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THERMAL BEHAVIOR OF THE HEART OF SHR AND WISTAR RATS

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Hypertension is a major and growing public health problem. It is responsible for the mortality of millions of people around world, and is increasing each year. Nevertheless, the understanding of the relationship between the composition of the heart's cells, hypertension and health diseases is still very incomplete. The present study focuses on the evaluation of the attributes of some hearts of Spontaneous Hypertension Rats (SHR), comparing with SHR which received additional amounts of polysaccharide (SHR+P) and with wistar rats (normal blood pressure) by Thermal Analysis. Some differences could be seen between groups, as the residue content after 800°C was different for rats from different groups, and of wistar rats hearts samples showed 5.3±0.3% of residues *vs.* 8.3±1.5% of the SHR. DSC profiles for wistar rats showed one intense endothermic event at 160°C, with enthalpy transition of 450 J g^{-1} and more three small events. Thermal analyses curves also showed some differences between freezing and no freezing samples, probably associated to the denaturation of proteins and degradation of organic materials.

Keywords: *heart, hypertension, thermal behavior*

Introduction

Cardiovascular diseases are the major cause of death in the world [1]. They are influenced and accelerated by the presence of cardiovascular risk factors such as hypertension, smoking, diabetes mellitus, genetic, hypercholesterol, aging and other environmental factors [2–6]. In 2001, cardiovascular diseases were responsible for 27.4% of deaths in Brazil [7]. According to the American Heart Association, cardiovascular disease was the cause of 950 thousand deaths in the USA during the same period. Myocardial infarction, for example, affects approximately 1.1 million American people each year, with a mortality rate of 50% [3].

Hypertension is a major and growing public health concern due to its high prevalence and complications [1, 5, 8]. Recently, data from the Framingham Heart Study showed that the risk of developing hypertension throughout life is nearly 90% among men and women from 55 to 65 years of age. Hypertension contributes to increasing cardiovascular problems and in accelerating the aging process of the heart often and is often caused by: significant myocyte loss, hypertrophy of remaining cells, and an increase in collagen content [2, 9]. Cell loss can be a critical variable in the development of heart dysfunction and failure, while at the same time, an important risk factor for sudden cardiac death. The incidence increases with rising in blood pressure [1, 2, 9].

The heart's muscular wall (myocardium) is composed of cardiac muscle cells arranged in complex spirals around the chamber of orifices [10, 11]. The cardiac muscle is classified as a striated muscle, which is composed of two types of myofilaments (thin and thick filaments) responsible for muscle cell contraction [12]. Thin filaments are composed of the proteins actinin, tropomyosin and troponin associated with α-actinin. The thick filaments are composed of the myosin protein, among others. Almost half the volume of the cardiac muscle cell is occupied by mitochondria, attesting to its great energy consumption [10]. Sarcoplasm is relatively abundant and rich in glycogen. In the regions of sarcoplasm, there are often a few droplets of lipid and, in older animals, deposits of lipofuchsin pigment [11]. Both the glycogen and the lipid may be used as sources for the contractile activity of the myocardium [10, 11]. Because the oxygen requirement of cardiac muscle cells is high, they contain an abundant supply of myoglobin. Calcium ions play an important role in the forces that bind cells together but the majority of Ca^{2+} is transported into the cardiac muscle cell from extra-cellular fluid compartments [10].

The understanding of the composition of the heart's cells, hypertension, and health diseases is still very incomplete. Rats have been widely used in studies on cardiovascular systems because there is a general similarity between the cardiovascular system of rats and men [13].

The present work aims to study the attributes of some hearts of Spontaneous Hypertension Rats (SHR), comparing them with SHR which received additional amounts of polysaccharide (SHR+P) and with wistar rats (normal blood pressure) by Thermal Analysis.

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Experimental

Rats

Male Spontaneous Hypertension Rats (SHR) and wistar rats (normal blood pressure) obtained from colonies maintained at the State University of Rio de Janeiro, Brazil were used in this study. The University Standing Committee on Animal Research had approved the protocols. The investigation conforms to the 'Guide for the Care Use of Laboratory Animals' published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985).

Rats were housed in polypropylene cages in three groups of five animals (total of fifteen animals): SHR animals (SHR group), SHR animals fed with an addition of polysaccharides by gavage (SHR+P group) and wistar rats (wistar group).

