Prostaglandin F$_{2\alpha}$ metabolite and F$_2$-isoprostane excretion rates in migraine

Johanna HELMERSSON*, Peter MATTSSON† and Samar BASU*

*Sections of Geriatrics and Clinical Nutrition Research, Department of Public Health and Caring Sciences, Faculty of Medicine, Uppsala University, Box 609, SE-751 25 Uppsala, Sweden, and †Department of Neurosciences, Neurology, Faculty of Medicine, Uppsala University, University Hospital, UAS, SE-751 85 Uppsala, Sweden

ABSTRACT

The pathophysiology theory of migraine postulates a local, neurogenic inflammation and the possible involvement of oxidative stress. We analysed the levels of 15-oxo-dihydro-prostaglandin F$_{2\alpha}$ (a metabolite of prostaglandin F$_{2\alpha}$) and 8-iso-prostaglandin F$_{2\alpha}$ (a major isoprostane), which are biomarkers for inflammation and oxidative stress respectively, in urine from 21 patients with migraine, with and without aura. Urine samples from migraine patients were collected during a migraine attack, and control samples were collected from the same subjects on a migraine-free morning. The mean basal levels of 15-oxo-dihydro-prostaglandin F$_{2\alpha}$ and 8-iso-prostaglandin F$_{2\alpha}$ in the morning control urine samples were 0.54 ± 0.11 and 0.31 ± 0.13 nmol/mmol of creatinine respectively. The mean levels of 15-oxo-dihydro-prostaglandin F$_{2\alpha}$ and 8-iso-prostaglandin F$_{2\alpha}$ in the urine samples collected during the migraine attack in the 21 patients were 0.53 ± 0.13 and 0.32 ± 0.11 nmol/mmol of creatinine respectively. Thus there were no differences in the 15-oxo-dihydro-prostaglandin F$_{2\alpha}$ and 8-iso-prostaglandin F$_{2\alpha}$ excretion rates during the migraine attack compared with on the migraine-free day. However, the basal 8-iso-prostaglandin F$_{2\alpha}$ excretion levels on the migraine-free day were significantly lower in pre-menopausal women (0.24 ± 0.08 nmol/mmol of creatinine; n = 11) compared with post-menopausal women (0.39 ± 0.14 nmol/mmol of creatinine; n = 7; P = 0.009). In conclusion, in this study we found no support for the involvement of inflammation and oxidative stress in migraine pathophysiology. Our results indicate, however, a lower level of oxidative stress in pre-menopausal compared with post-menopausal women.

INTRODUCTION

Migraine is a common disorder characterized by attacks of headache. Some patients experience a preceding visual disturbance (migraine with aura). Many authors believe that sterile inflammation within the trigeminovascular system is of great importance in the pathophysiology of migraine pain. This model (the neurogenic inflammation model) postulates release of the neurotransmitter substance P, vasodilatation, increased permeability, oedema of cranial blood vessels and sensitization to pain [1].

Several biochemical mediators have been implicated in the pathogenesis of migraine, such as calcitonin-gene-related peptide [2], nitric oxide [3] and prostaglandins [4]. Only a few attempts have been made so far to measure prostaglandin-related compounds during the migraine pain phase. However, prostaglandin E$_{2\alpha}$ (PGE$_{2\alpha}$) and thromboxane A$_2$ levels in saliva have been found to be elevated during migraine attacks [5]. Increased levels of plasma PGE$_{2\alpha}$ were found during the pain phase in women with menstrual migraine [6]. Plasma levels of non-esterified fatty acids, in particular linoleic acid (a pre-
cursor of arachidonic acid), have been found to be elevated during migraine attacks [7,8], possibly indicating an increased involvement of pathways using arachidonic acid as a substrate during a migraine attack. Cyclooxygenase inhibitors, e.g. acetylsalicylic acid and other NSAIDs (non-steroidal anti-inflammatory drugs), are effective in relieving migraine pain, suggesting an important role for eicosanoids in the pathogenesis of migraine [9].

