Immunomodulatory effect of NSAID in geriatric patients with acute infection: effects of piroxicam on chemokine/cytokine secretion patterns and levels of heat shock proteins. A double-blind randomized controlled trial. (ISRCTN58517443)

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Abstract Inflammation in older persons is associated with frailty, cachexia, and disability. We hypothesized that NSAID treatment in addition to antibiotics in older patients with acute infection might rapidly reduce inflammatory cytokines and might be of therapeutic potential to improve outcomes. A double-blind controlled trial was conducted in geriatric patients admitted for acute infection. Patients were randomized to receive either 10 mg piroxicam or placebo. Patients ≥ 70 years with CRP levels >10 mg/L of acute infectious origin were eligible. Twenty-five cyto-/chemokines as well as heat shock proteins Hsp27 (HSPB1) and Hsp70 (HSPA1A) were measured the first 4 days and then weekly until discharge, with a maximum of 3 weeks. Thirty

Caucasian patients were included (median age 84.5 years, 67% female, median CRP 87.5 mg/L). In the piroxicam group, IL-6 and IP-10/CXCL10 decreased significantly during the study period. Relationships between cytokines were disrupted in the piroxicam group: for 12 out of 20 cytokines the number of correlations between changes in serum levels was significantly lower compared to placebo. Serum Hsp70 levels decreased significantly in the piroxicam group, but not in the placebo group. Without heat challenge, intracellular levels of Hsp70 in monocytes decreased in both groups, whereas Hsp27 in monocytes increased with piroxicam with a significant difference compared to placebo at 3 weeks. Piroxicam in this setting cannot be considered merely as an anti-inflammatory drug, but rather as an immunomodulator. Further studies are needed to establish whether these effects can change functional outcomes in geriatric patients.

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Introduction

The physiological of inflammation is complex and includes host defense, tissue repair and maintenance, or restoration of homeostasis. The pathological potential of inflammation, however, is important (Medzhitov 2008). Small but persistent increases in pro-inflammatory cytokines, especially interleukin (IL)-6, in older persons have been associated with disability and frailty (Ershler and Keller 2000). Acute infection-induced inflammation contributes to

cachexia and functional decline in older hospitalized patients (El Solh et al. 2006). We and others have previously shown that elevated levels of inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor α (TNF- α) are associated with poor physical function in older persons with (Morley et al. 2006; Bautmans et al. 2005) or without (Bautmans et al. 2008; Schaap et al. 2006) acute disease.

Endogenous down-regulation of inflammation is achieved mainly by IL-10, IL-1 receptor antagonist (IL-1RA), and by glucocorticosteroids. Another ubiquitous reaction to inflammation consists of increased heat shock protein (Hsp) expression, acting as chaperones protecting intracellular proteins (Stryer 1995). Our group has demonstrated higher baseline (Njemini et al. 2007b) levels of some intracellular Hsps in healthy older subjects compared to young ones, but less increase after heat challenge (Njemini et al. 2002, 2003, 2008). Intracellular (Njemini et al. 2003) and circulating (Njemini et al. 2004, 2011) Hsp70 concentrations are related to IL-6 and TNF- α , both in geriatric patients and normal elderly persons.

Pharmacological intervention with non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to influence pro-inflammatory cytokines. One week of piroxicam treatment decreased IL-1, IL-6, TNF- α , and interferon gamma (IFN γ) in healthy individuals (Rosenstein et al. 1994). Intravenous lornoxicam, however, had no effect on these cytokines in sepsis patients (Memis et al. 2004).

In the present placebo-controlled RCT, we investigated the effect of piroxicam on a large set of circulating cytokines and chemokines, and on intra- and extra-cellular Hsps in hospitalized geriatric patients with acute infection. We hypothesized that NSAID treatment would reduce infection-induced inflammation faster than antibiotics alone. We further aimed at documenting the interrelationship between inflammation-related mediators with respect to the study intervention.

