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Title:

Adriamycin-induced cardiomyopathy can serve as a model for diabetic cardiomyopathy-A hypothesis

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Abstract

Diabetic cardiomyopathy is one of the life threatening complications of diabetes. A number of animal models are being used for studying diabetic cardiomyopathy. In laboratory animal models, induction of cardiomyopathy happens in two stages: first being the induction of diabetic condition and the second being the induction of cardiomyopathy by prolonging diabetic condition. It takes a longer time to develop diabetes with the limited success rate for development of cardiomyopathy. Adriamycin is an effective anti-cancer drug limited by its major side-effect cardiomyopathy. A number of features of Adriamycin treatment mimics diabetes. We postulate that Adriamycin-induced cardiomyopathy might be used as a model system to study diabetic cardiomyopathy in rodents since a number of features of both the cardiomyopathies overlap. Left ventricular hypertrophy, systolic and diastolic dysfunction, myofibrillar loss, and fibrosis are hallmarks of both of the cardiomyopathies. At the molecular level, calcium signaling, endoplasmic reticulum stress, AGE activation, mitochondrial dysfunction, inflammation, lipotoxicity and oxidative stress are similar in both the cardiomyopathies. The signature profile of both the cardiomyopathies shares commonalities. In conclusion, we suggest that Adriamycin induced cardiomyopathic animal model can be used for

studying diabetic cardiomyopathy and would save time for researchers working on cardiomyopathy developed in rodent using the traditional method.

1. Introduction

Diabetes is one of the alarming diseases in the developing and developed world. It affects a number of organ systems including kidneys, liver, eyes, reproductive system, heart *etc.* Diabetic cardiomyopathy is one of the leading causes of death compared to other complications of diabetes [1]. The incidence of diabetic heart failure is correlated with increase in age, blood pressure, weight and cholesterol levels [2]. It is characterized by a series of alterations in structure and functions of the heart, without a coronary artery disease and hypertension, ultimately leading to heart failure. Pathophysiology includes left ventricular hypertrophy, systolic and diastolic dysfunctions [3].

Adriamycin is an anthracycline class of anti-cancer drug. In a rodent model, the drug induces a variety of symptoms which coincide with type II diabetes [4]. One of the major side effects of the drug is cardiomyopathy [5]. Since cardiomyopathy being a complication of diabetes, we compared both the cardiomyopathies (*i.e.*, Diabetic cardiomyopathy and Adriamycin-induced cardiomyopathy). Surprisingly, we observed striking commonalities between both. In the current article, we discuss similarities between both the cardiomyopathies and therefore we postulate that Adriamycin cardiomyopathy could serve as a model system to study diabetic cardiomyopathy. Adriamycin induced cardiomyopathy has several advantages over the currently used model systems to study diabetic cardiomyopathy.

2. Diabetic cardiomyopathy

Diabetic cardiomyopathy is characterized by changes in cardiac functions such as systolic and diastolic dysfunction, left ventricular hypertrophy, fibrosis [3], increased left ventricular mass [6] *etc.* At the cellular level, there is a profound change in expression of NF κ B [7], increased level of cardiac troponin 1 levels are prominent [8]. Mitochondrial dysfunction [3] and myofibrillar disarray [8] are also observed in diabetic cardiomyopathy.

3. Adriamycin-induced cardiomyopathy (AiC)

The ultrastructural changes in cardiomyocytes during AiC include swelling of mitochondria [9], cytoplasmic vacuolization [9], loss of myofibril [9], changes in lysosomal number [10], chromatin decondensation [11], reduction in contractile force [11], shrinkage of nucleoli, disruption of cytoskeleton, and disruption of mitochondrial network [12]. Some of the crucial genes involved in myocardial functions such as desmin, troponin- I , troponin-C, α -actin, α -tropomyosin, myosin light and heavy chains gets deregulated [13,14], leading to the changes in the physiology and metabolic function of heart such as left ventricular dysfunction, systolic and diastolic dysfunction, increased left ventricular thickness [15-17].

