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Short- and long-term modulation of microvascular responses in streptozotocin-induced diabetic rats by glycosylated products

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Abstract

Objective: This study aimed to determine the role of early and late glycation products in modulating inflammation in early diabetes. **Materials:** Sprague–Dawley rats (130–170 g) were injected with streptozotocin (75 mg/kg, ip) and treated with daily aminoguanidine (AG, 25 mg/kg, ip) or vehicle for 2 or 4 weeks. **Methods:** The base of a vacuum-induced blister raised on the hind paw was perfused with substance P (SP, 1 μM) and sodium nitroprusside (SNP, 100 μM). Changes in blood flow and plasma extravasation (PE) were measured. Amadori (1 mg/ml), advanced glycation end products (AGEs, 10 mg/ml), and anti-RAGE IgG (antibody against AGE receptors, 100 μg/ml) were individually perfused prior to SP. **Results:** In diabetic rats, responses to SNP and SP were reduced by 60% and 70%, respectively ($P<.05$). Amadori increased responses to SNP by 50% and 90% and to SP by 70% and 80% in control and diabetic rats, respectively (both $P<.05$). SP responses were significantly increased after anti-RAGE IgG (70%) or AG treatments (175%) with PE responses normalized. **Conclusion:** Amadori and anti-AGE agents enhance peripheral vascular responses in diabetes and may ameliorate microvascular damage.

Keywords: Streptozotocin; Neurogenic inflammation; AGEs; Amadori; Aminoguanidine

1. Introduction

The glycation process is initiated when proteins and lipids react with aldose sugars nonenzymatically to form Schiff bases. These intermediate products undergo reversible rearrangement to form amadori products, which can further rearrange, though irreversibly, to form advanced glycation end products (AGEs). This natural biochemical reaction occurs in a slow rate under normal ambient sugar concentrations but is enhanced in diabetes (Ahmed & Ahmed, 2006; Wautier & Schmidt, 2004). AGEs and high levels of early glycation products are present in the skin of diabetic patients (Yu et al., 2006) and in multiple tissues of diabetic rats (Crijns, Struijker Boudier, & Wofflenbuttel, 1998; Rumble et al., 1997; Ryle, Leow, & Donaghy, 1997).

Previous studies suggested that diabetic vascular and neuronal abnormalities may be linked to early and late glycation products (Cameron, Gibson, Nangle, & Cotter, 2005). It is postulated that late glycation products (or AGEs) promote many of the chronic diabetic vascular complications (Forbes, Fukami, & Cooper, 2007; Nawale, Mourya, & Bhise, 2006; Stitt, 2005). This notion is supported by the induction of cardiac and renal damage resembling that of diabetes in nondiabetic rats and rabbits (Li et al., 1996) by the short-term administration of AGE-modified albumin; AGE interactions with widespread receptors for AGEs (RAGE) can generate a pro-inflammatory environment, causing functional impairment and vascular damage (Lalla, Lamster, Stern, & Schmidt, 2001; Ramasamy et al., 2005).

Glycation products are capable of generating reactive oxygen species either themselves or by interacting with RAGE (Ramasamy et al., 2005; Wautier & Schmidt, 2004). Amadori products are a potent source of hydrogen peroxide in vitro (Elgawish, Glomb, Friedlander, & Monnier, 1996), and when AGE interacts with RAGE on vascular matrix,

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superoxide anions are formed (Ramasamy et al., 2005). Superoxide anions and products of hydrogen peroxide cause nitric oxide (NO) inactivation and endothelial dysfunction (Wautier & Schmidt, 2004).

AGEs can also impair cellular antioxidant defense mechanisms (Shin, Oh, & Park, 2006; Ulusu et al., 2003). It has been suggested that the increased vascular permeability in diabetes induced by AGE–RAGE interactions involving erythrocytes could be mediated by oxygen free radicals (Vallejo et al., 2000) as this effect was inhibited by antioxidants both in vitro and in vivo (Bonnardel-Phu, Wautier, & Vicaut, 2000). Further endothelial function defects that can be induced by AGEs are induction of vasoconstrictory endothelin-1 (Quehenberger et al., 2000) and pro-inflammatory vascular cell adhesion molecules (Schmidt, Crandall, Hori, Cao, & Lakatta, 1996).

Evidence that both RAGE receptor antagonists [i.e., soluble RAGE (sRAGE) and antibody against RAGE (anti-RAGE IgG)] can block AGE–RAGE interaction and ameliorate vascular damage also exists (Guo et al., 2005; Wautier et al., 1996). Indeed, the presence of sRAGE for 48 h, in vivo, corrected the enhanced vascular permeability in diabetic animals (Wautier & Guillausseau, 1998).

