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Chronic type 1 diabetes in spontaneously hypertensive rats leads to exacerbated cardiac fibrosis

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Abstract

Introduction: Diabetes in human subjects is often associated with hypertension. The aim of this study was to examine the development of cardiac fibrosis following induction of type 1 diabetes in genetically hypertensive rats. **Methods:** Diabetes was induced by streptozotocin (STZ) injection in 8-week-old normotensive Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs) for a duration of 16 or 24 weeks. Aged-matched, nondiabetic WKY and SHRs were used as controls. At termination of treatment, the rats were anaesthetized, hearts arrested in diastole and perfusion fixed. A comprehensive examination of cardiac fibrosis throughout the right and left ventricles was undertaken in picrosirius red-stained sections, using image analysis and by undertaking collagen type I and type III immunohistochemistry. **Results:** Induction of diabetes in the SHRs led to a marked increase in the levels of interstitial fibrosis in the left ventricle plus septum (LV+S) at both 16 and 24 weeks duration (59% and 43% increase, respectively) and also in the right ventricle after 24 weeks duration of diabetes (35% increase compared to the nondiabetic SHR). Exacerbated perivascular fibrosis was also observed in the LV+S in the diabetic-hypertensive rats at the later time point. These effects of induction of diabetes were not observed in the normotensive strain. **Conclusions/Interpretation:** Our findings clearly demonstrate elevations in cardiac fibrosis when type 1 diabetes is combined with hypertension. Our findings thus stress the importance of closely monitoring both blood pressure and glucose levels in type 1 diabetic patients in order to prevent myocardial collagen deposition.

Keywords: Cardiac fibrosis; Diabetes type 1; Heart; Hypertension; SHR; WKY

1. Introduction

The prevalence of cardiac failure in patients with diabetes is about fivefold higher than in nondiabetic patients [1,2]. This is thought to be due, in part, to the comorbidity of hypertension in many of these patients with the incidence of hypertension in the diabetic population approximately twice that found in the nondiabetic population [3]. In patients with type 1 diabetes, hypertension is often secondary to the development of diabetic nephropathy [4]. Diabetes alone can lead to adverse effects on the heart, but when combined with hypertension, the severity of these conditions is greatly increased [5–7]. The combination of these two diseases can lead to marked adverse changes to the structure of the heart, which detrimentally impacts on cardiac function and contributes to the increased risk of heart failure [8,9]. In human and in experimental animal studies, hypertension combined with diabetes leads to exaggerated left ventricular hypertrophy [10,11] which is a known predictor of cardiac dysfunction and mortality [12].

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It is important to determine whether the structural remodeling that occurs in the hypertensive-diabetic heart is due to increased levels of fibrosis, as this can lead to accumulation of advanced glycation end products (AGEs) [13,14] and multiple adverse effects on the mechanical and electrophysiological properties of the myocardium [15–18]. Cardiac fibrosis can manifest as interstitial, perivascular, or reparative fibrosis [19]. Induction of myocardial fibrosis ultimately leads to diastolic stiffness of the cardiac muscle [20,21], impairment of electrical conductivity [15,16], and after prolonged periods of collagen accumulation, systolic dysfunction and reduced ejection fraction may also result [22,23]. In addition, it has been shown that collagen is particularly susceptible to irreversible AGE modification in the setting of hyperglycemia [24]; increased production and accumulation of AGEs are strongly linked to end-organ damage in diabetes [25].

Hence, in order to prevent the end-organ damage and cardiac dysfunction in hypertensive-type 1 diabetic subjects, it is imperative to determine the combined effects of type 1 diabetes and hypertension on the development of cardiac fibrosis. Importantly, in previous experimental studies, a marked increase in reparative fibrosis, associated with myocardial infarcts, has been reported in rats, when streptozotocin (STZ) type 1 diabetes is combined with renovascular hypertension [26]. However, it must be kept in mind when interpreting the data from these studies that induction of two kidney–one clip renovascular hypertension leads to a reactive rise in circulating angiotensin II levels [27]. Indeed, it is well known that high levels of angiotensin II can induce cardiac fibrosis in both the right and left ventricles [20,28]. Whether type 1 diabetes combined with non-renin-dependent hypertension also leads to exacerbated cardiac fibrosis is currently unknown and is of clinical importance.