4. Comparison Diabetic cardiomyopathy VS. Adriamycin induced cardiomyopathy

4.1. Similarities in circulatory profile of diabetic cardiomyopathy and Adriamycin cardiomyopathy

During diabetic cardiomyopathy there is elevated glycemic level, lipidemic level [3] and pro-inflammatory cytokines [18]. Correspondingly, the notable changes found in circulation in doxorubicin treated condition would cause hyperglycemia, hyperlipidemia [4] and elevated pro inflammatory cytokines level includes IL-1 and TNF- α [19].

4.2. Similarities between diabetic cardiomyopathy and Adriamycin cardiomyopathy at molecular level

In both type 1 and type 2 diabetes condition, ER stress is one of the factors leading to apoptosis of cardiomyocytes, ultimately resulting in cardiomyopathy. Elevated levels of unfolded protein response (UPR) signaling proteins such as glucose regulated protein and caspase12, act as a biomarker for ER stress, have been observed in diabetic cardiomyopathy [20,21]. Similarly, there is also an elevated level of UPR signaling protein as well as caspase 12 in AiC [22].

The altered expression of micro RNA is found in both diabetes and doxorubicin cardiomyopathies. In diabetic cardiomyopathy, following miRNAs have been found to be elevated which includes miR-1, miR-133, miR-206, miR-320, miR-21, miR-223, miR-141, miR-195, miR-199a-3p, miR-700, miR-142-3p, miR-24, miR-21, miR-221, miR-499-3p, miR-208a, miR-705 and decreased level of miR-195, miR-199a-3p, miR-700, miR-142-3p, miR-24, miR-21, miR-221, miR-499-3p, miR-208a and miR-705 [23,24]. Under doxorubicin treatment, the upregulation of miR-146a, miR-367, miR-215, miR-216b, miR-208b, miR-34c cause apoptosis in cardiomyocytes, further it leads to form cardiomyopathy [25,26].

Epigenetic modifications are found in both the histone 3 and CpG island in diabetic cardiomyopathy, whereas in case of doxorubicin cardiomyopathy, there is no evidence of epigenetic modification. Autophagy and mitophagy are commonly observed in diabetic cardiomyopathy. Mitochondria are the power house of the cell. It has the capability to produce more ROS during diabetic cardiomyopathy which would lead to mitochondrial damage further is cleared by autophagy. Some of the atg genes are altered in both diabetic and doxorubicin-induced cardiomyopathy conditions [27,28].

In both cases of cardiomyopathies, oxidative stress is one of the contributing factors of the pathophysiology. NADPH oxidase, xanthine oxidase and some of the enzymes of electron transport chain in mitochondria are increased during both the cases of cardiomyopathies [29,30]. Advance glycation endproduct (AGE) enhances NADPH oxidase through a series of events. In both the cases of cardiomyopathies elevated the level of AGE has been observed [31,32]. Apparently, ROS induced by hyperlipidemia and dyslipidemia, plays the crucial role in the development of both the cardiomyopathies [3,4]. Thus, ROS leads to enhanced cell death. The outcome of oxidative stress is fibrosis, hypertrophy, apoptosis, alterations in calcium homeostasis, endothelial dysfunction, accumulation of extracellular matrix, and lipotoxicity [5,33].

In type 1 and type 2 diabetes, there is a reduction in glucose uptake, glycolysis and pyruvate oxidation [34]. Therefore, heart switches to enhanced fatty acid metabolism to meet its energy demands [35]. Adipose derived fatty acids serve as the substrate during this metabolic shift [36]. It is almost the same series of events during Adriamycin treatment [37,38]. The circulatory profile during both diabetic and Adriamycin cardiomyopathies exactly overlap.

Triglyceride, long-chain fatty acid, cholesterol and transporters of lipids such as LDL and VLDL are elevated during both the conditions [39,40]. In both the cases, lipotoxicity is observed in cardiomyocytes along with ceramide accumulation [41,42].