All of the rats were fed with a standard rat diet (Nuvilab) and unlimited water. They were kept in a controlled temperature (21±1°C) and a humidity-controlled (60±10%) room while being submitted to a 12 h light, a dark cycle (artificial lights) and to an air exhaustion cycle (15 min h^{-1}). From 3 to 6 months of age months blood pressure (PA) was measured by plethysmography. At six months of age rats were sacrificed after anesthesia with ether. The thorax was opened exposing the heart; where it was injected with 1 mL of 10% KCl. Small parts of the rats' heart (part of left ventricle) were analyzed by Thermal Analysis.

Thermal analysis

The thermal behavior – Thermogravimetry (TG), Derivative thermogravimetry (DTG) and Differential thermal analysis (DTA) – was observed in a TA Instruments; model SDT 2960, at a heating rate of 10°C min^{-1} in nitrogen atmosphere, from 30 to 800°C.

Differential scanning calorimetry (DSC) was performed on a TA Instruments, model 2010. Samples were heated from 30 to 400°C and used in a heating rate of 10°C min^{-1} in a nitrogen atmosphere.

Statistical analysis

Differences between groups, two by two, were tested with nonparametric *t* test (unpaired test) and Welch's correction with the significant level of $P = 0.05$ [9].

Results and discussion

Blood pressure was measured during the three months of experiments with SHR rats shown in Fig. 1. We observed that rats from SHR group had higher systolic blood pressure (170–190 mmHg) than rats from the SHR+P group (160 mmHg) which received additional amounts of polysaccharide by gavage. Wistar rats presented the lowest pressure among all the groups (130 mmHg) during all the experiment.

We carried out TG/DTG and DTA analyses for five heart samples per group and picked out one of them to show here. Figure 2 shows TG/DTG and DTA curves for a single heart sample of rat from SHR group. The TG curve shows two decomposition stages. The first stage occurs at 70% of mass loss until 150°C and can be related to serum decomposition. The second degradation stage occurs at 240 (Tonset) to 400°C, with 20% of mass loss, suggesting the decomposition of proteins, lipids, glycogen, and polysaccharides, among others. At 800°C, it was observed a residue of 8.3±1.5% was observed, probably due to inorganic substances like calcium, iron, magnesium and others. A DTG curve exhibited three mass degradation stages until 140°C (70, 120 and 140°C) and various degradation stages above of 300°C. The DTA profiles showed two endothermic events close to 70 and 120°C.

TG/DTG and DTA curves for a single heart sample of rat from SHR+P group are illustrated in Fig. 3. TG curves also shows two decomposition stages. However, there was 70% of mass loss at the first stage, which occurs until 170°C. At 800°C, only 6.6±1.2% residue was observed, which could be linked with the presence of less calcium amount in the heart cells of rats showing

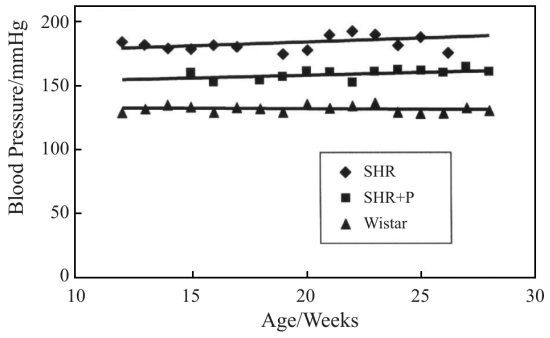

Fig. 1 Medium blood pressure measured of SHR group, SHR+P group (rats fed with an addition of polysaccharide) and wistar group

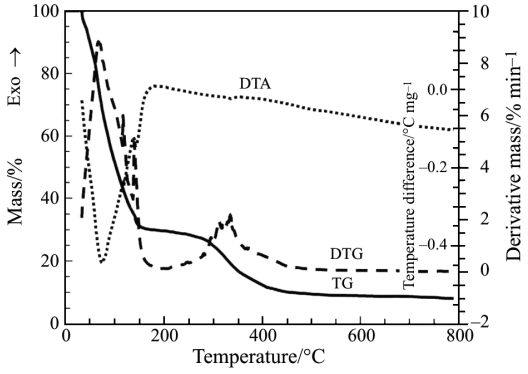

Fig. 2 TG/DTG and DTA curves for SHR heart samples (SHR group)

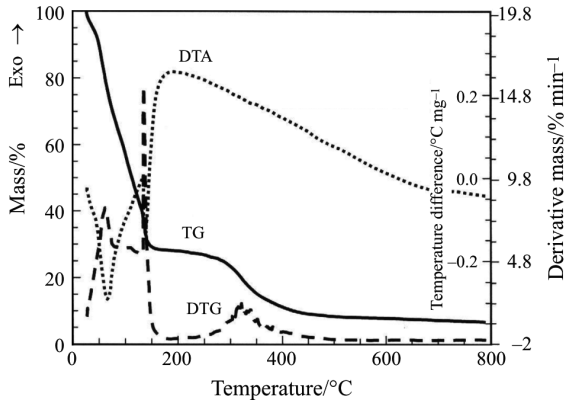

Fig. 3 TG/DTG and DTA analyses of the gavaged SHR heart samples (SHR+P group)

