Int J Microcirc Clin Exp* 1989; 8: 293-302.
15. MUSCARA MN, VERGNOLLE N, LOVREN F *et al.*: Selective cyclo-oxygenase-2 inhibition with celecoxib elevates blood pressure and promotes leukocyte adherence. *Br J Pharma - col* 2000;129: 1423-30.
16. HARRIS A, SCHROPP A, MESSMER K: Effects of oxaceprol on the microcirculation in ischemia/reperfusion injury. *Eur J Med Res* 1998; 3: 182-8.
17. GEARING AJ, NEWMAN W: Circulating adhesion molecules in disease. *Immunol Today* 1993; 14: 506-12.
18. COMINACINI L, PASINI A, GARBIN U *et al.*: Zofenopril inhibits the expression of adhesion molecules on endothelial cells by reducing reactive oxygen species. *Am J Hypertens* 2002; 15: 891-5.
19. NEEDLEMAN P, ISAKSON PC: The discovery and function of COX-2. *J Rheumatol* 1997; 24 Suppl 49: 6-8.
20. MIGITA K, YAMASAKI S, IDA H *et al.*: The role of peroxynitrite in cyclooxygenase-2 expression of rheumatoid synovium. *Clin Exp Rheumatol* 2002; 20: 59-62
21. NAKAMURA Y, KOZUKA M, NANIWA K *et al.*: Arachidonic acid cascade inhibitors modulate phorbol ester-induced oxidative stress in female ICR mouse skin: differential roles of 5-lipoxygenase and cyclooxygenase-2 in leukocyte infiltration and activation. F*ree Radic Biol Med* 2003; 35: 997-1007.
22. HOCHBERG MC: New directions in symptomatic therapy for patients with osteoarthritis and rheumatoid arthritis. *Semin Arthritis Rheum* 2002; 32: 4-14.
23. WILGUS TA, ROSS MS, PARRETT ML, OBERYSZYN TM: Topical application of a selective cyclooxygenase inhibitor suppresses UVB mediated cutaneous inflammation. *Prostaglandins Other Lipid Mediat* 2000; 62: 367-84.
24. ANDERSON GD, HAUSER SD, MCGARITY KL, BREMER ME, ISAKSON PC, GREGORY SA: Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. *J Clin Invest* 1996; 97: 2672-9.
25. OCHI T, OHKUBO Y, MUTOH S: Role of cyclooxygenase-2, but not cyclooxygenase-1, on type II collagen-induced arthritis in DBA/1J mice. *Biochem Pharmacol* 2003; 66: 1055-60.