EFFECT OF HYPERGLYCAEMIC STATES ON VASCULAR REACTIVITY

Ph.D. thesis

Erzsébet Kocsis, MD

tutor: †Mária Zsófia Koltaí, MD, Ph.D, D.Sc

György Gottsegen National Institute of Cardiology

Semmelweis University School of Ph.D. Studies
School of Theoretic Medicine Program Nr. 5
Budapest 2002.
1. Introduction

Cardiovascular alterations are the principal causes of morbidity and mortality in patients with diabetes mellitus. Accelerated and premature atherosclerosis of the coronary arteries had previously been considered as the major pathogenic factor in diabetes but it has become evident, that the increased cardiovascular risk develops in diabetic patients without any signs of coronary sclerosis. Thus, myocardial alterations due to the pathological changes of the vasculature and the autonomic innervation of the heart should also be considered, suggesting a specific heart disease secondary to diabetes mellitus, determined as diabetic cardiopathy. There is experimental evidence for the development of microangiopathy in the myocardial vasculature. Furthermore, cardiac autonomic neuropathy – a diabetic complication of microvascular origin – proved to be associated with a poor outcome in patients with diabetes. The above data suggest, that both macroangiopathy and microangiopathy could contribute to the increased cardiovascular risk in diabetes.

Clinical studies suggested a major pathogenic role for hyperglycaemia in the development of the specific, microvascular diabetic complications. In case of the macrovascular alterations, other factors of the complex metabolic disorder should also be considered as pathogenic contributors. Numerous data have lead to the conclusion, that metabolic and circulatory disorders due to the insulin-resistant state - such as atherogenic dyslipidaemia, hypertension, rheological changes – are strongly associated with the development of the macrovascular complications and cardiovascular risk is further increased with hyperglycaemia.

In the development of vascular disease, structural changes are preceeded by the pathologic alterations of the vascular reactivity. In experimental, as well as in clinical diabetes before the onset of the detectable morphological changes of the vessel wall characteristic of atherosclerosis and microangiopathy, functional disorders may develop. Resulting in increased contractile responsiveness with a possible concomitant decrease in the vasodilation potential of the different areas of the vasculature. The above changes could result in increased risk of tissue ischaemia in diabetes.

There is experimental and clinical evidence for the pathogenic role of the altered endothelial function in the development of the above early changes in vascular
reactivity in diabetes. Extended investigations suggest that diabetes and hyperglycaemia per se could result in an imbalance of endothelial vasoconstrictor (endothelin-1, angiotensin II, vasoconstrictor prostanoids) and vasodilator (nitric oxide, prostacyclin, endothelium derived hyperpolarizing factor-EDHF) factors. However, in case of the coronary and femoral vasculature – vascular regions with a great clinical importance considering the development of cardiovascular diabetic complications - further investigations are needed to characterize the pathological changes of vascular reactivity due to the endothelial dysfunction induced by diabetes or hyperglycaemia. Moreover, the contribution of the distinct endogenous endothelial vasoactive mechanisms to these functional alterations still aims to be clarified.

2. Objectives

The present study aimed to investigate the pathological changes of vascular reactivity owing to the altered endothelial function in experimental diabetes mellitus. Furthermore, *in vivo* and *in vitro* studies were designed in order to elucidate the possible pathogenic role of hyperglycaemia in the development of the functional changes characteristic of diabetic vascular alterations.

We aimed to study the effects of the alloxan-diabetic state, as well as of a high glucose concentration environment on the endothelium-dependent relaxation of isolated femoral arteries. As a corresponding in vivo model, we aimed to study the effect of acetylcholine on the conductivity of the femoral arterial bed under metabolically healthy, alloxan-diabetic or acute, topical hyperglycaemic conditions. In order to elucidate the possible interaction of the endogenous vasoactive mechanisms (the L-arginine-NO-cyclic guanosine monophosphate: cGMP and the cyclooxygenase pathways) involved, investigation of the effects of cyclooxygenase inhibition on the acetylcholine-induced relaxation was also aimed.