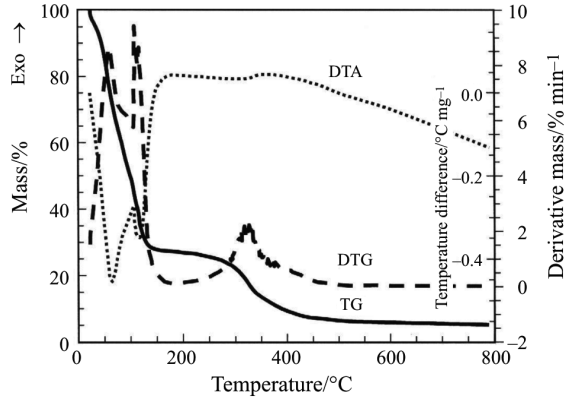

Fig. 4 TG/DTG and DTA curves for wistar rats heart samples (wistar group)

low hypertension rates. The DTG curve shows mass degradation stages at 62, 108 and 135°C and various degradation stages at 280 to 400°C.

Figure 4 shows TG, DTG and DTA curves for a single heart sample of a rat from the wistar group. The TG curve for heart samples of wistar rats also shows two decomposition stages, however, at 800°C only 5.3±0.3% of residue can be seen, which can be linked with the presence of less calcium (or other inorganic

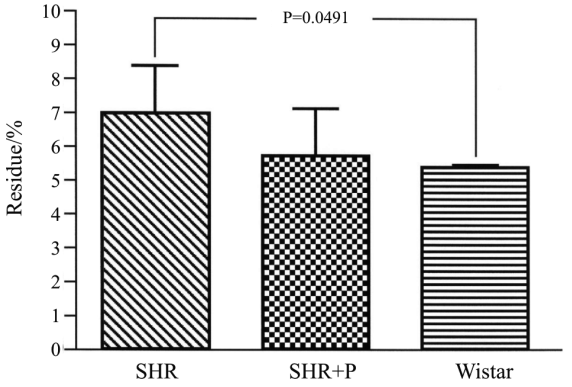

Fig. 5 Plot for SHR, SHR+P and wistar rats heart samples of the residue at 800°C after thermal analyses. Differences between SHR and wistar groups were statistically significant (P<0.05)

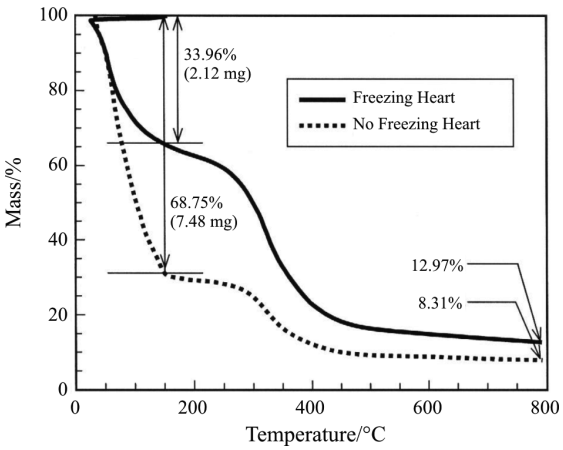

Fig. 6 Comparison of TG curves for freezing SHR and no freezing hearts samples

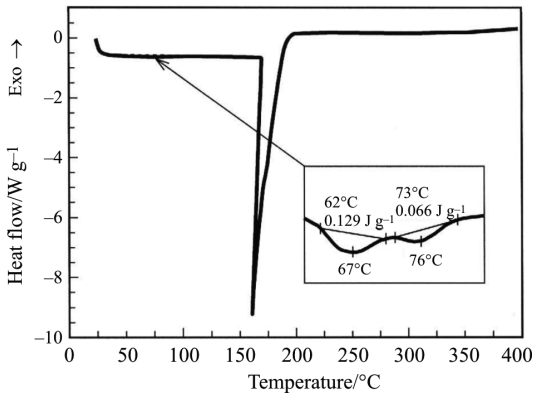

Fig. 7a DSC analysis of wistar rat heart samples . Zoom: endothermic events (proteins)

