Heart Failure & Cardiomyopathy

C57BL/6 Mice are More Appropriate than BALB/C Mice in Inducing Dilated Cardiomyopathy with Short-Term Doxorubicin Treatment

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Objective: To develop an appropriate method to induce the model of dilated cardiomyopathy in mice with doxorubicin.

Methods and Results: Twenty C57BL/6 mice and twenty BALB/C mice were divided into four groups. group A: C57BL/6 (n = 10), administered doxorubicin 2.0 mg/kg, every other day for 8 days, then once a week for 6 weeks, for a total cumulative dose of 20 mg/kg; group B: C57BL/6 (n = 10), administered doxorubicin 2.5 mg/kg, every other day for 12 days for a total cumulative dose of 15 mg/kg; group C: BALB/C (n = 10), administered doxorubicin 2.5 mg/kg, every other day for 12 days for a total cumulative dose of 15 mg/kg; group D: BALB/C (n = 10), administered doxorubicin 2.0 mg/kg, every other day for 8 days, then once a week for 6 weeks, for a total cumulative dose of 20 mg/kg. Echocardiography was done before and after the study. There is no significant difference in survival between group B and group C (90% vs 90%). However, survival was significantly lower in group D compared with group A (30% vs 90%, p < 0.05). The ejection fraction was markedly decreased and the left ventricular end-diastole diameter was markedly increased in groups A, B and D after DOX injection, but not in group C.

Conclusion: C57BL/6 mice are more suitable than BALB/C mice for inducing dilated cardiomyopathy with short-term doxorubicin treatment. Intraperitoneal injection of doxorubicin in C57BL/6 mice (2.5 mg/kg, every other day for 12 days, for a total cumulative dose of 15 mg/kg) is a reliable method to induce dilated cardiomyopathy.

Key Words: BALB/C mice • C57BL/6 mice • Dilated cardiomyopathy • Doxorubicin

INTRODUCTION

Doxorubicin (DOX) is a widely used and effective chemotherapeutic agent in the treatment of a variety of cancers. Despite its effectiveness, the clinical use of DOX is limited by a dose-dependent and cumulative cardiotoxicity that may cause irreversible myocardial damage, leading to dilated cardiomyopathy (DCM) with congestive heart failure. This cardiotoxicity of DOX has been exploited to generate an in vivo animal model of DCM. There is still no standard method as to how to induce chronic DCM with DOX. The method of injection may be by intraperitoneal or via vena caudalis. Total doses vary from 10 mg/kg to 25 mg/kg. The total time taken to induce DCM can be divided into 2 groups: the long-term method and the short-time method. The most common method is the long-term method (DOX was...
administered by intraperitoneal injection over 6-10 weeks for a cumulative dose of 15-20 mg/kg). And in recent years, the short-term method (DOX was administered by intraperitoneal injection every other day for 12 days, for a cumulative dose of 15 mg/kg) was adopted by many experimenters for the benefit of its short period. But there is still no head-to-head comparison between the long-time method and the short-time method. Also, little is known about what kind of mice should be used in the induction of DCM with DOX. So we conducted this research to compare the short-time method and the long-term method, and to compare between the C57BL/6 mice and the BALB/C mice (two most common species of experimental mice), to select an appropriate method to induce DCM with DOX.

METHODS

Male BALB/C (n = 20) and C57BL/6 (n = 20) (6-8 weeks, weighted 18-22 g) mice were purchased from B&K Universal Group Limited (Shanghai, China). The mice were housed in temperature- and humidity-controlled rooms with 12-h light/dark cycles and with free access to food and water. All protocols were approved by the Animal Care and Use Committee of Zhongshan Hospital, and were in compliance with “Guidelines for the Care and Use of Laboratory Animals” published by the National Academy Press (NIH Publication No. 85-23, Revised 1996).

The mice were divided into 4 groups:
- **Group A**: C57BL/6 (n = 10), DOX 2.0 mg/kg, administered every other day for 8 days, then once a week for 6 weeks, for a total cumulative dose of 20 mg/kg;
- **Group B**: C57BL/6 (n = 10), DOX 2.5 mg/kg, administered every other day for 12 days, for a total cumulative dose of 15 mg/kg, then waiting for 4 weeks after the last DOX injection;
- **Group C**: BALB/C (n = 10), DOX 2.5 mg/kg, administered every other day for 12 days, for a total cumulative dose of 15 mg/kg, then again 4 weeks after the last DOX injection;
- **Group D**: BALB/C (n = 10), DOX 2.0 mg/kg, every other day for 8 days, then once a week for 6 weeks, for a total cumulative dose of 20 mg/kg;

GROUP A and GROUP D belonged to the long-term groups, GROUP B and GROUP C belonged to the short-term groups. DOX (Shenzhen Main Luck Pharmaceuticals Inc., CHINA) was dissolved in sterile saline and administered intraperitoneally.

Echocardiography

At the beginning of the DOX treatment, and on the day that mice were sacrificed, cardiac function was assessed by echocardiography. In brief, mice were anesthetized with 1.5% isoflurane mixed with oxygen until the heart rate stabilized at 400 to 500 beats per minute. Two-dimensional long-axis images were obtained with a high resolution Micro-Ultrasound system (VEVO 770, Visual Sonics Inc, Toronto, Canada) equipped with a 30-MHz mechanical scan probe. Ejection fraction (EF) and left ventricular end-diastole diameter (LVEDD) were calculated with VEVO Analysis software (version 2.2.3).

