Original Article

An Ovine Model of Dilated Cardiomyopathy Induced by Doxorubicin

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Abstract

Background: Dilated cardiomyopathy is associated with a progressive deterioration in cardiac function and eventually death. Initial interest in this hypothesis was to create another large animal model for dilated cardiomyopathy in addition to pigs and dogs.

Methods: After the induction of anesthesia to 10 female sheep, a carotid-jugular shunt was created in all the animals via a 1-cm fistula between the carotid artery and jugular vein. Six sheep out of the total of 10, were given intravenous Doxorubicin. Echocardiographic studies were performed before surgery and 3 months after that. The 4 animals not injected with Doxorubicin were evaluated for echocardiographic parameters after one year.

Results: There was no abnormality in echo parameters in the 4 sheep that had not received Doxorubicin; in addition, their valves and cardiac output were normal. As regards the six sheep injected with Doxorubicin, 4 received a dose of 2 mg/kg weekly and expired after the second injection due to the toxicity of the drug, 1 was given Doxorubicin 1 mg/kg and died after one week, and 1 had Doxorubicin 0.5 mg/kg but showed no abnormality in terms of dilated cardiomyopathy.

Conclusion: We conclude that the sheep is sensitive to Doxorubicin and that the dosage that is enough for creating dilated cardiomyopathy in dogs is very toxic for the sheep.

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Keywords: *Sheep • Dilated cardiomyopathy • Doxorubicin • Carotidojugular shunt*

Introduction

Dilated cardiomyopathy is associated with a progressive deterioration in cardiac function and eventually death.[1] Heart failure is an unresolved problem in the human despite advances in pharmacological therapy. Initial interest in this hypothesis was to create another large animal model for dilated cardiomyopathy besides pigs and dogs.

Methods

Ten adult female Iranian sheep at a mean weight of 40±5 kg were selected for this study. The study was approved by the ethical committee of Tehran University of Medical Sciences. All the experiments received humane care in accordance with the "Guide for the Care and Use of Laboratory Animals", published by the US National Institute of Health (NIH

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During the study, the animals were held in metabolic cages, had free access to water, and were fed with a mixed diet of hay and sheep pellets. All the sheep were housed for one week in the animal house for adaptation. They were examined by a veterinarian and a cardiologist both clinically and echocardiographically and excluded out of the study if any serious morbidity was detected.

The sheep were NPO (nil per os) from 24h prior to surgery. The animals received intramuscular xylazine, 0.2 mg/kg to become sedated for hair shaving and instrumentation. Neck hair was shortened and then shaved. The saphenous vein was cannulated with a #20 gauge (pink) intravenous catheter. A central venous cannula was placed in the jugular vein using the Seldinger technique. An intravenous infusion of lactated Ringer's solution (20 cc/kg in 1h) was delivered before anesthesia and was maintained at the rate of 10 cc/kg per hour. The urethra was catheterized with a #10 Foley catheter connected to a urine bag. A pulse oximeter transducer was connected to the ear to monitor O_2 saturation. Five electrocardiogram (ECG) electrodes were connected to the extremities and on the chest. Anesthesia was induced via an intravenous injection of sodium thiopental, 5 mg/kg and was maintained with halothane (2.0-3.0%) in oxygen.[2] The animals were then immediately intubated with a 7.5-mm endotracheal tube and were mechanically ventilated (Draeger Ventilog3®) with 100% O_2 at a respiratory rate of 12-14/min and in-to expiratory cycle ratio of 1:1 and a tidal volume of 10 mL/kg. Gastric decompression was accomplished by the insertion of an orogastric tube. An anticholinergic (atropine, 2 mg) to prevent hypersalivation and an antibiotic (cefazolin, 1g) for prophylaxis were administered intravenously upon the induction of anesthesia. Prophylactic antibiotic was repeated 8 and 16 hours after surgery.

After surgical prep/drape, heparinization was performed by injecting 100 IU/kg heparin intravenously and then a 5-cm longitudinal skin incision was made anterior to sternomastoid muscle. The carotid artery and jugular vein were dissected and exposed. Between the cross clamps, a 1-cm incision was created on both jugular vein and carotid artery. The vessels were, thereafter, anastomosed together with a 7/0 Prolene suture and a carotidojugular shunt was created (Figure 1). Muscles and skin were sutured with a Vicril 3/0 suture. Echocardiographic studies were performed before surgery and 3 months after that.

In 6 animals Doxorubicin was injected intravenously (IV) in the following regimens: 4 animals 2 mg/kg weekly; 1 animal 1 mg/kg one dose; and 1 animal 0.5 mg/kg one dose.

Results

All the animals had a carotid-jugular shunt. The four sheep not injected with Doxorubicin were evaluated for echocardiographic parameters after one year, which showed no abnormality (Table 1).

