COX-inhibitors promote nasal polyps or bronchial asthma in individuals susceptible to an alteration of the pattern of the eicosanoids, especially leukotrienes and prostaglandins. This is associated with an abnormal release of eicosanoids from white blood cells. Since COX-inhibitors also protect from colorectal cancer an analogous association may be suggested. The study was performed to detect abnormal patterns of eicosanoids in white blood cells of patients with intestinal cancer compared to healthy controls. Seventy patients with intestinal cancer (stomach = 5; colon = 25; sigma = 18; rectum = 22) were compared to 62 healthy controls. Blood leukocytes from patients in complete long-lasting remission were incubated with diluent, arachidonic acid or acetylsalicylic acid. The synthesis of prostaglandin E\(_2\) and peptido-leukotrienes was quantified using competitive enzyme-immuno-assays and calculated for individual eicosanoid patterns. The mean basal and arachidonic acid- or acetylsalicylic acid-modulated PGE\(_2\) synthesis in patients was significantly higher than in controls (4.8-fold, 9.4-fold, 3.7-fold, respectively) whereas pLT was generally less elevated. We conclude that the eicosanoid-pattern of white-blood-cells from patients with intestinal cancer differs significantly from that in healthy individuals. This abnormal cellular metabolism may contribute to the manifestation of cancer and help to detect individuals at risk.

**Key words: Gastrointestinal cancer; eicosanoids; white-blood-cells**

**INTRODUCTION**

Eicosanoids are of substantial importance for the organism. They are involved in many biological mechanisms, for instance inflammation. Therefore the formation of arachidonic acid and its conversion into eicosanoids, mainly leukotrienes (LT), prostaglandins (PG) or thromboxans (TX), are essential. This
statement includes the appropriate enzymes lipoxygenase (LOX) and cyclooxygenase (COX).

Any shift within this system results in the manifestation of various disorders especially in the respiratory tract. This can be induced by the ingestion of COX-inhibitors. Individuals experiencing nasal polyps or asthma upon aspirin or NSAID are designated intolerant (1) showing abnormal eicosanoid patterns in affected tissue (2). It is striking that white blood cells from patients render analogous results (3). The same coincidence was demonstrated in patients suffering from gastroduodenal ulcer (4). This is pertinent with an artificially shift regarding the eicosanoid-complex.

The observation that aspirin and NSAID protect from intestinal cancer and adenoma when taken over a long time implicates the importance of eicosanoids also in this concern (5, 6, 7, 8). This is underlined by investigations of the expression of COX even within familial adenomatous polyposis of the human colon (9). In addition, an increased level of salicylic acid is found in the serum after consumption of dietary fruits and vegetables which may at least contribute to the protective effect of such food and explain its benefit (10, 11). The importance of PG referring to intestinal cancer is generally accepted although not yet completely understood. The suggested protective mechanisms of COX-inhibitors are attributed to the reduction of angiogenesis (12), to the increased apoptosis of malignant cells (13) and to a direct inhibition of cancer cell growth (14). Since the eicosanoid-pattern is an individual biological trait and hence can be found in affected tissues as well as in white blood cells a well-established functional test was applied to detect abnormal patterns using white blood cells of patients with intestinal cancer.

MATERIAL AND METHODS

Patients

Seventy patients with gastrointestinal cancer were investigated (31 female; 39 male; mean age 67.4 years). The diagnosis was assessed by endoscopy and histology. The localization was as follows: stomach 5, duodenum 0, colon 25, sigma 18, rectum 22. All patients underwent surgical therapy and chemotherapy according to the actual guidelines. There was a complete remission of at least 2 years at the moment of investigation omitting any therapy in this concern. Individuals suffering from other gastrointestinal diseases or disorders basing on eicosanoid imbalance were excluded as well as those taking drugs interfering with the generation or metabolism of eicosanoids like NSAID or steroids. The protocol and informed consent were approved by Independent Board of Ethical Committee at the Erlangen-Nuremberg University and informed consent was obtained from each subject studied.

