Clinical Biochemistry 43 (2010) 882–886

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Soluble receptor for advanced glycation end-products (sRAGE) and polymorphisms of RAGE and glyoxalase I genes in patients with pancreas cancer

Tomáš Krechler [a], Marie Jáchymová [b], Oto Mestek [c], Aleš Žák [a], Tomáš Zima [b], Marta Kalousová [b,*]

[a] Department of Medicine IV-Department of Gastroenterology and Hepatology, 1st Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic
[b] Institute of Clinical Chemistry and Laboratory Diagnostics, 1st Faculty of Medicine and General University Hospital, Charles University, Karlovo nám. 32, 121 11 Prague 2, Czech Republic
[c] Institute of Chemical Technology, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 30 December 2009
Received in revised form 10 March 2010
Accepted 1 April 2010
Available online 14 April 2010

Keywords:
Cancer
Diabetes mellitus
Glyoxalase
Pancreas
Polymorphism
RAGE
Receptor for advanced glycation end products
Soluble receptor for advanced glycation end products
sRAGE

ABSTRACT

Objectives: The receptor for advanced glycation end-products (RAGE) takes part in the pathogenesis of many diseases, including diabetes mellitus and cancer. AGE-precursors are detoxified by glyoxalase (GLO). sRAGE, soluble RAGE, is an inhibitor of pathological effects mediated via RAGE. The aim was to study sRAGE and polymorphisms of RAGE (AGER) and GLO genes in patients with pancreas cancer (PC).

Design and Methods: The studied group consisted of 51 patients with PC (34 with impaired glucose tolerance—IGT, 17 without IGT), 34 type 2 DM and 154 controls. For genetic analysis, the number of patients was increased to 170. Serum sRAGE was measured by ELISA and all polymorphisms (RAGE −429T/C, −374T/A, 2184A/G, Gly82Ser and GLO A419C) were determined by PCR-RFLP and confirmed by sequencing.

Results: Soluble RAGE is decreased in patients with PC compared to patients with DM and controls (975 ± 532 vs. 1416 ± 868 vs. 1723 ± 643 pg/mL, $p<0.001$). Patients with PC and IGT have lower sRAGE levels compared to patients with PC without IGT (886 ± 470 vs. 1153 ± 616 pg/mL, $p<0.05$). No relationship of sRAGE to the stage was found. We did not show any difference in allelic and genotype frequencies in all RAGE and GLO polymorphisms among the studied groups.

Conclusion: This is the first study demonstrating decreased sRAGE in patients with pancreas cancer. Its levels are even lower than in diabetics and are lowest in patients with PC and IGT. Our study supports the role of glucose metabolism disorder in cancerogenesis. Further studies are clearly warranted, especially with respect to potential preventive and therapeutic implications.

Introduction

The receptor for advanced glycation end-products (RAGE), a member of the immunoglobulin super-family, takes part in the pathogenesis of many diseases, among them diabetes mellitus and cancer. RAGE is a multi-ligand receptor, first described as receptor for advanced glycation end products [1], however, binding mediators like proinflammatory S100 proteins/calgranulins (EN-RAGE), High Mobility Group proteins including HMGB1/amphoterin and amyloid β peptide [2]. RAGE–ligand interaction is followed by generation of oxidative stress and triggering of inflammatory and proliferative processes which critically contributes to tissue injury [3].

Advanced glycation end products have many pathological effects and may contribute to malignancies [4,5]. They modify biological structures and change their physical and chemical properties and metabolism. Some of them might cause changes in DNA—glycation of DNA gives rise to characteristic nucleotide adducts, which are associated with mutagenesis and carcinogenesis [6,7]. Stimulation of RAGE probably potentiates the process of growth, infiltration and metastases of tumor via activating nuclear factor κB. Precursors of advanced glycation end products, methylglyxal and glyoxal, are metabolized predominantly by a metalloenzyme glyoxalase I (GLO I). Decreased glyoxalase I activity due to the aging process and oxidative stress results in increased glycation and tissue damage [8]. There is recent evidence that activation of RAGE by S100A12 protein decreases the expression of glyoxalase I and so impairs the enzymatic defence against glycation [9,10].

Another RAGE ligand, HMGB1 is highly expressed in pancreas. In pancreas cancer, enhanced expression of RAGE and HMGB1 was observed for the setting of metastases [2].

sRAGE, soluble RAGE, the extracellular domain of RAGE, is a naturally occurring inhibitor of pathological effects mediated via RAGE. It is a valuable biomarker in many pathological states—it increases in patients with decreased renal function [11], but decreases in diabetic complications [12,14], coronary artery disease [14]. Recently, it was demonstrated as a new biomarker for lung cancer [15].