Analysis of the haemodynamic effects of angiotensin II in the coronary circulation, as well as of the effect of this agonist on the prostanoid synthesis of the coronary arteries under metabolically healthy and alloxan-diabetic conditions was also aimed. Previous findings suggested that vascular prostanoid synthesis is modulated by α-
adrenergic mechanisms, thus we aimed to study the effect of \( \alpha \)-adrenergic blockade on the angiotensin II action in these experimental settings.

We aimed to study the possible alterations in the endothelium-dependent relaxation of the coronary arteries in experimental diabetes by analyzing the acetylcholine-induced relaxation of isolated coronaries and by evaluating the coronary conductivity changes \textit{in vivo} in response to this agonist. In order to elucidate the involvement of the distinct endogenous vasoactive mechanisms in the functional changes in coronary reactivity, indirect investigative tools – measurement of the cGMP release, NO synthase and cyclooxygenase inhibition – were also included. We aimed to investigate whether insulin treatment started a week after the diabetes induction or the \textit{in vitro} substitution with L-arginine could prevent or normalize the diabetic changes in coronary reactivity.

We aimed to assess the acetylcholine-induced relaxation in coronary arteries subjected to high glucose concentration \textit{in vitro} or acute, topical hyperglycaemia \textit{in vivo}, in order to elucidate the possible pathogenic role of hyperglycaemia in the alterations characteristic of the diabetic changes in coronary reactivity.

3. Experimental design

3.1. Experimental animals

- studies were conducted in 66 untreated and 17 insulin-treated (0.5-1.5 IU/kg/day sc.) alloxan-diabetic (560 \( \mu \)mol/kg alloxan-monohydrate iv.) and 101 metabolically healthy mongrel dogs of either sex
- investigations were performed 3 months after diabetes induction

3.2. \textit{In vitro} methods

- acetylcholine-induced (3 nml/l-10 \( \mu \)mol/l) endothelium-dependent relaxation was studied by isometric tension recording (F-30 transducer, Hugo Sachs) in isolated vessel preparations. Indirect experimental methods - cyclooxygenase blockade (indomethacin, 3 \( \mu \)mol/l) and NO synthase inhibition (N\( \text{\textsuperscript{o}} \)-nitro-L-arginine, 100 \( \mu \)mol/l) - were used in order to elucidate the pathogenic role and
the possible interactions of the endogenous vasoactive mechanisms contributing to the alterations in vascular reactivity

- basal and acetylcholine-stimulated (0.1-10 µmol/l) prostanoid synthesis of the coronaries isolated from metabolically healthy and diabetic dogs was studied by radioimmunoassay (Amersham)
- basal and angiotensin II-stimulated (50 nmol/l) prostanoid synthesis of the coronaries isolated from metabolically healthy and diabetic dogs was also studied by radioimmunoassay

3.3. In vivo methods

- in pentobarbital-anaesthetised (133 µmol/kg iv.) dogs mean arterial blood pressure, heart rate (Statham P23Db), femoral and coronary blood flow (Gould SP 2202 electromagnetic flowmeter) were monitored
- vasoactive substances and glucose were delivered as intraarterial infusions (STC 526 pump, Terumo) via a catheter (Vygon 13210) introduced directly into the femoral artery (Seldinger’s technique) or inserted through a diagonal coronary branch

4. Results

4.1. Summary of the in vitro findings

- under cyclooxygenase blockade, 3 µmol/l prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) induced significantly stronger vasoconstriction in diabetic femoral arteries compared with metabolically healthy controls
- high in vitro glucose concentration (25.5 mmol/l) resulted in increased PGF<sub>2α</sub>-induced contractions in femoral arteries isolated from normoglycaemic control dogs, either before or after indomethacin administration
- acetylcholine-induced endothelium-dependent relaxation of the femoral arterial rings did not differ under diabetic and control conditions, however, high in vitro
glucose concentration resulted in diminished relaxation to acetylcholine; indomethacin had no effect on this phenomenon.

