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Original article

Structural modifications in the arterial wall during physiological aging and as a result of diabetes mellitus in a mouse model: Are the changes comparable?

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Abstract

Aim. – Vascular accelerated aging represents the major cause of morbidity and mortality in subjects with diabetes mellitus. In the present study, our aim was to compare premature functional and morphological changes in the arterial wall resulting from streptozotocin (STZ)-induced diabetes mellitus in mice over a short-term period with those that develop during physiological aging. The effect of aminoguanidine (AG) on the prevention of these alterations in the diabetic group was also analyzed.

Methods. – The vascular relaxation response to acetylcholine (ACh) in the mouse was tested in isolated segments of phenylephrine (Phe)-precontracted aorta at 2, 4 and 8 weeks (wk) of STZ-induced diabetes and compare to 12- and 84-wk-old mice. Aortic structural changes were investigated, and receptor for AGE (RAGE) aortic expression was quantified by western blot.

Results. – Compared to the 12-wk control group ($76 \pm 5\%$), significant endothelium-dependant relaxation (EDR) impairment was found in the group of 12-wk-old mice, which underwent a 4-wk diabetes-inducing STZ treatment (12wk-4WD) ($52 \pm 4\%$; $P < 0.01$) and was yet more apparent in the group of 16-wk-old mice, which underwent an 8-wk diabetes-inducing STZ treatment (16wk-8WD) ($34 \pm 4\%$; $P < 0.001$). The alteration in EDR was relatively comparable between the diabetic 12wk-4WD group and the 84-wk-old group (52.7 ± 4 vs. $48 \pm 4\%$). Intima/media aortic thickening and aortic structural changes were significantly increased in the diabetic 12wk-4WD group and were even more apparent in the 84-wk group compared to the 12-wk controls. AG treatment in the 12wk-4WD + AG diabetic group significantly improved EDR, decreased RAGE expression and showed an aging preventive effect on the structural changes of the arterial wall.

Conclusion. – Our study compared EDR linked to physiological aging with that observed in the case of STZ-induced diabetes over a short-term period, and demonstrated the beneficial effect of AG.

Keywords: Vascular aging; Diabetes; Endothelium; Intima/media; Vasodilatation

Résumé

Les modifications de la paroi vasculaire liées au vieillissement et celles liées au diabète sont-elles comparables ?

But. – Le vieillissement vasculaire accéléré induit par le diabète représente la principale cause de mortalité et de morbidité chez le patient diabétique. Notre travail avait pour but de comparer les anomalies fonctionnelles et morphologiques artérielles chez des souris rendues diabétiques par la streptozotocine (STZ) à celles observées au décours du vieillissement physiologique. L'effet préventif de l'aminoguanidine sur ces anomalies a également été analysé.

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Méthodes. – L'analyse de la vasorelaxation induite par l'acétylcholine a été testée sur des anneaux aortiques précontractés à la phényléphrine issus de souris âgées après deux, quatre et huit semaines de diabète, et issues de souris âgées de 12 et 84 semaines. Les modifications morphologiques aortiques ont été évaluées parallèlement et l'expression du récepteur des produits de glycation avancée (RAGE) au niveau aortique a été quantifiée par western blot.

Résultats. – Comparativement aux souris témoins âgées de 12 semaines (12 s) (76 ± 5 %), la relaxation endothélium dépendante (*endothelium-dependant relaxation* [EDR]) était significativement diminuée chez les souris après quatre semaines de diabète (12 s, 4SD) (52 ± 4 % ; $P < 0{,}01$) et dans le groupe huit semaines de diabète(16 s, 8SD) (34 ± 4 % ; $P < 0{,}001$). Les anomalies d'EDR étaient comparables entre ces souris (12 s, 4SD) et celles observées chez des souris âgées de 84 semaines (52,7 ± 4 vs 48 ± 4 %). L'épaisseur intima/média et les modifications morphologiques étaient significativement augmentées chez les souris (12 s, 4SD) et plus encore chez les souris âgées de 84 semaines par rapport aux souris témoins (12 s). Le traitement par aminoguanidine des souris diabétiques âgées de quatre semaines a amélioré les anomalies d'EDR, diminué la surexpression de RAGE et prévenu partiellement l'apparition des modifications morphologiques.