Methods

Participants

The study protocol was approved by the IRB and all patients (or proxy) gave written informed consent. Patients aged ≥ 70 years, consecutively admitted to an acute geriatric ward of a general teaching hospital for acute infection (documented by C-reactive protein (CRP) serum level >10 mg/L and/or fibrinogen >400 mg/dL) were eligible. Patients presenting inflammation of non-infectious origin, using corticosteroids or NSAID within the past 7 days, or showing contraindication to treatment were excluded. The use of inhalation corticosteroids, low-dose aspirin (as anti-aggregating medication)

and paracetamol was allowed. Patients were included within 72 h after admission.

Intervention and treatment

Patients were randomized, using numbered containers, to receive piroxicam 10 mg, or placebo daily for 10 days. All patients, except those already using proton pump inhibitors, also received oral ranitidine 150 mg daily to avoid gastrointestinal side effects. The medical staff was blinded to treatment allocation and patients received standard care. Patients were evaluated until discharge with a maximum of 21 days after treatment allocation.

Measurements

Primary outcomes were changes in levels of cyto-/chemokines and Hsps. Secondary outcomes were overall clinical evolution and length of stay. Explanatory variables included comorbidity and general patient parameters such as age, gender, height, weight, and body temperature, as well as basal activities of daily living (bADL; modified scale according to Katz et al. 1963), instrumental ADL (iADL; 7-point Lawton scale) (Lawton and Brody 1969) and complete biochemical and hematological profile, including renal function (Cockcroft and Gault formula; Cockcroft and Gault 1976). Safety outcomes were evolution of renal function and occurrence of adverse events.

Samples were taken at baseline, after 1, 2, and 3 days, and after 1, 2, and 3 weeks. Serum was stored at -20°C until determination. For technical reasons intracellular Hsps were not determined on day 3.

Cytokine determinations

Serum levels of 25 different cyto-/chemokines were measured simultaneously by multiplex bead immunoassay (Human Cytokine Twenty-Five-Plex, Biosource International, Nijvel, Belgium) according to the manufacturer's indications, including: IL-1 β , IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-17, CCL2/(MCP-1), CCL3/(MIP-1 α), CCL4/(MIP-1 β), CCL5/(RANTES), CCL11/(eotaxin), CXCL8/(IL-8), CXCL9/(MIG), CXCL10/(IP-10), TNF α , IFN- α , IFN- γ , and GM-CSF (for full names see legend of Table 2).

Sandwich ELISA for Hsp determination in serum

Hsp27 and Hsp70 in serum were detected using commercial enzyme-linked immunosorbent assay (ELISA) kits (StressGen, Victoria, Canada). All reagents, dilutions, and calculations were applied according to the manufacturer's instructions. The standard curve ranged

from 0 to 12.5 ng/mL (sensitivity of 0.09 ng/ml) for Hsp70 and from 0 to 25 ng/ml (sensitivity 0.39 ng/ml) for Hsp27.

Flow cytometric determination of intracellular Hsp27 and Hsp70

Hsp27 and Hsp70 were quantified in peripheral blood cells using flow cytometry as previously reported (Njemini et al. 2007b). Briefly, cells were incubated with anti-CD14 antibody (Becton Dickinson, San Jose, CA) for 15 min, washed in PBS containing 1% BSA, and fixed in reagent A (IMTEC Diagnostics, Antwerpen, Belgium) for 15 min. After washing, the cells were permeabilized with reagent B (IMTEC Diagnostics) and at the same time incubated with the primary Hsp-specific antibody (clone G3.1, SPA-800FI for Hsp27, and clone C92F3A-5, SPA-810 for Hsp70; StressGen, Victoria, Canada). In negative controls, an isotype-matched goat IgG (Becton Dickinson, San Jose, CA) was used as the primary antibody. After 15-min incubation, cells were washed, and 500 µl of facsflow solution (Becton Dickinson, San Jose, CA) were added in the case of Hsp27. For Hsp70, the labeled cells were further re-suspended in reagent B and the secondary antibody (goat anti-mouse IgG, IMTEC Diagnostics), and incubated for 15 min. Cells were washed and 500 µl of Facsflow solution were added. The samples were analyzed immediately or within a few hours (stored at 4°C) using the flow cytometer Epics-XL-MLC.

