Medical Hypotheses 93 (2016) 16–20

Contents lists available at ScienceDirect

Medical Hypotheses

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

Could angiotensin-(1–7) be connected with improvement of microvascular function in diabetic patients? Angiotensin-(1–7) iontophoresis may provide the answer

Aleksandar Kibel *

Department for Heart and Vascular Diseases, Internal Medicine Clinic, Osijek University Hospital, Croatia
Department of Physiology and Immunology, Faculty of Medicine, University of Osijek, J.Huttlera 4, 31000 Osijek, Croatia

ARTICLE INFO

Article history:
Received 30 November 2015
Accepted 12 May 2016

Keywords:
Diabetes mellitus
Angiotensin-(1–7)
Iontophoresis
Endothelial dysfunction
Microcirculation

ABSTRACT

Diabetes mellitus, a metabolic disorder with significant global health care burden, causes chronic microvascular and macrovascular complications that still comprise a therapeutic challenge. Angiotensin-(1–7), a heptapeptide with vasodilatory properties, has been found to restore vascular reactivity and endothelial cell function, mostly in experiments on larger isolated animal vessels and in cell cultures. The presented hypothesis suggests that angiotensin-(1–7) might have beneficial effects on microvascular function that is damaged in diabetic patients, alleviating endothelial dysfunction and increasing microvascular reactivity to various vasoactive agents in diabetes. It is further proposed that iontophoresis with angiotensin-(1–7) might be used to explore this potential beneficial effect, as well as provide a possible future therapeutic delivery method for angiotensin-(1–7). Since other peptides and proteins have been previously tested and used in iontophoretic transdermal delivery, it is plausible that angiotensin-(1–7) would be a suitable candidate for transdermal iontophoretic application for research (and potentially therapeutic) purposes. If confirmed, the delineated hypothesis would have immense implications for more effective care of diabetic patients, as well as for better understanding of microcirculatory pathophysiological mechanisms in diabetes.

Introduction

Diabetes mellitus, a complex metabolic disorder with a stunningly significant global burden that has risen from 30 million in 1985 to 382 million in 2014 [1], represents a continuing challenge to health care providers worldwide. Leading to microvascular and macrovascular complications, diabetes can cause tissue hypoxia and ischemia, accelerate atherosclerosis and is a well-known risk factor for coronary artery disease and stroke [2–5]. Numerous studies documented that diabetes leads to impaired vascular function – increased reactivity to physiological vasoconstrictors and reduced reactivity to vasodilators [6–12], with chronic disturbances in cerebral blood flow autoregulation [13]. In diabetic rat models, vasodilation to acetylcholine is impaired, as well as arteriolar flow-dependent dilation [11,14,15], whereas contraction to noradrenalin in aorta, skeletal arteries and mesenteric arteries in such rat models is increased [7,16,17]. Studies in diabetic human subjects found microvascular dysfunction (such as reduced responses to acetylcholine or sodium nitroprusside), as evidenced by methods investigating changes in skin microcirculatory blood flow [18].

The heptapeptide angiotensin-(1–7) (ANG-(1–7)), with amino acid sequence Asp-Arg-Val-Tyr-Ile-His-Pro, is mainly a metabolite of angiotensin II, formed primarily by *angiotensin converting enzyme 2* (although there are alternative pathways, for instance through neutral endopeptidase). It is a vasodilatory peptide, with opposite signaling functions to angiotensin II [19–22]. Endogenously and exogenously applied ANG-(1–7) was demonstrated in animal experimental models to improve the damaged vascular reactivity to various constrictors and dilators in diabetes (such as on carotid and renal vascular rings and in the mesenteric artery) and to facilitate recovery from ischaemia–reperfusion injury in hearts of diabetic rats [23,24]. ANG-(1–7) reduces oxidative stress-induced DNA damage in diabetes [25] and attenuates renal nephropathy and inflammatory responses [26]. The synthetic ANG-(1–7) agonist NorLeu(3)-ANG-(1–7) accelerates healing of diabetic wounds [27]. However, there don't seem to be any studies investigating a potential beneficial effect of ANG-(1–7) on

* Address: Department for Heart and Vascular Diseases, Internal Medicine Clinic, Osijek University Hospital, J.Huttlera 4, 31000 Osijek, Croatia.
E-mail addresses: alekibel@mefos.hr, aleksandar_mf@yahoo.com

http://dx.doi.org/10.1016/j.mehy.2016.05.010
0306-9877/

improvement of microvascular function in diabetes mellitus patients.

To test changes of microvascular function in human subjects, iontophoretic techniques have been used to deliver certain agents into the skin and to measure resulting blood flow alterations. For instance, iontophoretic application of vasodilators acetylcholine (ACh) or sodium nitroprusside (SNP) and measurement of blood flow changes using laser-doppler flowmetry, is being readily used in investigations of microvascular reactivity and endothelial dysfunction [28–30]. Iontophoresis is a process in which ions flow through a medium driven by an applied electric field. Except for research purposes, iontophoresis has a prospect to be clinically used for transcutaneous drug delivery, by utilizing a minimal amount of current (which was found to affect the skin permeation process) on ionized molecules [31–33]. By iontophoretically administering vasoactive substances and measuring real-time blood flow changes, it is possible to non-invasively assess microcirculatory function [28]. Laser-doppler measurements of post-occlusive reactive hyperemia (for instance after temporary external compression of the arm with a blood pressure cuff) give insight into endothelium-dependent vasodilation, analogous to flow-mediated brachial artery vasodilation – but at the capillary and arteriolar level [28,34].

