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The significance of lipid peroxidation in cardiovascular disease

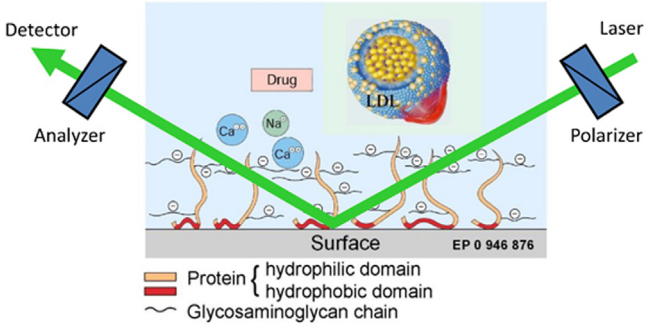
Günter Siegel[a,b,*,1], Eugeny Ermilov[a,1], Axel R. Pries[a], Karl Winkler[c], Annette Schmidt[d], Lovisa Ringstad[e], Martin Malmsten[b,a], Björn Lindman[f,g]

[a] Charité – University Clinic Berlin, Institute of Physiology, 10117 Berlin, Germany
[b] University of Uppsala Biomedical Center, Department of Pharmacy, 751 23 Uppsala, Sweden
[c] University Clinic Freiburg, Institute of Clinical Chemistry, 79106 Freiburg, Germany
[d] University of Münster, Leibniz Institute of Arteriosclerosis Research, 48149 Münster, Germany
[e] Ytkemiska Institutet, Institute for Surface Chemistry, 114 86 Stockholm, Sweden
[f] University of Lund, Physical Chemistry 1, 221 00 Lund, Sweden
[g] University of Coimbra, Department of Chemistry, 3004-535 Coimbra, Portugal

HIGHLIGHTS

- In a clinical trial, metabolic syndrome (MS) patients were treated with *Ginkgo biloba*.
- Ellipsometry, photometric methods and ELISAs were applied for biosensor profiling of MS risk, status and treatment outcome.
- After medication, biomarkers of oxidative stress, inflammation and arteriosclerosis were significantly diminished.
- Multiple correlations unraveled the network of biomarker interactions and demonstrated its usefulness in personalized medicine.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The metabolic syndrome describes a cluster of cardiovascular risk factors that frequently appear together. Its diagnosis is generally based on several well-recognized indicators in clinical practice, such as abdominal obesity, elevated triglycerides, reduced high-density lipoprotein, raised blood pressure, and elevated fasting plasma glucose. Today, decisive importance must be attached to the metabolic syndrome since it leads to increased morbidity and mortality, and thus to a decreased life expectancy, and to higher direct and indirect healthcare costs. This is also due to the fact that its symptomatology irradiates on many organs of the body, which may thereby be damaged.
Methods: In the present clinical trial on 11 metabolic syndrome patients treated with *Ginkgo biloba* (EGb 761, 2 × 120 mg/d) for two months, ellipsometry, fluorescence microscopy, photometric methods, ELISAs and EIAs were applied for biosensor profiling of metabolic syndrome risk, status and treatment outcome.
Results: A spectrum of more than 20 arteriosclerotic, cytokinic, inflammatory, lipidic, and oxidative stress biomarkers served for a detailed diagnosis and therapy monitoring. After medication, the ratio oxLDL/LDL

Abbreviations: ALP, alkaline phosphatase; BP_{dias}, diastolic blood pressure; BP_{sys}, systolic blood pressure; cAMP, adenosine 3′,5′-cyclic monophosphate; cGMP, guanosine 3′,5′-cyclic monophosphate; CREA, creatinine; CS, chondroitin sulfate; GPx, glutathione peroxidase; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; HS, heparan sulfate; HS-PG, heparan sulfate proteoglycan; IDL, intermediate-density lipoprotein; IL-6, interleukin-6; 8-*iso*-$PGF_{2\alpha}$, 8-*iso*-prostaglandin $F_{2\alpha}$; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; oxLDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; SOD, superoxide dismutase; URAC, uric acid; VLDL, very low-density lipoprotein.

* Corresponding author at: Charité – University Clinic Berlin, Institute of Physiology, Charitéplatz 1, D-10117 Berlin, Germany. Tel.: +49 30 450 528 521; fax: +49 30 450 528 920.
E-mail address: guenter.siegel@charite.de (G. Siegel).
[1] These authors contributed equally to this article.

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was reduced by 21.0%, 8-*iso*-$PGF_{2\alpha}$ 39.8%, MPO 29.6%, IL-6 12.9%, hs-CRP 39.3%, Lp(a) 26.3%, MMP-9 32.9%, insulin 9.4%, HOMA-IR 14.0%, ALP 14.8%, CREA 11.3%, URAC 10.6%, *in vitro* modeled nanoplaque formation 14.3% and size 23.4%, whereas SOD was augmented by 17.7%, GPx 11.6%, cAMP 43.5%, and cGMP 32.9%. Special focus was concentrated on the significance of lipid peroxidation for cardio-cerebro-vascular diseases. Through multiple correlations between the biomarkers and clinical parameters, their significance for and involvement in several clinical pictures could be elucidated.

Conclusion: The present clinical observational study was helpful in unraveling this network of biomarker interactions and demonstrated its usefulness for theranostics. For personalized medicine, the selection of the biomarkers is of decisive importance. On the background of a growing obesity among children and adolescents with an increase in prevalence of the metabolic syndrome, diagnosing this syndrome in young subjects may be helpful in identifying a population of risk for increased subclinical arteriosclerosis.