Expression of intracellular cell adhesion molecule-1 such as interleukin-1 β and interleukin-6 are increased in both the cases of cardiomyopathies [43,44]. Interestingly, proinflammatory transcription factor NF- κ B is elevated [45,46] with elevated macrophage infiltration in both the cases [19,47].

5. Rodent models of diabetic cardiomyopathy

There are several rodent models being used to investigate diabetic cardiomyopathy. Beta cell over-expression of calmodulin [48], non-obese diabetic [49], BB rat [50], Streptozocin [51], Alloxan [8], Akita [52], ob/ob mice [43], db/db mice [53], Otsuka Long-Evans Tokushima Fatty rat [54], Zucker fatty/Zucker diabetic fatty rats [55], Goto-Kakizaki rats [56] are among the frequently used models.

Developing diabetic cardiomyopathy involves two stages: (1) inducing diabetes (2) developing cardiomyopathy. Diabetic cardiomyopathy using Streptozocin follows the same rule. Similarly, Akita diabetic mice model also takes 5-6 weeks to develop hyperglycemia [57]. Ob/Ob mice also take as high as 15 weeks just to develop hyperglycemic condition [53]. Further, it would take a few more weeks to develop cardiomyopathy. The db/db mice take at least 8 weeks to develop the diabetic condition and would, therefore, take further time to show the signs of cardiomyopathy [53]. In the Western diet-induced diabetic model it takes 20 weeks to develop cardiac dysfunction [58]. In Otsuka Long-Evans Tokushima Fatty rats it takes 18 weeks to induce hyperglycemia [54]. The Bio Breeding rats take 60-120 days to induce diabetes. WBN/Kob rat takes 21 weeks of age to induce glucose intolerance and glucosuria [59]. Zucker diabetic fatty rat model takes 6 to 8 weeks to induce diabetes in males and 9 to 11 for females [60,61]. The disruption of islets and fibrosis is found in six-month-old ESS-rat [62,63]. In Bureau of Home Economics rats, to induce diabetic conditions like hyperglycemia and

hyperinsulinemia, it takes 50 days of age and the diabetic condition occurs only at maturity level [54]. Non-obese diabetic mice take 100 to 200 days to induce diabetic condition [64]. Sand rat forms diabetes in 2-3 months [65]. In summary, all the diabetic rodent models take longer period of time to develop the diabetic condition and further prolongation may induce cardiomyopathy.

Therefore, Adriamycin would be a better alternative to the aforementioned cardiomyopathic models. Most importantly at 4th week of Adriamycin administration, there is a pronounced incidence of cardiomyopathy [66].

6. Conclusion

Diabetic cardiomyopathy is one of the life threatening complications associated with diabetes. Even though a number of animal models for diabetic cardiomyopathy are available, each one of them has its own limitations. Previously we reported that doxorubicin treatment mimics type 2 diabetic condition. Literature survey reveals striking similarities in the key hallmarks of diabetic cardiomyopathy and Adriamycin induced cardiomyopathy. The circulatory profile in both the cases is marked with hyperlipidemia with prominent elevation in fatty acid levels. Inflammatory cytokines are elevated in both the cases which seem to have important roles in the progression of both the types of cardiomyopathies. At the organ level, literature survey reveals left ventricular dysfunction as a result of left ventricular hypertrophy, which is a significant feature of both the cardiomyopathies. Cardiac relaxation is hampered in both the cases. At molecular mechanistic level also there are quite a lot of similarities such as endoplasmic reticulum stress, impaired calcium signaling, Renin angiotensin aldosterone system activation, lipotoxicity. Therefore, we hypothesize that doxorubicin in rodents might also be used for studying the complications of diabetic cardiomyopathy. Relatively, there are a number of advantages in using doxorubicin such as rapid induction of cardiomyopathy and cost effectiveness. The simple and effective model would enable a number of research groups to explore the disease and therefore, a better understanding of the complication which would speed up the process of identifying better treatment strategies.

Conflict of interest statement

We declare that we have no conflict of interest.

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