* Corresponding author. Fax: +420 224962848.
E-mail addresses: marta.kalousova@seznam.cz, mkalousova@hotmail.com (M. Kalousová).

0009-9120/$ – see front matter
doi:10.1016/j.clinbiochem.2010.04.004

The RAGE gene (AGER—gene encoding RAGE) [16] as well as that for GLO I [17] are located on chromosome 6, close to the major histocompatibility complex. Several studies have focused on the genetic background of RAGE and have demonstrated that some gene polymorphisms are implicated in various pathological states, e.g. diabetic complications [18,19], amplification of the inflammatory response [20], non small cell lung cancer [21], gastric cancer [22] or breast cancer [23]. Additionally, the A419C (E111A) polymorphisms of the GLO I gene was studied in patients with breast cancer and the higher presence of the C allele was found in patients with worse prognosis [24].

The aim was to study sRAGE and polymorphisms of RAGE (AGER) and GLO genes in patients with pancreas cancer (PC).

Materials and methods

Study population and design

Fifty one patients with pancreas cancer (34 with impaired glucose tolerance—IGT, 17 without IGT) and thirty four patients with type 2 diabetes mellitus were enrolled in this cross-sectional study. For genetic analysis, the number of patients was increased to 170 (99 patients with PC and 71 patients with type 2 diabetes mellitus).

All patients with pancreas cancer had newly diagnosed tumor. The diagnosis for PC was based on recommended guidelines [25] and all the PC patients had the tumor presence verified by histology (from aspiration biopsy or surgical resection sample). The tumor staging was evaluated by the combination of criteria issued by Union International Contre le Cancer (UICC) and American Joint Committee on Cancer [26]. The studied group of 51 patients contained 8 patients in stage T2, 22 patients in stage T3 and 21 patients in stage T4. In the enlarged group for genetic analysis (99 patients), 17 patients were in stage T2, 34 patients in stage T3 and 48 patients in stage T4.

The diagnosis for type 2 diabetes mellitus was based according to the previously published criteria [27]. All type 2 diabetes mellitus patients had normal renal functions and had not serious cardiopulmonal complications. Their therapy was based on the peroral-antidiabetic drugs: metformin in average daily dose 1700 mg.

One hundred and fifty four unrelated healthy Caucasian subjects served as controls.

Table 1 depicts laboratory characteristics of patients and their comparison with controls.

The study was performed in adherence to the principles of the Declaration of Helsinki and approved by the Institutional Ethical Committee. All patients gave their informed consent prior to entering the study.

Table 1
Laboratory characteristics of studied patients, comparison with healthy controls.

Characteristic	PC	DM	Controls
Number of patients (men/women)	51 (31/20)	34 (24/10)	154 (57/97)
Age (years)	64 ± 11	60 ± 10	57 ± 10***
BMI (kg/m^2)	24.9 ± 5.0	33.2 ± 4.3	25.7 ± 3.3***
Hemoglobin (g/L)	136 ± 17.5	153 ± 14.4	142 ± 10.8***
Leukocytes (×10^9/L)	8.0 ± 2.4	7.8 ± 1.6	6.3 ± 1.5***
Thrombocytes (×10^9/L)	297 ± 82	264 ± 64	261 ± 59*
Bilirubin (μmol/L)	48 ± 66	14 ± 8	12 ± 6***
ALT (μkat/L)	1.4 ± 1.5	0.7 ± 0.4	0.4 ± 0.2***
AST (μkat/L)	0.9 ± 0.8	0.6 ± 0.3	0.4 ± 0.1***
ALP (μkat/L)	3.6 ± 2.7	1.1 ± 0.4	1.2 ± 0.4***
GGT (μkat/L)	6.0 ± 7.6	1.3 ± 1.4	0.4 ± 0.3***
Albumin (g/L)	41 ± 6	48 ± 4	45 ± 3***
Glucose (mmol/L)	7.1 ± 3.2	8.1 ± 2.2	5.1 ± 0.9***
Insulin (mIU/L)	9.2 ± 9.1	14.0 ± 12.3	8.7 ± 5.1 (*N* = 58)**
CRP (mg/L)	31.9 ± 48.4	5.0 ± 4.2	3.5 ± 3.3 (*N* = 64)***
Creatinine (μmol/L)	70 ± 21	81 ± 17	82 ± 15***
Urea (mmol/L)	4.9 ± 2.3	5.6 ± 1.3	4.9 ± 1.2 n.s.
Cholesterol (mmol/L)	5.4 ± 2.5	4.4 ± 1.2	5.5 ± 1.0**
LDL-cholesterol (mmol/L)	3.7 ± 2.4	2.2 ± 0.7	3.2 ± 0.8**
HDL-cholesterol (mmol/L)	0.9 ± 0.4	1.2 ± 0.3	1.7 ± 0.4***
Triacylglycerols (mmol/L)	1.7 ± 0.7	3.2 ± 6.4	1.3 ± 0.8***
AFP (μg/L)	3.4 ± 1.7	3.7 ± 2.2	3.1 ± 1.5 (*N* = 18) n.s.
CEA (μg/L)	16.7 ± 52.7	1.5 ± 1.2	0.8 ± 0.6 (*N* = 18)***
CA 19-9 (kU/L)	10689 ± 42770	8.6 ± 11.1	8.2 ± 7.9 (*N* = 18)***
CA 72-4 (kU/L)	30.1 ± 89.8	1.7 ± 1.4	7.0 ± 13.6 (*N* = 18)***