Another well-described model of combined type 1 diabetes and hypertension is the induction of STZ type 1 diabetes in the spontaneously hypertensive rat (STZ-SHR) [29]. The SHR is a genetic model of hypertension where circulating levels of angiotensin II are usually not elevated above normal [30]. The STZ-SHR model closely resembles hypertensive type 1 diabetic patients whereby there is cardiac remodeling (hypertrophy and dilation) [29] as well as concomitant albuminuria and nephropathy [31]. We have previously shown in this model that the development of cardiac hypertrophy is independent of angiotensin II [32].

The aim of the present study was to examine levels of perivascular, interstitial, and reparative fibrosis in the hearts of SHRs following long-term exposure (16 and 24 weeks duration) of STZ-induced diabetes. In this model, Kubota et al. [33] reported increased perivascular fibrosis and focal necrosis in the diabetic-hypertensive heart at 12 weeks of age, whereas Pijl et al. [29] reported no change in the relative cardiac collagen content compared to nondiabetic SHRs after 8 weeks duration of diabetes. However, to date, the long-term effects of combined type 1 diabetes and hypertension on the development of cardiac fibrosis in the adult heart have not been investigated in this model and are the focus of the current study. Importantly, in this study, we have undertaken a comprehensive examination of cardiac fibrosis by systematically sampling both the right and left ventricles, beginning at the top of the ventricles through to the apex. In using this approach, the right ventricle (RV) has acted as an internal negative control for hemodynamic factors such as elevated ventricular systolic and/or diastolic pressures. Alternatively, the right ventricle acts as a positive control for circulating substances that might mediate a response independent of ventricular pressure.

Immunolocalization of monocyte/macrophages within the hearts of the experimental rats was also investigated at the early experimental time point, since infiltration of macrophages into tissues is implicated in the induction of fibrosis [34].

2. Methods

2.1. Animals

Male SHR and Wistar–Kyoto (WKY) rats were obtained at 7–8 weeks of age (weighing between 200 and 250 g) from the Australian Resource Centre, Perth. All rats were housed in groups of two or three with a 12-h day/night cycle. Food and water were administered ad libitum. The animal experiments were approved by the Austin and Repatriation Medical Centre Animal Ethics Committee. Care and treatment of the animals conformed with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.2. Streptozotocin-induced diabetes

Diabetes was induced through a single tail-vein injection of STZ (Sigma Chemical Co., St Louis, MO, USA) in randomly assigned fasting WKY rats and SHRs aged between 7 and 8 weeks. The SHRs received 45 mg/kg and the WKY rats received 50 mg/kg STZ in sodium citrate buffer at pH 4.5. Nondiabetic control WKY rats and SHRs received sodium citrate buffer only.

Rats that received STZ were tested 2–3 days after the injection for an increase in blood glucose. Rats with a blood glucose reading above 15 mmol/L were classified as diabetic and any rat failing to become diabetic was withdrawn from the study.

Diabetic WKY and SHRs received 2 and 4 U, respectively, of Ultratard insulin (Novo Nordisk, Bagsvaerd, Denmark) daily to increase the rate of survival for the time period of the study, without normalizing their blood glucose levels. The amount of insulin given was adjusted to maintain blood glucose levels between 25 and 30 mmol/L. Rats were killed at either 24 or 32 weeks of age after 16 and 24 weeks duration of diabetes, respectively (in the diabetic treatment groups). The number of rats in each treatment group ranged between six and eight animals per group (eight rats in the