Conclusion. – Notre étude a comparé les anomalies EDR liées à l'âge et celles liées à un diabète induit par la STZ chez la souris et suggère un effet bénéfique de l'aminoguanidine dans la prévention de ces anomalies.

Mots clés : Vieillissement vasculaire ; Endothélium ; Intima/média ; Diabète ; Vasodilatation

1. Introduction

The incidence of cardiovascular diseases (CVD) tends to increase with age, and risk factors such as smoking, hypertension, hypercholesterolemia, renal failure, obesity and diabetes may be implicated in the initiation and progression of atherosclerotic lesions [1]. Diabetic patients are up to four times more likely than non-diabetics to suffer from CVD [2]. In terms of CVD risk, it has recently been reported that diabetes is associated with the equivalent of a 15-year aging risk [3]. While a strong association between glycemic control and CVD has been reported in certain epidemiological studies [4] the mechanisms whereby diabetes leads to an increased risk of premature CVD are still not fully understood.

Endothelial dysfunction (ED), which has been described both in the case of diabetes and also physiological aging, may play an early key role in the pathogenesis of CVD. The presence of ED in the coronary or peripheral circulation constitutes a risk factor for CV events independent of the development of arteriosclerosis or other vascular complications [5–7]. Endothelial-dependent relaxation (EDR) in response to acetylcholine (ACh) decreases in the rat aorta and in the large vessels in humans during aging [8,9]. The age-related decrease in EDR varies from one vessel to another, and from one species to another [10]. In both human and animal models of type-1 and type-2 diabetes mellitus, ED is characterized by an impaired endothelium-dependent vasodilatory response to different agonists [11]. In a previous study on physiological aging carried out on C57B/J6 mice, which do not develop hypertension, hypercholesterolemia, renal failure, obesity or diabetes during aging, we demonstrated that the maximal ACh-induced relaxation decreased significantly between the 12- and the 84-week (wk) mice [12]. This functional age-related ED was associated with major histological changes of the arterial wall.

In the present work, we aim to compare premature functional and morphological arterial wall changes brought about by STZ-induced diabetes over a short-term period with those that develop in the course of physiological aging. Because physiological aging and diabetes share common metabolic pathways including advanced glycation end products (AGEs) and oxidative stress pathways, we also investigated the effect of aminoguanidine (AG), which is known for its anti-oxidative and AGE inhibitory properties.

2. Materials and methods

2.1. Animals

C57B/J6 male mice were obtained from Janvier Laboratories (Le Genest-St-Isle, France). Six mice per group were analyzed for the study of aortic reactivity, and five different mice per group were used for the immunohistological investigations. The mice were housed and fed (water was provided ad libitum) as previously described [12]. All experiments had been authorized by the local ethical committee and were performed in strict accordance with the guidelines issued by the National Institutes of Health and the French Department of Agriculture.

Diabetes was induced by administering 180 mg/kg i.p. streptozotocin (STZ) (Sigma-Aldrich, Saint Quentin Fallavier, France) in pH 4.5 citrate buffer. After 72 hours, fasting blood glucose levels were monitored by a One-Touch blood glucose meter (Lifescan, Milpitas, CA, USA). If blood glucose levels were < 200 mg/L, the mice then received a second similar injection of STZ. Blood glucose and blood HbA_{1c} (high performance liquid chromatography, Bio-Rad Laboratories, Munich, Germany) were measured at sacrifice.

The different experimental groups consisted of the following:

- Control group: 12-wk-old mice: 12 wks;
- Physiological aging group: 84-wk-old mice: 84 wks;
- STZ-induced diabetes group: 8-wk-old mice were treated with STZ and left in stand by, during the development of STZ-induced diabetes over a period of 2, 4 or 8 wks: group (10wk-2WD), group (12wk-4WD), group (16wk-8WD);
- STZ-induced diabetes + AG treatment group: 8-wk-old mice were treated with STZ-induced diabetes and AG treatment and left in stand by over a period of 4 wks, (12wk-4WD + AG). AG (Sigma-Aldrich) was added to the drinking water (to obtain the relevant administration of 50 $mg.kg^{-1}day^{-1}$) 48 hours after the induction of diabetes by STZ.