Functional eicosanoid test (FET)

Blood was drawn during control-examination. Five ml of venous blood were collected by S-monovette® (Sarstedt, Hannover, Germany), including heparin to prevent clotting. Thereafter blood
was further processed using the LiPiDoC©-FET (SIAT, Bad Essen, Germany) according to the instruction of the manufacture. In brief, the heparinised blood samples were diluted by a standardised protocol. Thereafter a defined number of cells was incubated at room temperature for 20 minutes with diluent, arachidonic acid (10⁻⁵ M), or acetylsalicylic acid (10⁻⁶ M), followed by centrifugation of the samples and storing of the supernatant at -20°C until further procession for analysis by enzyme-immuno-assays.

Quantification of Eicosanoids

Prostaglandin E₂ (PGE₂) and peptide-Leukotrienes (pLT) were quantified using a commercial available competitive enzyme-immuno-assay (SPI-bio, Paris, France), which was specifically produced und validated for use in combination with LiPiDoC©-FET. The assays were performed according to the instructions of the manufacturer. Samples were analysed in duplicates. Optical density was measured using a Dynatech MR5000 and BioLinx 2.0 software package. Results were presented as arithmetic mean.

Evaluation of Data

The data were processed as published recently (4). Briefly, the individual enzymatic capacity of cyclooxygenases (EC₉Oₓ) and 5-lipoxygenase (EC₅LOₓ) were calculated from arachidonic acid-induced and basal eicosanoid synthesis using formula (1). Furthermore, the individual arachidonic acid-induced PGE₂/pLT ratio (RA) as well as the acetylsalicylic acid-mediated PGE₂/pLT ratio (RS) were calculated using formula 2 and 3, respectively). Thereafter EC₉Oₓ, RA; and RS were integrated using the equation (2), revealing the individual eicosanoid pattern score (EPS):

(1) ECₓ = Aₓ / Bₓ;  \hspace{2cm} A = arachidonic acid -induced synthesis; 
B = basal synthesis of PGE₂ or pLT; 
X = COX or LOX; 
e = PGE₂ or pLT
(2) RA = A₉PGE₂/A₉pLT ; \hspace{2cm} A₉PGE₂ = arachidonic acid -induced PGE₂ synthesis; 
A₉pLT = arachidonic acid -induced pLT synthesis 
(3) RS = S₉PGE₂/S₉pLT ; \hspace{2cm} S₉PGE₂ = acetylsalicylic acid-mediated PGE₂ synthesis; 
S₉pLT = acetylsalicylic acid-mediated pLT synthesis 
(4) EPS = (EC₉PGE₂ ) * RA / RS)⁰.⁷⁵.

The EPS cut-off for healthy controls was calculated <3.4 using the linear regression model of statistical analysis as published recently (4). According to the alteration of the EPS compared with normal subjects each patient was assigned to a "functional eicosanoid type" (FET) revealing "normal", "borderline", "abnormal", and "severe abnormal" eicosanoid patterns as summarised in Table 1.

Statistical Analysis

The values of synthesised PGE₂ and pLT were given as arithmetic mean ±SD. Patients and controls were compared using the two-tailed U-test. A continuity correction of 0.5 was included because of some paired scores. EPS values were compared by the paired t-test. The t-test of Satterthait was performed if homogeneity of variance of values of both groups was not achieved. The linear regression model was used for controlling the integrated eicosanoid pattern score. 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 p<0.001 (highly significant).

RESULTS

Clinical Characterisation of Intestinal Cancer Patients

The 70 patients suffering from intestinal cancer and the 62 healthy controls were homogenous with regard to sex (41% females, 59% males) and a slightly younger group of controls (controls: 48.2 ±14.5 years; patients: 66.9 ±23.7 years). The location of the cancer was as follows: stomach (n=5), colon (n=25), sigma (n=18), rectum (n=22) (Fig.1). Statistical evaluation of EPS differentiated by cancer location was omitted because of low numbers.

Synthesis of Eicosanoids

The individual PGE$_2$ and pLT values featured high variability in controls as well as patients concerning basal, arachidonic acid-induced and acetylsalicylic acid-modulated eicosanoid synthesis.