Data are expressed as mean ± SD (standard deviation).
In healthy controls, laboratory parameters were measured in about 100 subjects if not stated else in the table. In diabetic patients, laboratory parameters were measured 24 patients.
***$p<0.001$, **$p<0.01$, *$p<0.05$ patients versus controls, ANOVA or Kruskal–Wallis test.
Abbreviations: AFP—alfa fetoprotein, ALP—alkaline phosphatase, ALT—alanine aminotransferase, AST—aspartate aminotransferase, BMI—body mass index, CEA—carcinoembryonic antigen, CRP—C-reactive protein, DM—patients with type 2 diabetes mellitus, GGT—gamma-glutamyl transferase, HbA_{1c}—glycated hemoglobin, HDL—high density lipoprotein, LDL—low density lipoprotein, n.s.—not significant, PC—patients with pancreas cancer.

Samples

Blood was collected after overnight fasting via puncture of the cubital vein, simultaneously with blood collection for routine control examinations. Blood count and serum concentrations of routine biochemical parameters were determined in fresh samples. For special biochemical analysis, blood was collected into tubes without anticoagulant, centrifuged for 10 min at 3000 rpm (rotations per minute) and serum was frozen at −80 °C.

For DNA analysis, blood was collected into tubes with EDTA (ethylene diamine tetraacetic acid). Tubes were stored at 4 °C and isolation of DNA was performed within 1 week and kept frozen at −80 °C.

Laboratory analyses

sRAGE

sRAGE was measured with ELISA (enzyme linked immunosorbent assay). Standard kits (Quantikine, RD Systems, Minneapolis, MN, USA) were used according to the protocol of the manufacturer. The plate is coated with monoclonal antibodies against sRAGE and polyclonal antibodies are used for detection. The results are expressed in pg/ml.

RAGE polymorphisms

The gene encoding RAGE (AGER) is located on chromosome 6p21.3 and comprises 11 exons (spanning 3.27 kb). Four single nucleotide polymorphisms (SNPs) of the RAGE gene −429T/C (rs1800625), −374T/A (rs1800624), Gly82Ser (557G/A, rs 2070600) and 2184A/G (rs13209119) were determined from DNA extracted from a sample of peripheral blood.

For amplification of the region containing the −374T/A and −429T/C polymorphisms, the following primers were used: forward primer 5′ GGG GCA GTT CTC TCC TCA CT 3′ and reverse primer 5′GGT TCA GGC CAG ACT GTT GT3′. Polymerase chain reaction (PCR) amplification was conducted in a 25 μL volume containing 100 ng of genomic DNA and 12.5 pmol of each primer. Annealing temperature was 59.5 °C and final extension occurred at 72 °C for 7 min. Restriction analysis was performed with all PCR products using 3 units of restriction nucleases, *Alu*I for −429T/C and *Mfe*I for −374T/A polymorphisms overnight at 37 °C. The restriction products were directly separated by electrophoresis in 3% agarose gel, and visualized in UV light after ethidium bromide staining. Digestion with *Mfe*I revealed fragments 215 and 35 bp for the wild type allele −374T and 250 bp for the mutated allele −374A. After the digestion reaction with *Alu*I fragments 250 bp for −429T allele (wild type) and 88 + 162 bp for −429C allele were detected.

The Gly82Ser (557G/A) polymorphism in exon 3 of the RAGE gene was amplified by PCR using primers 5′GTA AGC GGG GCT CCT GTT GCA′3 and 5′GGC CAA GGC TGG GGT TGA AGG 3′ [28]. After digestion of PCR fragment (397 bp) with *Alu*I we obtained fragments of 149 and