1. Introduction

The metabolic syndrome with its multifacet symptoms is an excellent example for targeting polyorganic impairment such as cardiovascular (cardiac infarction), cerebral (stroke, Alzheimer's), pancreatic (diabetes), hepatic (cholestasis), and gastrointestinal (irritable bowel syndrome) diseases. In the present clinical trial, photometric methods, ELISAs, EIAs and ellipsometry were applied. A wide spectrum of more than twenty oxidative stress, cytokinic, inflammatory, lipidic and arteriosclerotic biomarkers, all interconnected with each other, and navigated by reactive oxygen species (ROS) served for a detailed diagnosis and point-of-care therapy monitoring [1]. Special focus is concentrated on the significance of lipid peroxidation for cardiovascular diseases. As one example, correlations between oxLDL/LDL ratio as well as *in vitro* modeled nanoplaque formation on the one side and various biomarkers on the other are presented.

At an HS-PG-coated silica surface representing a receptor site for specific lipoprotein binding through basic amino acid-rich residues within their apolipoproteins, the binding process was studied by ellipsometric techniques [2]. LDL proved to be deposited strongly at the proteoglycan-coated surface, particularly in the presence of Ca^{2+}, apparently through complex formation 'lipoprotein receptor (HS-PG)–LDL–calcium'. This ternary complex build-up may be interpreted as arteriosclerotic nanoplaque formation on the molecular level before any cellular reactivity, possibly responsible for the arteriosclerotic primary lesion. HDL privileged by a high binding affinity compared with LDL and therefore strongly bound to HS-PG, protected against LDL deposition and completely suppressed calcification of the proteoglycan-lipoprotein complex. In addition, HDL was able to decelerate the ternary aggregational complex deposition. Therefore, HDL attached to its proteoglycan receptor sites is thought to raise a multidomain barrier, selection and control motif for transmembrane and paracellular lipoprotein uptake into the arterial wall.

The processes described take place under *in vitro* and *in vivo* conditions. *In vivo*, the polyanionic and hydrophilic glycosaminoglycan chains dominate the physical properties of the proteoglycans such as endothelial and vascular smooth muscle cell membrane syndecan (HS/CS-PG) and vascular matrix perlecan (HS-PG). Both these macromolecules belong to a class of transmembrane and interfacial matrix polyelectrolytes that control and regulate the transendothelial and paracellular trafficking of blood lipids at the strategic boundary layers of blood–cell (glycocalyx), cell–cell (paracellular adhesive ground and cement substance) and cell–matrix (basal lamina and subbase matrices) barriers [3].

From a series of previous preclinical and clinical studies it appeared, that many biomarkers, e.g., hs-CRP, cytokines, and liver values are up- or down-regulated in the subthreshold range, but so much the more have an important early diagnostic, preventive and prognostic significance [4–9]. The picture is the more complicated in that some inflammatory biomarkers, e.g., MPO and Lp(a) behave inversely with regards to cytokine balance. Nevertheless, it is a general rule for the metabolic syndrome that oxidative stress with its lipid peroxidation dominates decisively nanoplaque formation and size as well as all biomarkers. Our clinical trials with statins [3,4], ginkgo [6,10], ω3-fatty acids [11] and blackcurrant extract [12] unraveled this network of biomarker interactions and demonstrated its usefulness for theranostics.

Moreover, these clinical studies allowed us to characterize the clinical picture of the metabolic syndrome in its facets as well as to represent and develop its clinical sequelae till the cardio-cerebro-vascular events on a time axis (cf. Fig. 1). The importance of oxidative stress (ROS) for this faulty development is clearly recognizable. Nanoplaque formation, precursor of a fully-blown arteriosclerotic plaque, is a biomarker which cannot be measured directly in the patient. Therefore, we tried to prove nanoplaque build-up ellipsometrically, first of all on living human endothelial cells (primary culture) after incubation with oxLDL, and we were successful (cf. Fig. 2). These endothelial cells express the syndecan superfamily as natural lipoprotein receptor. As a next step, we coated the silica surfaces for ellipsometry with the isolated lipoprotein receptor (HS-PG), and could measure atherogenic nanoplaque formation with the lipoprotein fraction VLDL/IDL/LDL derived from the blood of patients [13]. Finally, the determination of additional biomarkers from the blood of the patients as well as the calculation of their interrelations made a further step to personalized medicine feasible.

2. Material and methods

2.1. Subjects and study design

A preventional, randomized, 3-month study comprising a 1-month dietary run-in phase followed by a study treatment period of 2 months was conducted in the Phase I-II study clinic of the UMHAPT "Zaritza Johanna" University Hospital, Sofia, Bulgaria. The project has been reviewed and approved by the local Ethics Committee and the Bulgarian Drug Agency. Eleven patients (2 male, 9 female) with metabolic syndrome aged 26–48 years were recruited, provided that they fell within the additional inclusion criteria smoking (all 11 patients were smokers) and blood lipoprotein(a) [Lp(a)] concentration >30 mg/dL (9 patients). The inclusion criterion smoking was enclosed to clearly demonstrate the antioxidative effect of ginkgo, and Lp(a) > 30 mg/dL was enclosed to confirm the Lp(a) lowering by 23.4% from a preceding ginkgo study in aortocoronary bypass patients (8 patients) [10]. After the first taking of a 45 mL blood sample, the standard therapy of the patients was 2×120 mg/d *Ginkgo biloba* special extract EGb 761 (Rökan novo®: Spitzner Arzneimittel, Ettlingen; Tebonin®: Schwabe Pharmaceuticals, Karlsruhe, Germany) over 2 months. No statins, no calcium antagonists and no nitrate compounds were given. No adverse events occurred and all the patients felt well during and after ginkgo intake [6]. After