The mean value of eicosanoids synthesised by blood cells form patients suffering from intestinal cancer of was 4.8-, 9.4-, and 3.7-fold higher for basal, arachidonic acid-induced acetylsalicylic acid-modulated synthesis of PGE$_2$ compared to controls (1587 vs. 326, 9387 vs. 1001, and 1592 vs. 422 pg/ml, respectively; s. Fig.2). Similar results were obtained for pLT synthesis revealing slightly elevated (1.4-fold), highly increased (12-fold), and not significantly (1.3-fold) elevated basal, arachidonic acid-induced, and acetylsalicylic acid-mediated pLT values (109 vs. 66.9, 4866 vs. 372, 107 vs. 71.2 pg/ml, respectively; s. Fig.3).

Fig. 1. Distribution of intestinal cancer
Seventy patients were included for investigation. Gastrointestinal cancer was identified by endoscopy and histology.
Fig. 2. PGE$_2$-Synthesis
Synthesis of PGE$_2$ by blood cells from controls (N=62) and patients with intestinal cancer (N=70); basal: basal PGE$_2$ synthesis, AA-induced: arachidonic acid-induced PGE$_2$ synthesis, ASA-mediated: acetylsalicylic acid-mediated PGE$_2$-synthesis; bars indicate arithmetic mean.

Fig. 3. pLT-Synthesis
Synthesis of pLT by blood cells from controls (N=62) and patients suffering from intestinal cancer (N=70); basal: basal pLT synthesis, AA-induced: arachidonic acid-induced pLT synthesis, ASA-mediated: acetylsalicylic acid-mediated pLT-synthesis; bars indicate arithmetic mean.
The individual enzyme capacity (EC) of cyclooxygenases calculated from synthesised PGE$_2$ (EC$_{PGE2}$) of blood cells from controls (N=62) and patients suffering from intestinal cancer (N=70) are presented; COX: cyclooxygenases; PGE$_2$: Prostaglandin E$_2$; bars indicate arithmetic mean.

**Fig. 4. Enzyme Capacity of Cyclooxygenases**

EC$_{PGE2}$ of blood cells from patients was 11.8-fold higher than from controls (EC$_{PGE2} = 42.5$ vs. 3.6; s. *Fig. 4*).

**Fig. 5. PGE$_2$/pLT-ratio**

The individual PGE$_2$/pLT ratio of blood cells from controls (N=62) and patients suffering from intestinal cancer (N=70) are presented for basal, arachidonic acid-induced (AA-induced), and acetylsalicylic acid-mediated (ASA-mediated) eicosanoid ratio; bars indicate arithmetic mean.
The mean basal PGE$_2$/pLT ratio was 3-fold elevated in patients compared to controls (RB: 14.5 vs. 5.9, p< 0.05); in addition the mean acetylsalicylic acid-mediated was 2.5-fold higher in patients than on controls (RS: 14.9 vs. 4.9, p<0.01). But the mean arachidonic acid-induced PGE$_2$/pLT ratio was slightly diminished in patients (RA: 1.9 vs. 2.7) (s. Fig.5).

**Eicosanoid Pattern Score and Functional Eicosanoid Type**

Upon integration of the individual eicosanoid patterns according to equation (4) the EPS from patients was characterised by a significantly elevated EPS (10.4, range: 0.1 to 4799; p < 0.05) compared to EPS from controls (1.8, range 0.14 to 8.9). For controls an upper cut-off value of the eicosanoid pattern score was calculated (EPS < 3.4) including 88.7 % (N = 55) of controls, whereas 11.3 % revealed slightly elevated EPS (EPS: 3.5 - 8.9). 64.3% of patients suffering from