Heat shock procedures

Intracellular Hsp levels were determined both with heat challenge (HC), and without (WHC), as previously described (Njemini et al. 2002). Briefly, for heat challenge, petri dishes containing the cells were heated at 42°C for 1 h. After heat challenge, adherent cells were allowed to recover for 16 h at 37°C in a 5% CO₂ incubator. Cells were then detached, washed, counted, and their viability assessed. At least 85% of the cells survived after exposure to 42°C for 1 h.

Statistical analysis

Analyses were done using IBM SPSS statistics 19 (SPSS Inc, Illinois, USA). Data are reported as median±interquartile range. As cytokines were not normally distributed non-parametric procedures (with exact testing) were used: Spearman's rho for correlations, Mann Whitney *U* test for unpaired comparisons, Friedman test for overall time effect, and Wilcoxon's signed-rank test for changes relative to baseline and preceding measurements. Differences in the patterns of relationships between changes in cytokines during hospitalization were analyzed using McNemar

test. Differences in proportions were analyzed using Pearson Chi-square test. Significance was set a priori at two-sided $p < 0.05$.

Results

Thirty patients aged 70 to 94 years (median 84.5 years IQR 78.0–88.3) were included (see Fig. 1). As shown in Tables 1 and 2, the baseline characteristics were similar for both groups. Patients were predominantly female, highly dependent in bADL and iADL, a majority was malnourished, had a moderately reduced renal function and showed multiple comorbidity. At admission, 13 patients (seven in the placebo and six in the piroxicam group ($p = 0.713$)) presented with a body temperature above 37.5°C, and none had a body temperature above 39°C.

Throughout the study, IL-5, IL-13, IL-17, and IFN γ levels remained below the detection limit in more than 70% of the patients and RANTES remained above the upper detection limit in both groups. Further analyses thus concerned the remaining 20 cyto-/chemokines.

At baseline

All patients presented with important inflammation as documented by CRP (median 87.5 mg/L; 40.5–156.3) and fibrinogen (median 594 mg/L; 435–700) serum levels. None of the 20 cyto-/chemokines was correlated to CRP, whereas IL-7 was positively correlated to fibrinogen ($p = 0.042$; $r = 0.437$). No significant correlation existed between serum or intracellular Hsps and CRP or fibrinogen levels. A high number of (exclusively positive) significant correlations between cyto-/chemokines was observed (Fig. 2). Baseline correlations between cytokines and Hsps are shown in Table 3. No correlation was observed between body temperature and intracellular or circulating levels of Hsp27 and Hsp70, and levels of Hsps were comparable between patients with and without fever.

Follow-up

CRP decreased significantly over time ($p < 0.001$) in both groups. MIP-1 α /CCL3 and MIP-1 β /CCL4 in the placebo group and IL-6 and IP-10/CXCL10 in the piroxicam group decreased significantly ($p < 0.05$) over the study period. For these cytokines, changes at each time point compared to baseline and to preceding measurements are indicated in Table 4. No significant change was observed for serum Hsp27, whereas serum Hsp70 decreased significantly in the piroxicam group ($p < 0.05$). Intracellular Hsp27 and Hsp70 after HC in both cell types and WHC in lymphocytes did not change over time. In monocytes WHC, Hsp70

Fig. 1 Flow chart. Numbers of patients at randomization, treatment allocation, and each time point during follow-up

decreased significantly in both groups ($p < 0.05$), whereas Hsp27 significantly increased over time with piroxicam, but not with placebo. When changes referring to baseline were

compared between the treatment groups, only the increase in Hsp27 in monocytes WHC at week 3 was significantly different ($p < 0.05$).