Morphology and histological analyses

The hearts were fixed in 10% neutralized formalin and embedded in paraffin. Serial sections (5 μm) were routinely stained with hematoxylin-eosin (H-E) and Mallory’s trichrome staining, examined under a light microscope (× 400), and photographed for morphological analysis.

Statistical analysis

Data are presented as means ± standard deviation. Paired samples t-test was used to compare between the same group before and after DOX injection. Statistical significance was determined using one-way ANOVA, followed by the Student-Newman-Keuls test among groups before and after the DOX injection. Statistical analysis was performed using software program, SPSS V11.5 (SPSS Inc., Chicago, IL). For survival analysis, survival percentage was analyzed using the Kaplan-Meier method, and the statistical significance of the survival experiments was determined using the log-rank test. A p value of < 0.05 was considered statistically significant.

RESULTS

Survival analysis

Survival of the four groups of mice was analyzed by
the Kaplan-Meier approach (Figure 1). In the short-term groups, 4 weeks after the last DOX injection, there was no significant difference between group B and group C (90% vs. 90%). In the long-term groups, survival was significantly lower in group D compared with group A (30% vs. 90%, p < 0.05).

**Echocardiographic assessment of ventricular remodeling**

After injection of DOX, most of the mice experienced ventricular remodeling characterized by enlarged cardiac dimensions (Figure 2), and decreased left ventricle ejection fraction (Figure 3). The EF was markedly decreased post-DOX injection in group A (79.57 ± 3.48 vs. 64.18 ± 10.6, p < 0.05), group B (79.43 ± 2.59 vs. 60.52 ± 7.35, p < 0.05), group D (80.81 ± 1.01 vs. 47.21 ± 13.81, p < 0.05), but not in group C (79.92 ± 2.3, 72.11 ± 12.64, p = 0.18) (Figure 3). The LVEDD was markedly increased in group A (3.06 ± 0.16 vs. 3.4 ± 0.26, p < 0.05), group B (3.13 ± 0.21 vs. 3.58 ± 0.27, p < 0.05) and group D (3.18 ± 0.04 vs. 3.47 ± 0.07, p < 0.05), but not in group C (3.13 ± 0.17 vs. 3.17 ± 0.46, p = 0.85) (Figure 4). However, there was no significant difference among groups A, B, D in EF and LVEDD both pre- and post-DOX injection.

**Histopathological changes**

After DOX injection, degeneration and loss of myocytes and disorganized myofibrils were obvious on HE staining under a light microscope (× 400), and myocardial fibrosis was seen on Mallory’s trichrome staining (Figure 5).

**DISCUSSION AND CONCLUSION**

DCM is a primary myocardial disease of unknown etiology. DCM is characterized by diffuse cardiac damage, with a loss of cardiomyocytes and an increase in fibroblasts. In the failing heart, hypertrophy, degeneration, and loss of cardiomyocytes and interstitial fibrosis...
are histopathologically characteristic.\(^1\)

There are several methods used to induce DCM, including rapid pacing, trans-aortic constriction, and drugs. DOX is the most common drug used to induce DCM. Though the precise mechanisms of DOX cardiotoxicity remain elusive, there is increasing evidence that DOX exposure can trigger cardiomyocyte apoptosis, which represents the predominant form of cardiomyocyte damage seen in this setting.\(^5,6\) The advantage of DOX-induced DCM is that it is non-invasive, technically simple and reproducible. The limitation is high incidence of mortality and non-cardiac effects.\(^7\)

The results presented here demonstrated that treatment of mice with DOX resulted in cardiotoxicity manifested by decreased cardiac systolic function, as well as enlarged ventricle cavity and myofiber disarray, which are the most important properties of DCM. EF was decreased and the left ventricle dilated, in accordance with results of previous studies.\(^8-11\) Mice treated with DOX showed histological alterations similar to those that occur in humans: degeneration and loss of myocytes and interstitial fibrosis, which were found previously in rats.\(^12,13\)

In the induction of DCM with DOX, mice play an important role because of the feasibility and availability of transgenic models, which are the most effective method to study the role of specific genes or proteins. But transgenic mice do not represent the normal status of disease. BALB/C and C57BL/6 are the most common mice that are used in animal studies. As was shown in our study, in the long-term groups, C57BL/6 mice (group A) showed better tolerability and survival rate than BALB/C mice (group D). In the short-term groups, C57BL/6 mice (group B) showed much lower EF than BALB/C mice (group C). And there was no significant difference between pre-DOX and post-DOX in group C, which mean the failure of induction of DCM. Though lower EF was achieved in group D, but the high mortality rate (70%) was unacceptable. To sum up, C57BL/6 mice is more appropriate than BALB/C mice to be used to induce DCM with DOX. As far as C57BL/6 mice were concerned, there were no significant difference in survival rate, EF and LVIDD between groups A and B. Considering that group B saved 2 weeks to induce DCM compared with group A, the short-term method (group B) was at least as good as the long-term method (group A) if not better than.

In conclusion, DOX was effective in promoting macro and microscopic alterations in the cardiac tissue of mice, and can therefore be used as a model for experimental studies in DCM. C57BL/6 mice are more appropriate than BALB/C mice, which can be used to induce DCM with DOX. Intrapерitoneal injection of DOX in C57BL/6 mice (2.5 mg/kg, administered every other day for 12 days for a total cumulative dose of 15 mg/kg, then waiting for 4 weeks after the last DOX injection) is a reliable method to induce DCM.

REFERENCES


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\caption{(A) HE (\times 400); (B) MALLORY (\times 400).}
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