Abnormal eicosanoid pattern by blood leukocytes in gastroduodenal ulcer

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Summary

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are implicated in several diseases showing altered tissue and leukocyte eicosanoid patterns, such as nasal polyps and asthma. NSAIDs are also associated with gastrointestinal lesions, but it is unknown whether there is an altered eicosanoid pattern.

Material/Methods: The ex vivo modulated syntheses of prostaglandin E2 (PGE2) and peptido-leukotrienes (pLT) by leukocytes from 41 patients with gastroduodenal ulcer were compared with those of 61 healthy controls. Samples were incubated with diluent, arachidonic acid, or acetylsalicylic acid. The individual syntheses of PGE2 and pLT were quantified using competitive enzyme-immuno-assays followed by calculation of individual eicosanoid patterns.

Results: Controls synthesized ~4.9-fold whereas patients only ~2.9-fold more PGE2 than pLT due to higher basal synthesis of pLT (67 and 125 pg/ml, respectively). The baseline PGE2/pLT ratio was slightly higher in patients (6.1) than in controls (5.7). The arachidonic acid-induced PGE2/pLT ratio in patients (14.2) was significantly higher than in controls (3.3). The acetylsalicylic acid-induced PGE2/pLT ratio in patients (3.5) was significantly lower than in controls (8.3) due to diminished PGE2 and elevated pLT. Integrated individual PGE2 and pLT values revealed a highly significantly altered eicosanoid pattern score in ~95% patients and ~12% controls.

Conclusions: There is strong evidence of an altered eicosanoid pattern generated by leukocytes of gastroduodenal ulcer patients, which became obvious upon in vitro modulation by arachidonic or acetylsalicylic acid. The phenomenon of an abnormal eicosanoid pattern in gastroduodenal ulcer is yet not fully understood, but may have implications in pathophysiology and diagnostics.

key words: eicosanoids • prostaglandins • leukotrienes • gastroduodenal ulcer • blood leukocytes


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**Background**

Eicosanoids such as prostaglandins and leukotrienes comprise a class of chemically related compounds derived from 20-carbon fatty-acids, including arachidonic acid [1]. Prostaglandin E (PGE), a major metabolite of the cyclooxygenase (COX) pathway, has multiple effects on the suppression of cytokine production, immune response, nitrogen responses, and cytotoxicity [2–4]. Peptido-leukotrienes (pLT, i.e. LTC, LTD, and LTE) are highly potent metabolites of the 5-lipoxygenase pathway. Both metabolites are involved in inflammatory diseases [5].

Inhibition of COX changes the eicosanoid pattern of tissue as well as of blood leukocytes, defined by the spontaneous and modified generation of PGE and pLT [6–9]. There is a significant difference between individuals intolerant to NSAIDs and healthy subjects [6–11]. NSAIDs also cause gastroduodenal ulcer [12,13], but it is unknown whether there is an association with an altered/abnormal eicosanoid pattern. Therefore, the generation of PGE and pLT by blood leukocytes of patients suffering from gastroduodenal ulcer compared with healthy individuals was examined. This was done using either arachidonic acid or acetylsalicylic acid for the ex vivo modulation of blood leukocytes and comparing the results with the baseline generation of PGE and pLT. The individual data were integrated to evaluate the individual eicosanoid pattern.

**Material and Methods**

Subjects studied

Forty-one patients (18 females, mean age 62.1.5 years, and 22 males, mean age 53.6 years) suffering from gastroduodenal ulcer verified by endoscopy and histology of biopsy-specimens were investigated. Only patients taking no drugs interfering with eicosanoid metabolism (e.g. corticosteroids, NSAIDs, leukotriene antagonists) were included.

Sixty-one healthy individuals (28 females, mean age 49.3 years, and 33 males, mean age 47.2 years) without history and symptoms of gastrointestinal ulcer or intolerance to NSAIDs served as controls. Healthy individuals had had no previous gastrointestinal intervention and no treatment interfering with eicosanoid metabolism. No regular medication other than oral contraceptives were taken. In addition, neither healthy individuals nor patients had a history of intolerance to COX-inhibitors, asthma, or allergy to airborne or food allergens. Furthermore, none of the individuals reported a known history of intolerance to COX-inhibitors (e.g. nasal polyps, asthma upon treatment with NSAID). The subjects gave their informed consent.