Table 1 Participants' clinical and standard biological baseline characteristics

	Placebo $N=15$	Piroxicam $N=15$
Age, years	81.0 (78.0–86.0)	85 (78.0–91.0)
Gender, female, %	73%	60%
BMI, kg/m ²	23.5 (18.7–26.2)	26.2 (20.1–28.2)
Proportion BMI < 18.5	20.0%	14.3%
Proportion BMI > 25	33.3%	57.1%
bADL, 24-point Katz	19.0 (11.0–20.0)	12.0 (10.3–19.3)
iADL, 7-point Lawton	1.5 (0.3–3.5)	2 (1.0–4.0)
CRP, mg/L	92.0 (41.0–155.0)	76.0 (31.0–235.0)
Proportion CRP > 100 mg/L	33.3%	35.7%
Fibrinogen, mg/dL	630.0 (452.5–735.0)	569.5 (430.0–700.0)
Hb, g/dL	12.7 (11.9–13.7)	12.5 (10.7–13.7)
Prealbumin, g/dL	0.14 (0.07–0.17)	0.12 (0.09–0.18)
Proportion prealbumin < 20 g/dL	86.7%	90.9%
GFR, mL/min	56.7 (39.1–65.5)	49.3 (31.9–56.7)
Type of infection and comorbidity		
Respiratory, number	9 (60.0%)	6 (40.0%)
Urinary, number	1 (6.7%)	4 (26.7%)
Gastrointestinal, number	3 (20.0%)	2 (13.3%)
Other, number	2 (13.3%)	3 (20.0%)
Number of diagnoses	7.0 (5.0–10.0)	7.5 (5.75–11.0)

Data represent median values (interquartile range) for continuous variables and as numbers (percentage) for categorical variables. There was no statistically significant difference between groups.

BMI body mass index, bADL basic activities of daily living, iADL instrumental activities of daily living, CRP C-reactive protein, Hb hemoglobin, GFR glomerular filtration rate calculated with the Cockcroft and Gault formula

Table 2 Baseline levels of cytokines and heat shock proteins

		Placebo <i>N</i> =15	Piroxicam <i>N</i> =15
<p>Data represent median values (interquartile range). There was no statistically significant difference between groups.</p> <p>For chemokines old and new names are indicated, separated by a forward slash.</p> <p><i>GM-CSF</i> granulocyte macrophage colony-stimulating factor, <i>IL</i> interleukin, <i>IL-1RA</i> IL-1 receptor antagonist, <i>TNF-alpha</i> tumor necrosis factor alpha, <i>IFN</i> interferon, <i>IL-2R</i> IL-2 receptor, <i>IP-10</i> interferon γ-inducible protein 10 <i>MCP</i> monocyte chemoattractant protein, <i>MIG</i> monokine induced by interferon gamma, <i>MIP</i> macrophage inflammatory protein, <i>RANTES</i> Regulated upon Activation, Normal T-cell Expressed, and Secreted, <i>Hsp</i> heat shock protein(s), <i>MFI</i> mean fluorescence intensity, <i>WHC</i> without heat challenge, <i>42°C</i> after heat challenge, <i>LO</i> below lower detection limit, <i>HI</i> above upper detection limit</p>	Cytokines in pg/mL		
	IL-1beta	31.5 (22.0–164.0)	14.9 (14.9–54.0)
	IL-1RA	212.0 (135.3–533.5)	366.5 (199.8–605.5)
	IL-2	14.9 (14.9–49.0)	14.9 (14.9–14.9)
	IL-2R	237.0 (145.5–342.3)	410.0 (210.0–594.0)
	IL-4	4.9 (4.9–16.3)	4.9 (4.9–10.3)
	IL-5	LO	LO
	IL-6	24.0 (14.8–78.3)	17.0 (4.9–138.0)
	IL-7	24.9 (24.9–28.7)	24.9 (24.9–38.0)
	IL-10	5.5 (3.0–25.5)	4.5 (3.0–6.0)
	IL-12	150.5 (105.5–232.0)	142.0 (118.0–268.0)
	IL-13	LO	LO
	IL-15	39.5 (24.9–175.3)	25.5 (24.9–53.0)
	IL-17	LO	LO
	GM-CSF	4.9 (4.9–44.8)	4.9 (4.9–6.4)
	TNF-alpha	4.9 (4.9–9.5)	5.0 (4.9–8.0)
	IFN-alpha	38.0 (25.7–61.3)	41.0 (24.9–54.0)
	IFN-gamma	LO	LO
	MCP-1/ CCL2	767.5 (571.3–1101.3)	706.5 (410.5–1003.0)
	MIP-1alpha/ CCL3	45.5 (24.7–125.5)	51.5 (30.5–69.5)
	MIP-1beta/ CCL4	127.0 (66.5–197.8)	111.0 (57.0–212.3)
	RANTES/ CCL5	HI	HI
	Eotaxin/ CCL11	85.5 (67.3–160.5)	74.0 (60.0–120.0)
	IL-8/ CXCL8	134.5 (70.0–417.8)	66.5 (54.3–360.0)
	MIG/ CXCL9	79.5 (61.0–205.3)	139.5 (90.5–214.3)
	IP-10/ CXCL10	44.5 (32.0–81.0)	61.0 (46.5–132.0)
	Serum Hsp, ng/mL		
	Hsp27	52.4 (15.5–69.9)	22.6 (16.0–48.6)
	Hsp70	1.3 (0.6–2.1)	1.4 (0.9–1.6)
	Intracellular Hsp, MFI		
	Hsp27		
	Lymphocytes	WHC	
		42°C	
	Monocytes	WHC	
		42°C	
	Hsp70		
	Lymphocytes	WHC	
		42°C	
	Monocytes	WHC	
		42°C	