The level of 15-oxo-dihydro-PGF$_{2\alpha}$, a major metabolite of PGF$_{2\alpha}$ in plasma, is increased during the inflammatory response, and is considered to be a reliable biomarker of inflammation [10]. Oxidative stress is known to be induced during acute inflammation in animal models [11,12]. Further, nitric oxide has the potential to induce oxidative stress by acting as a free radical through the peroxynitrite pathway. Isoprostanes are formed by the free radical-catalysed peroxidation of arachidonic acid in the presence of oxidative stress. 8-Isoprostan-8,9-dienoic acid PGF$_{2\alpha}$ is a major isoprostane that is formed, and this metabolite is considered to be a reliable marker of oxidative injury in both animals and humans [13,14].

The aim of the present study was to investigate if inflammation and oxidative stress are involved in the pathological process of migraine. We analysed the excretion of 15-oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ in urine samples, as indicators of inflammation and oxidative stress respectively, collected during a migraine attack and on a migraine-free morning.

**MATERIALS AND METHODS**

**Study design**

A total of 21 patients with a clinical diagnosis of migraine, with or without aura [15], from the Outpatients Clinic of the Centre of Clinical Neuroscience, Uppsala University Hospital, agreed to participate in the study. The participants were asked to collect a urine sample 20–30 min after their migraine headache had started, and one control urine sample on a migraine-headache-free morning, with at least 2 days between collection of the samples. They were informed not to take any NSAIDs prior to the urine collections. The samples were stored frozen immediately at approx. –20°C and delivered to our laboratory as soon as possible. The urine samples were then stored frozen at –70°C until analysis.

The participants were asked to fill in a questionnaire about medication, menstrual cycle/pregnancy, diseases other than migraine, physical training 24 h prior to the urine collections, and the symptoms of the migraine attack during which the urine sample was collected. The study was approved by the Ethics Committee, Faculty of Medicine, Uppsala University, and all subjects gave informed consent.

**Patients**

The participants were 18 women and three men with a mean age of 47 years (range 24–63 years). None of the patients smoked, had diabetes mellitus or displayed clinical symptoms of ischaemic heart disease. Seven women were post-menopausal and 11 were pre-menopausal. Among the pre-menopausal group, three women were close to the menopause and were taking regular hormone replacement therapy (medium-potency oestrogens). Three women were taking combined contraceptive pills (ethinylestradiol and desogestrel/norethisterone), one woman had a hormone spiral (levonorgestrel) without ovulations, one woman was taking minipills (low norethisterone dose) with ovulations, and the rest of the pre-menopausal women had menstrual cycles and no hormone preventatives.

**Chemicals**

Unlabelled 15-oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ were purchased from Cayman Chemicals (Ann Arbor, MI, U.S.A.). Tris/HCl, Tris base, EDTA disodium salt and bovine γ-globulin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Instagel scintillation cocktail was from Packard Instruments (Meriden, CT, U.S.A.), and polyethylene glycol (MW 4000) was from Merck. Unlabelled 15-oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ standards, $^3$H-labelled tracer and working antibody dilutions were prepared in RIA buffer. $^3$H-labelled 15-oxo-dihydro-PGF$_{2\alpha}$ (specific radioactivity 6.77 TBq mmol$^{-1}$) was obtained from Amersham (Little Chalfont, Bucks., U.K.). Antibodies against 15-oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ were raised at our laboratory and well characterized [10,16].

