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Effect of indomethacin on cerebrovascular reactivity in patients with type 2 diabetes mellitus

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ABSTRACT

Aim: Impaired cerebral vasoreactivity to endothelium-dependent stimuli were described in type 2 diabetes mellitus (T2DM), but the mechanisms underlying that impairment are still unclear. The aim of this study was to investigate the role of cyclooxygenases' metabolites in response to acute hypercapnic stimulus in cerebral vessels, in patients with T2DM.

Methods: Vascular responses in the breath-holding test (BHT) were assessed in the absence/presence of a non-selective, reversible-inhibitor of cyclooxygenases, indomethacin (INDO), by functional transcranial Doppler sonography of the middle cerebral artery (*N* of patients = 50; 33 men and 17 women). The functional hemodynamic parameter mean flow velocity (MFV) was assessed at rest, before and 90 min after 100 mg of INDO, and during the BHT. Breath holding index (BHI) [(MFV at the end of BHT minus MFV at rest)/MFV at rest) × 100/s of breath-holding] was calculated after BHT performed before and 90 min after INDO.

Results: MFV at rest significantly decreased after INDO administration compared with a control condition before INDO (at rest before INDO from 49.36 ± 15.09 to 36.72 ± 8.45 after INDO, $p < 0.001$) However, overall cerebral vessel vasoreactivity to hypercapnia, evaluated with BHI, was significantly improved after INDO administration compared with the BHI before INDO administration (from 0.68 ± 0.4 to 1.27 ± 0.42, $p < 0.001$).

Conclusions: The improvement in cerebral vasoreactivity in response to BHT after INDO administration suggests that the production of a vasoconstrictor metabolite of cyclooxygenase in diabetic patients was reduced by indomethacin consumption.

1. Introduction

Previous studies have shown that diabetes mellitus is a major risk factor for stroke due to the development of macroangiopathy and microangiopathy [1–4]. Patients with type 2 diabetes mellitus (T2DM) exhibit impaired cerebral vasoreactivity in response to a vasodilatory stimulus, such as hypercapnia [5] and other vasodilators [6–9], as well as enhanced vascular responsiveness to vasoconstrictors [6–9]. The mechanisms of the impaired ability of the endothelium to properly maintain vascular homeostasis (endothelial dysfunction) in T2DM are still unclear. The nitric oxide (NO) – dependent pathway has been studied the most, in diabetes mellitus, but has also been reviewed in a number of other conditions, such as hypertension [10,11]. In addition to NO, arachidonic acid's metabolites

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produced via cyclooxygenase (COX) pathway [12] could have an important role in endothelial function underlying the hypercapnia-induced cerebrovascular vasodilatation, but also in many other vascular beds, as previously shown in hypertension [13–17].

The functional transcranial Doppler sonography (TCD) in conjunction with hypercapnic stimulus via the Breath holding test (BHT), has been used to evaluate cerebral vasoreactivity in many pathological conditions, such as in detection of vascular aging, asymptomatic carotid artery stenosis or obstructive sleep apnea [5,18–26]. In addition, the functional Transcranial Doppler ultrasound has been shown as a good, noninvasive and accurate method for the evaluation of the changes in mean flow velocity (MFV) in the cerebral arteries in response to various stimuli, e.g. hypercapnia. The hypothesis of this study was that there is significant impairement of the vascular response in cerebral circulation to acute hypercapnia in diabetic patients, that is restored with non-selective COX1,2 inhibitor indomethacin, possibly via indomethacin's blockade of vasoconstrictive prostaglandin production.

The aim of this study was: (a) to assess the response of the resistance cerebral artery to hypercapnia induced via BHT, and (b) to evaluate the potential role of cyclooxygenases' metabolites in the cerebral vasoreactivity in patients with type 2 diabetes mellitus. According to all existing previously published studies, this is the first study which investigates the influence of COX metabolites in cerebral vasoreactivity to hypercapnia using the Breath holding test in patients with type 2 diabetes mellitus.