### Table 1.

<table>
<thead>
<tr>
<th>&quot;Functional Eicosanoid Type&quot; (FET)</th>
<th>Eicosanoid Pattern Score (EPS)</th>
<th>Interpretation</th>
<th>Control % (N=62)</th>
<th>Patient % (N=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FET-0</td>
<td>0 &lt; 3.4</td>
<td>normal</td>
<td>88.7 (55)</td>
<td>35.7 (25)</td>
</tr>
<tr>
<td>FET-1</td>
<td>&gt;3.4 - 10</td>
<td>borderline</td>
<td>11.3 (7)</td>
<td>21.4 (15)</td>
</tr>
<tr>
<td>FET-2</td>
<td>&gt;10 - 300</td>
<td>abnormal</td>
<td>-</td>
<td>38.6 (27)</td>
</tr>
<tr>
<td>FET-3</td>
<td>&gt;300</td>
<td>severe abnormal</td>
<td>-</td>
<td>4.3 (3)</td>
</tr>
</tbody>
</table>
intestinal cancer (N = 45) revealed EPS >3.4. Furthermore, 17.1% of patients (N = 12 of 70) revealed an EPS lower than those of the EPS value of controls (s. Fig.6). The altered eicosanoid pattern was further classified and described as "normal"," borderline", "abnormal", or "severe abnormal" eicosanoid pattern, which were entitled as "Functional Eicosanoid Type" (FET). 21.4 % of the patients revealed FET-1 with borderline EPS, 38.6 % patients were classified as FET-2 showing abnormal EPS, and only 4.3 % revealed FET-2 showing severe abnormal EPS (for details s. Table 1).

DISCUSSION

The applied functional eicosanoid test detected significant alterations of the eicosanoid-pattern in white blood cells from patients with gastrointestinal malignancy compared to healthy controls. This was similar to former studies enrolling patients suffering from other diseases also caused by aspirin or NSAID. So the suggested coincidence between the protection from gastrointestinal cancer by COX-inhibitors and an innate altered eicosanoid-pattern of white blood cells has been confirmed.

The results of our study demonstrate an increased capacity to synthesise prostaglandin in white blood cells from patients. This was observed in the samples after addition of arachidonic acid as well as when omitting it. The demonstrated shift was enhanced by aspirin due to its specific effect. Regarding pLT there was a less increase. However, the applied functional test used peripheral leukocytes and therefore is of restricted relevance to the situation within the malignant transformed tissue. Generally speaking, the results explain the protective efficiency of COX-inhibitors since the generally elevated PG-synthesis in patients is reduced compared to normal.

The similarity of all results of our study regarding the location of the cancer, the age or the sex, points to a systemic peculiarity. Since complete elimination of intestinal cancer equals a normal healthy state it has to be suggested that the results after successful treatment are the same as before the manifestation of the malignancy. In fact, former studies revealed a long-lasting stability of the individual eicosanoid-patterns (4). So the FET could be of some predictive value and useful for the detection of subjects at risk to get eicosanoid-related disease including gastrointestinal cancer. Of course, these investigations were not appropriate to elucidate the mechanism of carcinogenesis.

It must be drawn attention to a considerable portion of patients (35,7%) which renders a normal eicosanoid-pattern. This might indicate another mechanism of malignant transformation. In contrast, a small portion (11,3%) of healthy controls show an abnormal eicosanoid-pattern possibly pointing at the risk to experience eicosanoid-related diseases later on. The difference between both groups is significant (p>0.05).
There are several techniques and methods available to detect malignancy. The early determination of the gene expression of COX on the local cellular level needs an invasive sampling of biopsy-specimens. The demonstration of aberrant cells in the stool detects malignancy after the manifestation. The FET reveals an actual abnormal activity of COX and LOX in white blood cells including the stimulatory or suppressive efficiency of arachidonic acid and aspirin which is obviously concomitant to the development of intestinal cancer in the majority of patients.

The demonstrated phenomenon regarding the intestinal malignancy has to be taken a real connexion since other situations altering or modifying the results were excluded. We are, however, aware that our test means an artificial situation. For confirmation of our suggestions prospective cohort studies are necessary. Further investigations have to observe healthy individuals with an abnormal eicosanoid pattern whether there is a correlation between the eicosanoid-pattern and future eicosanoid-related diseases including the manifestation of gastrointestinal malignancy. Moreover, the eicosanoid-pattern of leucocytes within malignant tissue should be investigated. The differentiation regarding the leucocytes or the COX-inhibitors is of minor importance.

In summary, the FET could prove an additional ordinary tool to detect individuals at risk to develop eicosanoid-related diseases including intestinal malignancy also offering the possibility to execute pharmacological prophylaxis.

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