**RIA of urinary 15-oxo-dihydro-PGF$_{2\alpha}$**

The urine samples (50 µl) were analysed for 15-oxo-dihydro-PGF$_{2\alpha}$ by a newly developed RIA in our laboratory, as described elsewhere [10]. In brief, an antibody was raised in rabbits by immunization with 15-oxo-dihydro-PGF$_{2\alpha}$ coupled to BSA at the carboxylic acid group using the 1,1′-carbonyldi-imidazole method. The cross-reactivity of the antibody with PGF$_{2\alpha}$, 15-oxo-PGF$_{2\alpha}$, PGE$_{2\alpha}$, 15-oxo-13,14-dihydro-PGE$_{2\alpha}$, 8-iso-15-oxo-13,14-dihydro-PGF$_{2\alpha}$, 11β-PGF$_{2\alpha}$, 9β-PGF$_{2\alpha}$, thromboxane B$_2$ and 8-iso-PGF$_{2\alpha}$ was 0.02 %, 0.43 %, < 0.001 %, 0.5 %, 1.7 %, < 0.001 %, < 0.001 %, < 0.001 % and 0.01 % respectively. The limit of detection was approx. 45 pmol/l.

**RIA of urinary 8-iso-PGF$_{2\alpha}$**

The urine samples (50 µl) were analysed for free 8-iso-PGF$_{2\alpha}$ without any prior extraction or purification by a newly developed RIA [16]. In brief, an antibody was
Inflammation and oxidative stress in migraine

raised in rabbits by immunization with 8-iso-PGF$_{2\alpha}$ coupled to BSA at the carboxylic acid by the 1,1-carbonyldi-imidazole method. The cross-reactivity of the antibody with 8-iso-15-oxo-13,14-dihydro-PGF$_{2\alpha}$, 8-iso-PGF$_{2\alpha}$, PGF$_{2\alpha}$, 15-oxo-PGF$_{2\alpha}$, 15-oxo-13,14-dihydro-PGF$_{2\alpha}$, thromboxane B$_{2\alpha}$, 11/8-PGF$_{2\alpha}$, 9/8-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ was 1.7%, 9.8%, 1.1%, 0.01%, 0.01%, 0.1%, 0.03%, 1.8% and 0.6% respectively. The limit of detection of the assay was approx. 23 pmol/l.

Urine creatinine assay

The creatinine concentration was determined in each urine sample by a colorimetric method using IL Test creatinine 181672-00 in a Monarch® 2000 centrifugal analyser (Instrumentation Laboratories, Lexington, MA, U.S.A.). The levels of 8-iso-PGF$_{2\alpha}$ and 15-oxo-dihydro-PGF$_{2\alpha}$ in urine were corrected for urine creatinine values.

Statistics

All results are expressed as means ± S.D. Paired $t$-tests were used to examine differences in the levels of 15-oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ between the control and migraine samples. Unpaired $t$-tests were used for testing differences between pre-menopausal and post-menopausal women with regard to basal levels of 15-oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$. Data for 8-iso-PGF$_{2\alpha}$, 15-oxo-PGF$_{2\alpha}$, and age had a skewed distribution according to the Shapiro–Wilks test, and were log-transformed. Since age data still had a skewed distribution after transformation, the Mann–Whitney test was used to test the difference between the two groups of women. All tests were two-tailed, and $P < 0.05$ was regarded as statistically significant. All calculations were performed using the statistical software package JMP (version 3.2; SAS Institute, Cary, NC, U.S.A.).

RESULTS

The symptoms reported during the migraine attack by each patient were in accordance with the migraine criteria [15]. Seven patients reported vision disturbances, in the form of scintillating scotomas, prior to the migraine headache. The migraine urine sample was collected on average 91 min (range 20–300 min) after the start of the migraine headache.

15-Oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ levels

The mean basal levels of 15-oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ in the control urine samples collected on a migraine-headache-free morning for all 21 patients were $0.54 ± 0.11$ and $0.31 ± 0.13$ nmol/mmol of creatinine respectively. The mean levels of 15-oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ in the urine samples collected during the migraine attack for all patients were $0.53 ± 0.13$ and $0.32 ± 0.11$ nmol/mmol of creatinine respectively. These levels measured in urine collected during a migraine attack were not different from the basal urinary levels of 15-oxo-dihydro-PGF$_{2\alpha}$ ($P = 0.87$) and 8-iso-PGF$_{2\alpha}$ ($P = 0.67$).