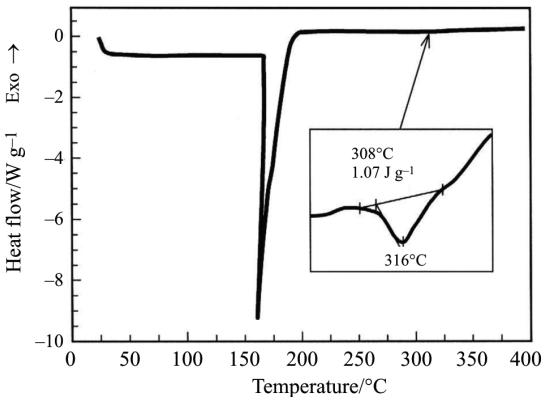

Fig. 7b DSC analysis of wistar rat heart samples. Zoom: endothermic events (lipids)

compound) amount in the heart cells of rats which shows low hypertension rates.

Some differences were observed between hearts samples from rats from the same group. Consequently, we did statistic tests for the residue results, which can be seen in Fig. 5. Differences were statistically significant only between SHR and wistar groups (P=0.0491). Nevertheless, we observed the tendency

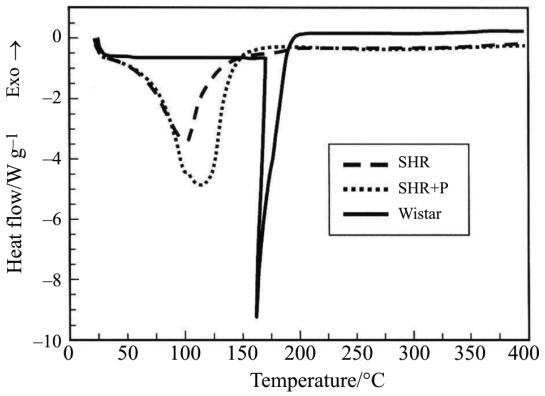

Fig. 8 DSC curves of SHR (freezing), SHR+P group and wistar rats heart samples

of having fewer amount of residue in groups that showed lower blood press.

A comparison of TG curves for SHR freezing (for 2 months) and no freezing heart samples is presented in Fig. 6. It also shows two decomposition stages at around 100 and 300°C. While for SHR freezing heart samples a mass loss of approximately 30% at 150°C was observed, for SHR no freezing heart it was at around 70%, suggesting the presence of a larger amount of blood serum in no freezing sample [14]. The loss mass at 300°C can be related to the glycogens and proteins. The curves SHR freezing heart shows 13% residue and SHR no freezing heart only shows 8.3±1.5 % of residue.

Figures 7a and 7b show DSC curves for heart samples of wistar rats. We observed one intense endothermic event at 160°C, with an enthalpy transition of 450 J g^{-1}. When we studied this curve (Fig. 7a) more carefully, we saw two other endothermic events at 67 and 76°C. These events were, probably, related to the thermal denaturation of protein compounds. This denaturation corresponds to an irreversible change of tertiary protein structure but it is not accompanied by the breakage of the polypeptide chain. A large number of hydrogen bonds were breaking at this temperature range which leads the proteins into the denaturation state [15]. Figure 7b shows the endothermic event that occurred at 316°C. This event suggests the presence of lipids and fats in the wistar rats' hearts samples [16].

Figure 8 presents DSC curves of SHR (freezing) and wistar rats' heart samples. Endothermic evenbe seen at 100, 120 and 160°C of SHR, gavaged SHR (SHR+P) and wistar rats hearts samples, respectively. These events may be attributed to organization systems, suggesting the melting temperature (T_m) of the glycogens, polysaccharides or proteins.

Conclusions

The hypertension effects for the heart cells have been widely discussed. Additionally, it is known that the higher the blood pressure, the more the injuries occur in the cardiovascular system. This study showed that TG/DTG, DTA and DSC profiles for the heart of SHR in comparison with SHR rats which received polysaccharide by gavage and wistar rats showed some differences, it was mainly in residue (wistar rats showed 5.3±0.3%, SHR+P 6.6±1.2% and SHR rats 8.3±1.5% of residue) and constituent content. Therefore, more studies need to be done to elucidate these variations. It was observed that the hearts showed one principal endothermic event (T_m) 160°C with enthalpy transition of 450 J g^{-1}; and other three small events related to protein denaturation and the degradation of lipids. Thermal analysis curves showed differences between freezing and non-freezing samples, probably associated to the denaturation of proteins and degradation of organic materials, so in the future it will be necessary to be more careful about freezing samples for posterior analysis. Undoubtedly, thermal analysis is a potential technique for this study.

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