Clinical investigation

All subjects were checked by standard techniques. Infection with Helicobacter pylori (Hp) was confirmed by endoscopic and histologic examination. Blood eosinophilia was excluded by the standard microscopic cytological technique. Ten ml venous blood containing 5% heparin sodium (Prometa, Hamburg, Germany) was drawn from all individuals during routine examination. All samples were anonymous.

**Functional eicosanoid test (FET)**

The FET was performed according to a recently published protocol [10]. Briefly, blood leukocytes were prepared by dextran sedimentation and adjusted to 106 cells/ml in RPMI-1640 (GIBCO, Hamburg, Germany). Aliquots were incubated in duplicate in humidified air (5% CO2, 37°C) for 20 minutes. Addition of diluent without any stimulants revealed basal mediator release. Induced mediator release was achieved by addition of arachidonic acid (106 M, Biomol, Hamburg, Germany) or acetylsalicylic acid (106 M, Sigma-Aldrich, Deisenhofen, Germany). Incubation was stopped by centrifugation (900 x g, 7 min, 4°C). Supernatants were collected and stored at –80°C for up to four weeks until quantification of PGE and pLT. Both eicosanoids were quantified simultaneously in duplicate for each sample using highly specific and sensitive competitive enzyme-immunoassays according to published protocol [7,8]. The individual arithmetical mean of each sample was calculated in pg/ml using an internal standard.

**Evaluation of data**

The ratios of the individual values of basal PGE (e1), arachidonic acid-induced PGE (e2), acetylsalicylic acid-induced PGE (e3), and basal pLT (t1), arachidonic acid-induced pLT (t2), acetylsalicylic acid-induced pLT (t3) were calculated by equations (1) to (3):

1. arachidonic acid-induced PGE index B = e2/e1;
2. arachidonic acid-induced eicosanoid pattern A = e2/t2;
3. acetylsalicylic acid-induced eicosanoid pattern S = e3/t3.

The individual PGE and pLT values were integrated using equation (4), producing the individual eicosanoid pattern scores (EPSs):

4. EPS = (BxAS)0.75.

The EPS cut-off for healthy subjects was calculated as <3.4, patients suffering from gastroduodenal ulcer rendered an EPS ≥3.4. The cut-off was calculated based on the sensitivity and specificity revealed by the linear regression model of statistical analysis.

**Statistical analysis**

Results of synthesized PGE and pLT are presented as arithmetic mean ± SD. Controls and patients suffering from gastroduodenal ulcer were compared using the two-tailed U-test. A continuity correction of 0.5 was included because of some paired scores. A comparison of EPS values was done using the paired t-test. If homogeneity of variance of values of both groups was not achieved, the test of Satterthait was performed. The integrated eicosanoid values were controlled using the linear regression model. Furthermore, other clinical and pathological variables were compared using the Chi-squared test. The statistical analysis was performed using the SHS statistic package version 8.1E. The levels of significance were established at p<0.05 (significant) and a P-value <0.001 (highly significant).

**Results**

Clinical characterization of gastrointestinal ulcer patients

The patients and healthy individuals were homogenous with regard to sex (46% females, 54% males) and the slightly
more elderly group of patients (patients: 57.5 ± 14.5 years; healthy individuals: 48.2 ± 12.8 years). Helicobacter pylori was detected in 97 (92.4%) persons tested. There was no correlation of Hp infection with age, sex, or other criteria. All patients suffering from gastroduodenal ulcer and those patients with negative Hp infection were classified with EPS ≥3.4.

Eicosanoid generation of blood cells

Basically, blood leukocytes from healthy individuals as well as from patients suffering from gastroduodenal ulcer synthesized significantly higher amounts of PGE_2 than pLT. Values of PGE_2 in relation to pLT differed in patients and healthy individuals depending on the in vitro modulation of the leukocytes.

**Basal eicosanoid pattern**

The mean basal synthesis of PGE_2 by leukocytes from healthy individuals (329.8 ± 274.9 pg/ml) was 4.9-fold higher than the basal synthesis of pLT (67.3 ± 64.4 pg/ml). Leukocytes from patients synthesized 2.9-fold more basal PGE_2 (360 ± 268.8 pg/ml) than basal pLT (125 ± 110.5 pg/ml). Basal PGE_2 and of pLT values from healthy individuals as well as patients were not normally distributed. In healthy individuals, the standard deviation of PGE_2 synthesis was higher than of pLT synthesis. Patients synthesized significantly more basal PGE_2 (P<0.05) and basal pLT (P<0.01) than healthy individuals. The diversity of PGE_2 and pLT values from healthy individuals was slightly higher than from patients. However, 76% of basal PGE_2 values and 64% of basal pLT values from patients did not differ from healthy individuals.