The pre-menopausal women (with either synthetic or physiological sex hormones) had a significantly lower basal level of excretion of 8-iso-PGF$_{2\alpha}$ compared with post-menopausal women (Table 1). The basal level of excretion of 15-oxo-dihydro-PGF$_{2\alpha}$ was not significantly different between post-menopausal and pre-menopausal women. Neither 8-iso-PGF$_{2\alpha}$ nor 15-oxo-dihydro-PGF$_{2\alpha}$ levels were correlated with age (results not shown).

DISCUSSION

In the present study, no altered excretion of a PGF$_{2\alpha}$ metabolite or an F$_{3\alpha}$-isoprostane was seen during a migraine attack, indicating no major involvement of inflammation or oxidative injury in these patients. The basal levels of PGF$_{2\alpha}$ metabolite and F$_{3\alpha}$-isoprostane in urine from patients with migraine were in the same range as described in our earlier study of healthy men and women [17].

The possibility exists that a local inflammatory response and subsequent eicosanoid release during the migraine attack may not have been extensive enough to be detected by measuring urinary excretion of metabolites. Further, minor increases in the levels of these compounds may be missed by the urinary measurement protocol unless frequent blood sampling is also performed. This is due mainly to the very short half-lives of

| Table 1 Age and basal excretion levels of 15-oxo-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ for seven post-menopausal and 11 pre-menopausal migraine patients
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Post-menopausal women ($n = 7$)</th>
<th>Pre-menopausal women ($n = 11$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54 ± 7</td>
<td>44 ± 10</td>
<td>0.02</td>
</tr>
<tr>
<td>8-Iso-PGF$_{2\alpha}$ (nmol/mmol of creatinine)</td>
<td>0.39 ± 0.14</td>
<td>0.24 ± 0.08</td>
<td>0.009</td>
</tr>
<tr>
<td>15-Oxo-dihydro-PGF$_{2\alpha}$ (nmol/mmol of creatinine)</td>
<td>0.61 ± 0.10</td>
<td>0.52 ± 0.11</td>
<td>0.08</td>
</tr>
</tbody>
</table>
explaining part of the well-known difference in the women found in the present study gives further support significantly lower basal levels of 8-iso-PGF\textsubscript{2\alpha} undetected. level of F\textsubscript{2\alpha} observed in epidemiological studies. The reduced pausal women prescribed non-contraceptive oestrogen compounds of medium potency, including conjugated ethinyl oestradiol and women prescribed oestrogen menopausal group of women in our study consisted of women given to post-menopausal women \cite{20} and added physiologically relevant concentrations for menstruating post-menopausal women \cite{19} and 17β-oestradiol in compounds, including conjugated equine oestrogens in has been described for various kinds of oestrogen inhibitors of oxidation of low-density lipoprotein suggest that oestrogens do possess antioxidative properties. This hypothesis that inflammation or oxidative stress is part of the pathogenesis of migraine. The basal levels of 15-oxo-dihydro-PGF\textsubscript{2\alpha} and 8-iso-PGF\textsubscript{2\alpha} in patients with migraine are not different from those in healthy humans. However, there is significantly decreased excretion of 8-iso-PGF\textsubscript{2\alpha} in pre-menopausal women compared with post-menopausal women, indicating a lower occurrence of oxidative stress in these women, possibly due to the antioxidative effect of oestrogen.

ACKNOWLEDGMENTS

This study was supported financially by the Geriatrics Research Foundation. We are indebted to Professor Bengt Vessby and Dr Eva Södergren for valuable discussions, and to Lars Berglund for statistical advice.

REFERENCES

inflammation and oxidative stress in migraine


Received 4 June 2001/10 July 2001; accepted 14 September 2001

© 2002 The Biochemical Society and the Medical Research Society