- endothelium-dependent relaxation of coronary arterial rings to acetylcholine proved to be diminished in alloxan-diabetes, furthermore, coronary rings from alloxan-diabetic dogs showed decreased basal, as well as acetylcholine- or L-arginine (1 mmol/l) potentiated cGMP release, compared with metabolically healthy controls.
- insulin treatment, initiated 1 week after the induction of diabetes, significantly improved the vasodilatory reactivity of coronary arteries; acetylcholine-induced relaxation of the coronary arterial rings isolated from the insulin-treated dogs was not different from the metabolically healthy controls.
- high in vitro glucose concentration resulted in a diminished acetylcholine-induced relaxation of the coronary arteries isolated from metabolically healthy dogs.
- cyclooxygenase inhibition further decreased the relaxation to acetylcholine in the untreated diabetic group only, in contrast, no significant effect could be detected in the normoglycaemic or hyperglycaemic preparations.
- angiotensin II potentiated the PGI₂ release of coronary arteries isolated from metabolically healthy but not from the alloxan-diabetic dogs; α-adrenergic blockade (phentolamine, 5 μmol/l) prevented the above increase in PGI₂ release in controls, but had no effect on the prostanoid synthesis of the diabetic coronaries.

4.2. Summary of the in vivo findings

- the dose-dependent increase in femoral conductivity induced by intraarterial acetylcholine infusion (2.25-36 pmol/kg/min) did not differ significantly among the alloxan-diabetic, normoglycaemic or topical hyperglycaemic metabolically healthy groups; cyclooxygenase inhibition (indomethacin, 10 μmol/kg) had no effect on this phenomenon.
- the dose-dependent increase in coronary conductivity induced by intraarterial acetylcholine infusion proved to be diminished in alloxan-diabetic, as well as in
topical hyperglycaemic dogs, compared with normoglycaemic, metabolically healthy controls

- in contrast, insulin-treated alloxan-diabetic dogs showed normal coronary vasodilation to acetylcholine

- intracoronary administered indomethacin diminished the vasodilation induced by acetylcholine in the untreated alloxan-diabetic group only, resulting in a decrease in coronary conductivity in response to intraarterial acetylcholine infusion in diabetic animals

- the dose-dependent increase in mean arterial blood pressure induced by intracoronary angiotensin II infusion (63-1000 pmol/kg) proved to be more prominent in diabetic animals compared with metabolically healthy controls; α-adrenergic blockade (phentolamine, 2 μmol/kg) further increased the pressor effect in the diabetic dogs, but had no significant effect on the reactivity of the healthy controls

- intracoronary angiotensin II infusion resulted in a dose-dependent increase in coronary conductivity and the responses were not different in the alloxan-diabetic and the metabolically healthy groups; however, α-adrenergic blockade diminished the angiotensin II-induced increase in coronary flow in controls, in contrast, no such effect could be seen in case of the diabetic animals

5. New findings

- PGF$_{2\alpha}$-induced vasoconstriction increased, and the endothelium-dependent relaxation to acetylcholine decreased in healthy femoral arterial rings subjected to high glucose concentration in vitro; cyclooxygenase inhibition failed to influence the above alterations

- hyperglycaemic insults – as well as the alloxan-diabetic state – resulted in a diminished acetylcholine-induced coronary vasodilation either in vitro or in vivo, however, different alterations could be seen after indomethacin administration under the above pathological conditions: cyclooxygenase inhibition further decreased the vasodilatory responses to acetylcholine in the untreated diabetic but not in the topical hyperglycaemic group
insulin treatment – initiated 1 week after diabetes induction - prevented the diabetic impairment in the acetylcholine-induced endothelium-dependent coronary vasodilation

- α-adrenergic blockade diminished the angiotensin II-induced increase in coronary conductivity in metabolically healthy dogs, in contrast, no such effect could be seen in case of the diabetic animals

- in concert, angiotensin II potentiated the PGI₂ release of coronary arteries isolated from metabolically healthy but not from the alloxan-diabetic dogs; α-adrenergic blockade diminished the above difference between the two investigated groups

The above findings indicate, that both experimental diabetes and acute, topical hyperglycaemia may lead to alterations in the endothelial modulatory function. Resulting in altered vasoconstictor responsiveness and vasodilatory potential. However, the pattern of the dysfunction seems not to be identical in the two investigated pathophysiological states, and its development could be dependent on the vascular region studied.
6.1. Related publications


6.2. Other publications