In a further approach, the ratios of basal PGE_2 and pLT were calculated. There was also a slightly higher basal eicosanoid ratio (6.1) accompanied by a high standard deviation (11.9) in patients compared with healthy individuals (5.7 and 5.2, respectively). About 67% of the basal eicosanoid ratios of patients overlapped with the basal eicosanoid ratios of healthy individuals. (Figure 1).

**Arachidonic acid-induced eicosanoid pattern**

Upon modulation by arachidonic acid, the synthesis of PGE_2 and pLT by leukocytes from healthy individuals (3929.6 ± 2727.1 and 979.9 ± 1356.9 pg/ml, respectively) was higher than from healthy individuals (1015.5 ± 855.8 and 372 ± 334.4 pg/ml, respectively). Furthermore, the arachidonic acid-induced eicosanoid ratio in patients (14.2 ± 19.7) was significantly higher (p<0.01) than in healthy individuals (3.2 ± 3.9). About 45% of the arachidonic acid-induced eicosanoid ratios from patients were in the range of healthy individuals. (Figure 2).

**Acetylsalicylic acid-induced eicosanoid pattern**

Acetylsalicylic acid-modulated synthesis of PGE_2 by blood leukocytes from healthy individuals was significantly (P<0.01) higher than from patients (428.6 ± 637.8 and 307.4 ± 245.3 pg/ml, respectively). In contrast, acetylsalicylic acid-modulated synthesis of pLT was significantly higher (P<0.01) in patients than in healthy individuals (174.7 ± 171.2 and 71.2 ± 71.6 pg/ml, respectively). Acetylsalicylic acid caused a 9.1% elevation of basal pLT synthesis by leukocytes from healthy individuals, but a 32% increase in pLT synthesis by leukocytes from patients. The PGE_2/pLT ratio and its standard deviation upon modulation by acetylsalicylic acid was significantly higher (p<0.01) in healthy individuals than in patients (8.2 ± 14.34 and 3.5 ± 3.3, respectively). 58% of the acetylsalicylic acid-mediated eicosanoid ratios from patients were in the range of healthy individuals. (Figure 3).
Individual eicosanoid pattern scores (EPSs) of patients and healthy individuals

Neither individual PGE₂ and/or pLT synthesis nor individual eicosanoid ratios revealed a discrimination of healthy individuals and patients suffering from gastroduodenal ulcer. Therefore, the individual eicosanoid patterns were integrated according to equation [4], taking into account the impact of the individual PGE₂ and pLT values caused by different modulation. The mean eicosanoid pattern score (EPS) for healthy individuals was 1.84 ± 1.8 (range: 0.14 to 8.9) and for patients 24.05 ± 26.8 (range: 0.9 to 101.5). The EPS of patients was increased highly significantly (P<0.001) compared with healthy individuals. Ten patients (4.8%) revealed EPS values from -0.03 to 0.93, which were in the range of the EPS values of the healthy controls. The EPS results were evaluated statistically using the linear regression model. Inclusion of the individual's age improved the predictive value from ~93% to ~94% for patients suffering from gastroduodenal ulcer. For this purpose, an EPS cut-off higher than 3.4 was chosen. This approach revealed a sensitivity and specificity of ~97.6 and ~72.1%, respectively. (Figure 4).

**DISCUSSION**

The suggested difference between healthy people and ulcer patients in the eicosanoid pattern of blood leukocytes was demonstrated (Figures 1–3). The underlying functional eicosanoid test determined the particular capacity of leukocytes to generate PGE₂ and pLT. This test also determined the modulating effect of arachidonic acid and acetylsalicylic acid, whereas acetylsalicylic acid shifted the synthesis of pLT in patients suffering from gastroduodenal ulcer by more than 39%. Modulation by arachidonic acid or acetyl salicylic acid revealed PGE₂ and pLT synthesis as single parameters sufficient to discriminate healthy individuals from patients. Integration of the individual eicosanoid patterns of *ex vivo* modulated eicosanoid synthesis resulted in individual eicosanoid pattern scores (EPS) discriminating both groups. The EPSs from patients suffering from gastroduodenal ulcer differed highly significantly (P<0.01) from the eicosanoid patterns of healthy individuals, accompanied by a specificity of ~97% and a sensitivity of ~72%. The discussion, therefore, will focus on the peculiar biochemical behavior of white blood cells with regard to the development of gastroduodenal ulcer.