2. Methods

2.1. *The study was a self-controlled, open-label and prospective study*

The study included 50 randomly selected T2DM patients from an outpatient clinic of the Department of Endocrinology, Diabetes and Metabolic Disorders at Dubrava University Hospital, Zagreb, Croatia. The inclusion criteria were type 2 diabetes mellitus diagnosed at least six months prior to entry of this study, ages ranging from 18 to 70. All patients abstained from alcohol and caffeine containing products 24 h prior to the study. Only T2DM patients, with well controlled hypertension (if existed) confirmed from medical reports and with blood pressures measured under 130/80 mmHg in the sitting position (results included in this study) were used in order to eliminate the effects of high blood pressure on vascular function. Patients were confirmed to be taking no known vasoactive medications except anti-diabetic and lipid-lowering medications, and their therapy was not changed in at least 3 months before testing. Patients did not take any anti-aggregation therapy. A list of medication is given in Table 1. All patients were tested in the morning and had instructed before not to eat anything at least 4 h and not to take any medication at least 12 h before the tests performed. They came to us 30 min before testing to be explained the procedures, measuring, taking blood samples for analyses and to prepare for testing. After first mean blood flow velocity (MBFV) assessment (basal test followed by Breath holding test (BHT, described below) patients received indomethacin and the same measurements were repeated 90 min after. Regular patients' medications could have been taken after the meal. The next blood sampling for glycaemia was 2 h after the meal.

Table 1 – Percentage of the consumed medications in T2DM patients (*N* = 50).

Medications	Number (%)
Oral anti-diabetic	28 (56)
Insulin	22 (44)
Lipid-lowering	30 (60)
Diuretic	40 (80)

Age, sex, body weight, body mass index (BMI), duration of diabetes, waist circumference and laboratory parameters (fasting glycemia and glycemia 2 h after meal, glycated hemoglobin (HbA1c), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, C-reactive protein, hematocrit, hepatic enzymes (AST – aspartate aminotransferase; ALT – alanine transaminase; GGT – gamma-glutamyl transpeptidase),creatinine clearance and albuminuria in a 24-h urine collection were recorded for each patient as it is custom in diabetic clinic.

The exclusion criteria were previous history of cardiovascular and/or cerebrovascular ischemic events (cerebral lesion recorded on CT scan in previous medical documentation) and use of anti-aggregation therapy. In addition, exclusion criteria were poor insonation via the temporal bone window, moderate or significant stenosis of the main blood vessels in the neck (more than 50%), excluded with extracranial Doppler ultrasound (Aloka 5500 Prosound, Tokyo, Japan), and non-cooperative, unconscious or demented patients. The study was approved by the Ethical Committees of the Dubrava University Hospital and the University Josip Juraj Strossmayer Osijek Faculty of Medicine. All patients provided written informed consent. All women were postmenopausal with no hormone therapies. The exercise status of tested patients was very bad due to not having any other extra activities (like fitness or similar) except normal daily activities which was assessed by taking personal anamnesis.

Breath holding test (BHT): responses of the middle cerebral artery (MCA) to hypercapnia were evaluated by transcranial Doppler ultrasound. The Breath holding test (BHT), was performed through trans-temporal window at a depth of 50 mm, on a TCD DWL Multidop X4 instrument with a 2 MHz hand-held pulsed wave probe (DWL Elektronische Systeme GmbH, Sipplingen, Germany), in the supine position after 5 min bed rest. Each middle cerebral artery was insonated separately using a standard protocol [18] and the mean flow velocity values were recorded during rest and breath holding (breath holding test) for 30 s at the end of normal inspiration. The patients did not hyperventilated before the test. If patients were not able to hold their breath for 30 s, they were suggested to hold it for as long as they could and that time would be taken into a subsequent calculation. Vascular reactivity to hypercapnia was evaluated by calculating the breath-holding index (BHI). The index is obtained by dividing the percentage increase in the mean blood flow velocity that occurs during