Aminoguanidine (AG) is a nucleophilic hydrazine compound that inhibits AGE formation (Chang et al., 2006). However, AG does not reduce the formation of early glycation products such as Schiff bases and amadori products; neither can it scavenge superoxide anions in the streptozotocin (STZ) diabetic rats (Nyengaard, Chang, Berhorst, Reiser, & Williamson, 1997). AG, in vivo, acts as an antioxidant, quenching hydroxyl radicals and lipid peroxidation in cells and tissues (Giardino, Fard, Hatchell, & Brownlee, 1998).

Retardation of AGE formation in experimental diabetic animals can prevent diabetic complications (Ahmed & Ahmed, 2006; Cameron et al., 2005; Chang et al., 2006; Jerums, Panagiotopolous, Forbes, Osicka, & Cooper, 2003). AG has been effective in diabetic rat models using short- and long-term treatment with AG (Soulis-Liparota, Cooper, Vranes, Bucala, & Jerums, 1996). In this study, the long-term treatment retarded metabolic and renal structural changes more than the short-term intervention. It was suggested that generation of AGEs in diabetic rat kidneys was time dependent and closely linked to the development of experimental diabetic nephropathy.

As poor wound healing is a feature (human and animal) of diabetes (Brem & Tomic-Canic, 2007), skin microvascular structure and function and AGE effects are relevant. Although reduction in skin blood flow in early diabetes has been previously documented (Hill & Larkins, 1989), no studies to date have examined the role of AGEs in modulating skin microvascular blood flow in early diabetes. In the present study, short- and long-term effects of amadori and AGEs (early and late glycation products) in modulating skin microvascular blood flow in 4 weeks diabetic rats were examined. It was hypothesized that AGEs may reduce microvascular blood flow in early diabetes and its deleterious effects may be inhibited by early AGE-based interventions. In this study, microvascular responses in diabetic rats are examined after short-term exposure of their microvasculature to amadori and AGEs and after long-term pretreatment with AG to prevent AGE formation.

We used exogenous perfusion of substance P (SP) to induce inflammatory responses. Exogenous SP acts directly on endothelium, activating the cGMP pathway and releasing NO, which diffuses into smooth muscle cells, leading to relaxation and vasodilation (Brain & Williams, 1988; Khalil & Helme, 1989). SP also degranulates mast cells, releasing histamine, which can act upon sensory nerves, causing the release of more neuropeptides. Vasodilatation responses and plasma extravasation (PE), when applicable, were used as outcome measures.

2. Materials and methods

2.1. Materials

Pentobarbitone sodium (Nembutal, 60 mg/ml) was from Boehringer Ingelheim Pty. Ltd. (Australia). SP (Auspep Pty. Ltd., Melbourne, Australia) and sodium nitroprusside (SNP; Sigma-Aldrich, St. Louis, MO, USA) were dissolved in Ringer's solution. AG hydrochloride from Sapphire Biosciences Pty. Ltd. (Alexandria, Australia) was dissolved in sterile saline. Amadori products, AGEs, and anti-RAGE IgG, provided by Peptech (Dee Why, Australia) and STZ (Sigma-Aldrich), were dissolved in 0.1 M sodium citrate buffer (pH 4).

2.1.1. AGEs, amadori, and anti-RAGE synthesis

Amadori, AGEs, and anti-RAGE IgG were provided by Peptech (Dee Why, Australia). The protocol for the synthesis of the above substances is briefly described.

2.1.1.1. Production of AGEs and amadori by Peptech. For the generation of AGEs, glucose-6-phosphate (G-6-P) and a 5 weeks incubation time were used, and for amadori production, D-glucose and a 1 week incubation were used; otherwise, they were made by the same protocol. The method was performed in a sterile environment. Glucose was filter sterilized using a 0.2 μm filter. Working in a tissue culture hood, the weighed out bovine serum albumin (BSA) powder was dispensed gradually into a sterile beaker of 200 ml sterile phosphate-buffered saline (PBS) and stirred into solution. Two hundred microliters of trasylol, 800 μl phenylmethylsulfonyl fluoride, 4 ml ethylenediaminetetraacetic acid, and 200 mg ampicillin were added and filter sterilized with a 0.2 μm filter. Five milliliters of filtered 2 M glucose was dispensed into three 50 ml tubes (set up in triplicates) and subsequently added up to 50 ml with the rest of the filtered ingredients. A fourth 50 ml tube was prepared without any glucose, that is, BSA only. Samples were incubated in a 50°C water bath for a required time according