Since no tested subject was intolerant to NASID and since there was no other confounder such as interfering diseases or medical intervention, the demonstrated abnormality is strictly correlated with the gastroduodenal lesion. Moreover, the adherence to the experimental design of the former experiments [8,10,11] proved successful in again omitting separation of the populations of blood cells or separation of isoforms of COX-inhibitors.

A notable fact is the infection with Hp in the majority of patients. This needs additional discussion. Studies on the synergism between Hp and NSAID have yielded conflicting results [12,14–16]. Baseline PGE₂ was significantly higher in infected mucosa and decreased after one week of Naproxen application, independent of the infection [16]. Limited data showed that COX-2 expression was also increased in human gastric ulcer regardless of Hp status [17]. Another study in patients with Hp noted that ingestion of NSAID was an independent variable affecting PGE₂ synthesis [14]. In Hp-
gastritis, the *ex vivo* PGE$_2$ synthesis of mucosa was increased, consistent with increased expressions of COX-1 and COX-2 [18]. Long-term low-dose Aspirin® caused more gastric damage than without Hp-gastritis despite similar degrees of gastric mucosal prostaglandin depletion [19]. This is in line with our observation of an incremental reduction (~28%) of PGE$_2$ (Figure 3) and increasing abnormality of the eicosanoid pattern (Figure 4). Recently, a meta-analysis distinguished the impact of infection with Hp from the application of NSAID on peptic ulcer disease. However, the relative importance of Hp in the pathogenesis of ulcer will increase due to the introduction of COX-2-inhibitors [13].

Studies on the influence of Hp are more relevant to several studies. Several effects have been reported regarding this matter. Most interesting is the stimulation of the different cellular populations [20–22] Moreover, Hp attracts blood leukocytes and induces inflammatory infiltration consisting mainly of neutrophils and monocytes [23]. Finally, a bacterial protein which activates neutrophils (Hp-NAP, *Helicobacter pylori* neutrophil-activating protein) has been identified as a result of the contact with Hp [24]. We emphasize that there was no influence of Hp on our test results, especially due to the complete eradication before the investigation. In addition, the designated abnormal EPS did not correlate with the interval between eradication and performing the functional eicosanoid test.

The elevated EPS of patients correlated with increased basal PGE$_2$ and pLT as well as slightly elevated basal eicosanoid ratio (Figure 1), dramatically increased arachidonic acid-mediated eicosanoid ratio (Figure 2), and diminished acetylsalicylic acid-mediated eicosanoid ratio (Figure 3). The investigation of the individual eicosanoid patterns, as performed in this study using the *ex vivo* functional test of intact blood leukocytes, revealed a sensitivity of ~97% accompanied by a specificity of ~72%. The interdependence of these different parameters has to be considered when interpreting an individual EPS value. The probability of an individual suffering from gastroduodenal ulcer was calculated as ~93%, which can be improved to ~94.5% by integrating the individual’s age. This points to a multiparametric pathogenesis of this disease which needs further investigation, including possible other parameters. It also has to be stressed that an abnormal eicosanoid pattern was found in some individuals without ulcer and without Hp while, on the other hand, an abnormal eicosanoid pattern could be detected in some individual patients with Hp. This also confirms a pathogenic event based on several factors.

We hypothesise that the *ex vivo* performed functional eicosanoid test using blood leukocytes depicts a biological prede-termination which can actually be detected before the development of gastrointestinal lesions, which becomes manifest upon the introduction of additional stress (e.g. inflammation or therapeutics, as experimentally caused by arachidonic acid or acetylsalicylic acid) due to a general increase in vulnerability and susceptibility of the mucosa.

The underlying biochemical mechanisms of the observed altered eicosanoid metabolism are yet not fully understood, but might point to a modified enzymatic environment/capacity, as discussed for intolerance to NSAIDs [8]. Further investigations have to deal with the mentioned questions by separate experiments using different populations of white blood cells and isoforms of COX-inhibitors as well as samples adding Hp. This will enable answering the questions whether EPS-abnormality promotes infection with Hp and whether infiltrating inflammatory cells respond like blood cells.

### Conclusions

In conclusion, the new phenomenon is of interest in different contexts. It could help to elucidate the pathophysiology and produce an additional tool to determine the individual risk for gastroduodenal ulcer.

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