The hypothesis

Firstly, ANG-(1–7) could have beneficial effects on microvascular function that is damaged in diabetic patients. ANG-(1–7) would, under this hypothesis, lead to a measurable improvement of microvascular function in diabetic patients, leading to alleviation of endothelial dysfunction and to increased microvascular reactivity to various vasoactive agents, such as acetylcholine.

Secondly, ANG-(1–7) iontophoresis in diabetic patients could be used to test this assumption and to measure a likely improvement of microvascular reactivity. By using iontophoresis to deliver the ANG-(1–7) peptide subcutaneously to microvasculature within a defined skin area, ANG-(1–7) could then influence microvascular reactivity. This can be subsequently measured using laser-doppler flowmetry and iontophoretic application of acetylcholine on the same area of the skin (detecting the increase in cutaneous blood flow after acetylcholine application) or using laser-doppler flowmetry after transient external vascular compression (reactive hyperemia). There seem to be no records of ANG-(1–7) being applied through iontophoresis, but it is possible since even larger peptides (such as insulin) have been previously readily used in iontophoretic transfer, as discussed later. Fig. 1 schematically depicts the transcutaneous iontophoretic use of ANG-(1–7) in testing improvement of microvascular function induced by ANG-(1–7).

Evaluation of the hypothesis

Lacking experimental evidence in diabetic patients, but considering previous data in animal experimental models in improvement of endothelial function [23–27], the hypothesis postulates that ANG-(1–7) – endogenous or exogenous – has a beneficial effect in human microvasculature, the function of which is damaged in diabetes. Perhaps because of yet unknown safety of clinical trials with systemic ANG-(1–7) application to diabetic patients, or possibly because of the relative recentness of the recognition of ANG-(1–7) as a potentially beneficial agent, it seems that to date there were no studies that investigated the effect of ANG-(1–7) on microvascular reactivity in diabetic patients. Furthermore, the second part of the hypothesis suggests that a very promising methodology might be the iontophoretic application of ANG-(1–7) – which would be a very useful initial evaluation because this proposed method limits a direct systemic effect of ANG-(1–7) and focuses on local (and therefore safer) effects in human patients (the quantity of locally applied ANG-(1–7) would be small to induce only a limited local effect in the monitored skin segment). It would be a non-invasive procedure, which contributes to patient compliance. On the one hand, the dilatory effect on microvasculature of ANG-(1–7) itself could be quantified with iontophoretic application and laser-doppler flowmetry (a possible way to test direct microvascular reactivity to ANG-(1–7) itself). On the other hand, after applying ANG-(1–7) iontophoretically into the patients skin, the standard acetylcholine and sodium nitroprusside tests of microvascular function can be performed (as well as reactive hyperemia tests) to investigate the improvement of endothelial function induced by the applied ANG-(1–7).

In addition to the aforementioned studies that determined improvement of endothelial dysfunction in diabetic animal models, there are some other investigations that speak in favor of the possible ANG-(1–7) beneficial effect on microvascular reactivity stipulated in the first part of the hypothesis. Namely, improvement of endothelial function mediated by ANG-(1–7) treatment was also demonstrated in other experimental models, such as in apolipoproteinE knockout mice, which are a model of accelerated atherosclerosis [35]. Even in animal models, most vascular functional studies have been performed using larger vessels. There are very limited hints on its potential effects in microcirculation. Intravenous infusion of ANG-(1–7) was found to recruit rat hind limb muscle microvasculature (it increases muscle microvascular blood volume and microvascular blood flow) and to exert insulin-sensitizing effects in rat muscle [36]. In studies on random pattern skin flaps with nicotine-induced ischemia in rats, ANG-(1–7) increased microvascular dilatation in nicotinized flaps, triggered angiogenesis, and contributed greatly to flap survival [37]. The ANG-(1–7) signaling axis prevents lipopolysaccharide-induced apoptosis of pulmonary microvascular endothelial cells, as recently shown in experiments on rat cell cultures [38]. It also improves function of mouse pancreatic islet microvascular endothelial cells (including an increase in activity of the Akt/endothelial NOS/nitric oxide pathway) [39] and it improves angiogenesis in corpus cavernosum of diabetic mice [40]. ANG-(1–7) counteracts angiotensin II-induced dysfunction and oxidative stress in human cerebral endothelial cells [41]. This data would be in concordance with the proposed hypothetic beneficial role of ANG-(1–7) in microvascular function of diabetic patients and form the basis on which the hypothesis evolved.

Although there don't seem to be studies using transdermal delivery of ANG-(1–7) yet, hypothetic use of ANG-(1–7) in this manner seems very feasible considering that various proteins and peptides have already been used in iontophoretic transcutaneous transfer, including insulin, luteinizing hormone–releasing hormone [42,43], growth hormone releasing factor analogs [44], botulinum toxin [45], arginine vasopressin [46] and others. Iontophoretic delivery of proteins is restricted mostly to molecules with a molecular weight of up to 10–15 kDa [32]. The amount of iontophoretic transdermal delivery of a certain protein/peptide is proportional to the applied current, but depends also on a variety of other parameters such as the physicochemical properties of the protein (size, overall charge, structure, and lipophilicity) and the experimental parameters employed (current density, duration of application, electrodes employed) [32]. The small size of the 7-amino acid ANG-(1–7) peptide certainly represents an advantage.

Depending on the isoelectric point of the used protein, it will have a potentially different overall charge in relation to the pH of the formulation [32]. Since anodal iontophoresis is more effective than cathodal iontophoresis (owing to the negatively charged surface of skin and the bulk solvent flow from anode to cathode), proteins with a high isoelectric point value are better for iontophoretic