Correlations between changes referring to baseline were computed at each time point. Relationships between cyto-/chemokines were disrupted in the piroxicam group with a significant loss of positive correlations for 12 out of 20 cytokines, and a significantly increased number for IL-8 compared to the control group (Fig. 3). Correlations between changes to baseline between Hsps, and between

Hsps and cyto-/chemokines showed no consistent patterns (data not shown).

No difference was observed for fever patterns between treatment groups. No significant change in renal function was observed in the piroxicam group. One patient on ranitidine in the piroxicam group presented with a gastrointestinal bleeding and was withdrawn from the study

[illegible]

Heat shock proteins	Cytokines									
	Serum Hsp27	Serum Hsp70	Hsp 27 lymphocytes WHC	Hsp 27 monocytes WHC	Hsp 27 lymphocytes 42°	Hsp 27 monocytes 42°	Hsp 70 lymphocytes WHC	Hsp 70 monocytes WHC	Hsp 70 lymphocytes 42°	Hsp 70 monocytes 42°
IL-1beta										
IL-1RA										
IL-2										
IL-2R										
IL-4										
IL-6										
IL-7										
IL-10										
IL-12										
IL-15										
GM-CSF										
TNF-alpha										
IFN-alpha										
MCP-1/ CCL2										
MIP-1alpha/ CCL3										
MIP-1beta/ CCL4										
Eotaxin/ CCL11										
IL-8/ CXCL8										
MIG/ CXCL9										
IP-10/ CXCL10										

Positive correlation $p < 0.05$ Positive correlation $0.05 < p < 0.095$ Negative correlation $p < 0.05$ Negative correlation $0.05 < p < 0.095$

Table 4 evolutions of significantly changing cytokines and heat shock proteins (Hsp) according to treatment group

		Day 1	Day 2	Day 3	Week 1	Week 2	Week 3
Cytokine							
MIP-1 α	Placebo*						↓
	Piroxicam		↓				
MIP-1 β	Placebo*				↓	↓	↓
	Piroxicam			↓	↓	↓	
IL-6	Placebo						
	Piroxicam*				↓		
IP-10	Placebo						
	Piroxicam*		↓	↓			
Serum Hsp							
Hsp70	Placebo						↓
	Piroxicam*				↓		
Cellular Hsp							
Hsp70 WBC monocytes	Placebo*						
	Piroxicam*		↓				
Hsp27 WBC monocytes†	Placebo					↑	
	Piroxicam*				↑		↑

*=significant change over 3 weeks study period (Friedman test $p<0.05$).

†=significant between groups difference at 3 weeks (Mann Whitney U test $p<0.05$).

Indicated are results of Wilcoxon signed rank tests as follows:

↓	Decreased compared to previous measurement; Wilcoxon signed rank tests $p<0.05$
↓	Decreased compared to previous measurement; Wilcoxon signed rank tests $0.05<p<0.09$
↑	Increased compared to previous measurement; Wilcoxon signed rank tests $p<0.05$
↑	Increased compared to previous measurement; Wilcoxon signed rank tests $0.05<p<0.09$
	Decreased compared to baseline; Wilcoxon signed rank tests $p<0.05$
	Decreased compared to baseline; Wilcoxon signed rank tests $0.05<p<0.095$
	Increased compared to baseline; Wilcoxon signed rank tests $p<0.05$
	Increased compared to baseline; Wilcoxon signed rank tests $0.05<p<0.095$

and treated accordingly. There was a tendency for better overall clinical evolution ($p=0.065$) and for a higher incidence in side effects ($p=0.079$) in the piroxicam group, but no difference in mortality or length of stay (see Table 5).

Discussion

The present study included very frail geriatric patients with serious inflammation of acute infectious origin. We aimed at examining the evolution of cyto-/chemokines and Hsps to evaluate whether an NSAID treatment attenuates acute infection-induced inflammation. Previously, we observed beneficial effects of treatment with celecoxib, a selective COX-2 inhibitor, in a similar group of patients (Mets et al. 2004). Here, we chose for piroxicam, a non-selective COX inhibitor in clinical use since many years, following the European Medicines Agency's press office warning (Doc. Ref. EMEA/117908/2004) about the use of COX-2 inhibitors.

At baseline, with the exception of a positive correlation between fibrinogen and IL-7, we could not find significant relationships of CRP or fibrinogen with inflammatory cytokines or Hsps. CRP significantly decreased throughout the study period in both groups. Contrary to our expectations, NSAID treatment did not decrease most inflammatory cyto-/chemokines and the significant decreases of IL-6 and IP-10/CXCL10 observed in the piroxicam group were not significantly different from the placebo group. However, a loss of correlations between changes in various cyto-/chemokines during hospitalization was noticed in the piroxicam group (Fig. 3). This might indicate that the NSAID treatment disrupts the interaction between inflammatory mediators, which are known to have a high degree of cross talk and redundancy. Previous observations concerning the effects of NSAID treatment on cytokines have shown conflicting results. Rosenstein et al. (1994) reported that 1 week of piroxicam treatment in healthy human volunteers decreased IL-1, IL-6, TNF α , and IFN γ in anti-CD3-stimulated peripheral blood monocytes, increased IL-2, and had no consistent effect on IL-4. Teeling et al. (2010) found no

Fig. 3 Correlations between changes in cytokine levels. For each time point Spearman's Rho correlations between changes (relative to baseline) in cytokine levels with a p value < 0.05 are indicated by red (positive) or blue bars (negative correlations). *Left panel* represents placebo and *right panel* piroxicam group. *Differences

(compared to placebo) in the pattern of relationships between changes in cytokines (McNemar test). A significant loss of positive correlations is observed for 12 out of 20 cytokines in the piroxicam group compared to controls

significant effect on lipopolysaccharide (LPS)-induced circulating IL-1 β , IL-6, TNF α after pretreatment with various NSAID in mice. These authors even observed a tendency towards increased serum levels of TNF α . Others reported increased TNF α in LPS-activated macrophages following 6-h incubation with piroxicam (Cho 2007).

NSAID appear to have immunomodulatory effects, independent from COX inhibition (Cho 2007). Anti-inflammatory effects of NSAID may be due to COX-inhibition and COX-independent mechanisms such as

inhibition of NF- κ B (Okamura et al. 2008). Therefore, our negative results may reflect the redundancy of the multiple mechanisms influencing cytokine production and might support the recent view of NSAID as immunomodulators, rather than pure COX inhibitors. Even though the high number of pairwise correlations computed in the present study bears a risk of a type-1 error, numerous persistent relationships between changes in cytokine levels were apparent over multiple time points: changes in some cyto-/chemokines like IL-1 β , IL-2, IL-15, MIP-1 α , and

Table 5 Clinical evolution

	Placebo	Piroxicam	<i>p</i> value
Clinical evolution (number of patients)			
Return to premorbid clinical status	6	11	0.065 ^a
Persistent decrease in clinical status	9	4	
Side effects (attributable to NSAID)			
None	14	9	0.079 ^a
Possible	1	3	
Probable	0	3	
Length of stay (days; median, range)	24.5 (19–64)	29.0 (9–75)	0.868 ^b
Death	1 (7.1%)	2 (14.3%)	0.543 ^a

^a Pearson Chi-square test

^b Mann Whitney U test

TNF α remained closely correlated in both groups. Significant relationships between changes in IFN α and MIP-1 α , IFN α and MIG, as well as eotaxin and IL-7 were observed exclusively in the placebo group, whereas relationships between changes in IL-6 and IL-7 as well as IL-8 and MIP-1 α appeared exclusively in the piroxicam group. These findings suggest that piroxicam interferes with the coordinated production of cytokines by immune cells and might reflect the above mentioned immunomodulatory effects of NSAID.

Besides IL-6 there was a significant decrease over time in IP-10/CXCL10 in the piroxicam group. CXCL10 is the prototype of the CXC chemokine superfamily (Antonelli et al. 2006), and a strong chemoattractant for Th1 lymphocytes and NK cells. It is a potent pro-inflammatory cytokine that increases with age (Antonelli et al. 2006) and its *ex vivo* monocytic expression has recently been correlated to IL-6 serum levels in frail community-dwelling older adults in contrast to matched controls (Qu et al. 2009). Furthermore, increased expression of CXCL10 has been described in various infectious diseases and might become a therapeutic target (Liu et al. 2011). Interestingly, in our patients CXCL10 significantly decreased with piroxicam, but not with placebo, and this decrease was independent of the changes in IL-6.

Immunosenescence has been associated with skewed polarization of T-helper function towards either Th1 (Sakata-Kaneko et al. 2000), Th2 (Ginaldi et al. 1999) or Th17 (Haynes and Maue 2009). In our patients, serum levels of IP-10/CXCL10, known to induce Th1 polarization (Kawaguchi et al. 2007), were high, but levels of cytokines produced by Th1, such as IFN- γ and IL-2, were undetectable or low. Th2 polarization is induced by MCP-1/CCL2, a chemokine mostly produced by macrophages, whose levels have been described to increase with aging (Antonelli et al. 2006). In our study, levels of MCP-1 were very high at baseline and throughout the hospitalization period. However, IL-4 and IL-5, reflecting Th2 polarization, were low or undetectable in our patients. The levels of IL-1 β , known to induce Th17 (Miossec et al. 2009), were only moderately increased and IL-17 was mostly undetectable, arguing against Th17 polarization.

As we included patients with bacterial infections and high neutrophil counts, we expected to find high levels of IL-8/CXCL8 (neutrophil-activating protein-1), GM-CSF and MIP-1 α (also known to activate neutrophils). GM-CSF levels were low in most of our patients. IL-8 was the only cytokine not related to any other at baseline, whereas relationships between changes in IL-8 and MIP-1 α exclusively appeared in the piroxicam group during the first 3 days of treatment. In contrast, MIP-1 α decreased significantly over time in the placebo group, but not in the piroxicam group. This differential evolution is surpris-

ing as MIP-1 α is known to be resistant to COX inhibition (Cruse and Lewis 2010) and might point to a COX-independent influence of piroxicam on the pathways of neutrophil chemoattraction.

Cellular Hsp production was comparable to previously observed levels in older patients with infection (Njemini et al. 2007a). We observed exclusively negative correlations between Hsp70 expression in peripheral blood mononuclear cells (PBMC) and several cytokines. Inflammatory cytokines have been shown to induce Hsp production. It has recently been suggested however, that Hsp70 may attenuate the expression of cyto-/chemokines and the expression of NF- κ B-dependent genes (Okamura et al. 2008). These seemingly contradictory observations might indicate a negative feedback loop between infection-induced inflammatory cytokines and subsequent production of Hsps down-regulating cytokine expression. Consequently, relationships between Hsps and cytokines might inverse, depending on the timing of the analyses with respect to the evolution of infection-induced inflammation. Our patients have been included within 72 h after admission for severe infection. During the first days and thereafter, CRP serum levels decreased significantly in both groups, suggesting that the inflammatory process was already full blown at the time of randomization. Hsp levels in PBMC were high and inflammatory cytokines did not increase any further during the study period. Thus the effect of piroxicam may not have been evident as the inflammatory process had already reached a state where Hsp production was maximal and possibly a negative feedback loop on cytokine production was sufficient even in the placebo-treated patients. These Hsp levels correspond to our results of production without heat challenge. The increase of Hsp27 in monocytes WHC with piroxicam, in opposition with the decrease in Hsp70 in both groups, was surprising, but our previous work suggests differential regulation of Hsp70 and Hsp27 (Njemini et al. 2007b) and it has been described that NSAID treatment may partially induce the human heat shock response (Tegeder et al. 2001). Heat challenge-induced levels of Hsps (which is a non-physiological condition) did not change over time in our patients.

Body temperature at admission was below 39°C in all patients and showed no relationship with circulating or intracellular levels of Hsps. Since NSAIDs are antipyretics and are known to lower the threshold for Hsp induction (Lee et al. 1995), we compared the fever pattern in both treatment groups, but found no differences. However, it has to be noted that administration of paracetamol (acetaminophen) was allowed in the study protocol, making the further evaluation of body temperature unreliable during hospitalization. Although differences have been described for Hsp27 and Hsp70 with regard to their reaction on fever (Brameshuber et al.

2010), the levels of the two Hsps were comparable in patients with and without fever.

A limitation of this study is the relatively small sample size, despite an inclusion period of 12 months. Even though exclusion criteria have been minimized (Ferrucci et al. 2004), many older patients were not eligible, because of safety reasons, or recent anti-inflammatory treatment. Furthermore, in order to avoid gastrointestinal side effects, ranitidine was added to the study medication or proton pump inhibitors (PPIs) were continued in the patients already receiving them. PPIs have been shown to raise Hsps in the gut in response to NSAIDs (Yeo et al. 2006). However, only four patients in each treatment group were on PPIs and it seems unlikely that this influenced our results. Another limitation is the still limited range of cyto-/chemokines analyzed. As cyto-/chemokines generally have various tissue sources and have effects on multiple target cells (Cruse and Lewis 2010), the cyto-/chemokine network is highly complex and redundancy limits the interpretation of individual levels. Nevertheless, following the evolution of the acute phase response by simultaneously assaying a large set of cyto-/chemokines, and cellular and circulating Hsps are unprecedented in this patient population.

Conclusion

We can conclude that piroxicam significantly decreases serum levels of IL-6, IP-10/CXCL10, and Hsp70, as well as the basal (without heat challenge) production of Hsp70 by monocytes in hospitalized geriatric patients with acute infection, while increasing basal Hsp27 in monocytes. However, only the increase in basal Hsp27 in monocytes was statistically different from placebo, whereas the observed decreases were not. Relationships between changes in cytokine levels were disrupted in the piroxicam group, suggesting an immunomodulatory effect rather than individual suppression of specific cytokines.

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Conflict of interest I Beyer and T Mets have been investigators in clinical trials sponsored by Pfizer. The present study was an investigator initiated trial receiving no sponsoring and the study medication was produced by the hospital's pharmacy.

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