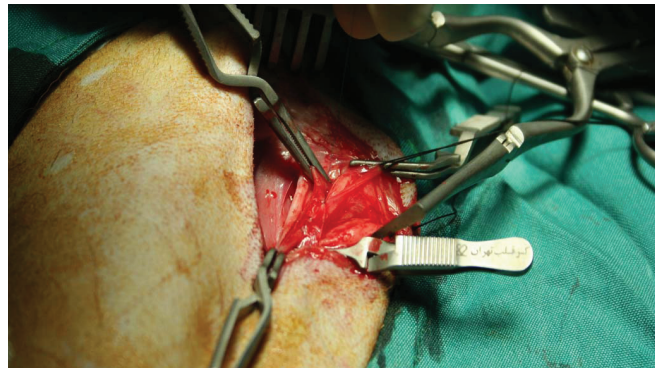

Figure 1 .Creating a carotid-jugular shunt

Table 1. Echo Parameters

Sheep No	Doxorubicin	EF (%) (primary)	EF (%) (3 months after surgery)
1	-	73	72
2	-	68	70
3	-	66	68
4	-	72	74
5	2 mg/kg	73	Died
6	2 mg/kg	68	Died
7	2 mg/kg	65	Died
8	2 mg/kg	75	Died
9	1 mg/kg	69	Died
10	0.5 mg/kg	70	69

EF, Ejection fraction

Furthermore, their valves and cardiac output were normal.

With respect to the six sheep injected with Doxorubicin, the 4 sheep that received a dose of 2 mg/kg weekly expired after the second injection due to the toxicity of the drug, the sheep that was given Doxorubicin 1 mg/kg died after one week, and the one that had Doxorubicin 0.5 mg/kg showed no abnormality in terms of dilated cardiomyopathy.

All the animals that had received Doxorubicin became weak and afflicted and showed alopecia due to the toxicity of Doxorubicin (Figure 2)

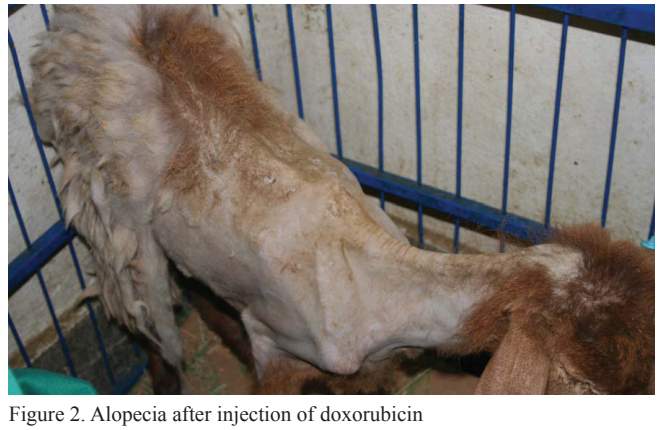

Figure 2. Alopecia after injection of doxorubicin

Discussion

Doxorubicin or hydroxyldaunorubicin is a DNA-interacting drug widely used in chemotherapy. It is an anthracycline antibiotic and structurally closely related to daunomycin and also intercalates DNA. It is commonly used in the treatment of a wide range of cancers.

The exact mechanism of action of Doxorubicin is complex and still somewhat unclear, although it is thought to interact with DNA by intercalation.[3] Doxorubicin is known to interact with DNA by intercalation and inhibition of macromolecular biosynthesis.[4] This inhibits the progression of the enzyme topoisomerase II, which unwinds DNA for transcription. Doxorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication

Doxorubicin is commonly used to treat some form of leukemias, Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others.

Combination therapy experiments with sirolimus (rapamycin) and Doxorubicin have shown promise in treating Akt-positive lymphomas in mice.[5]

Recent animal research coupling a murine monoclonal antibody with doxorubicin has created an immunoconjugate able to eliminate HIV-1 infection in mice. Current treatment with antiretroviral therapy (ART) still leaves pockets of HIV within the host. The immunoconjugate could potentially provide a complimentary treatment to ART to eradicate antigen-expressing T cells.

Acute side-effects of Doxorubicin can include nausea, vomiting, and heart arrhythmias. It can also cause neutropenia (a decrease in white blood cells), as well as mild alopecia. The risks of developing cardiac side effects, including congestive heart failure, dilated cardiomyopathy, and death, dramatically increase by the administration of Doxorubicin. Doxorubicin cardiotoxicity is characterized by a dose-dependent decline in mitochondrial oxidative phosphorylation.[6] Reactive oxygen species, generated by the interaction of Doxorubicin with iron, can then damage the myocytes, causing myofibrillar loss and cytoplasmic vacuolization.

Melissa J. Byrne et al. developed a model of long-term progressive heart failure in sheep.[7] They induced tachycardia with rapid ventricular pacing for 21 days at 160-190 bpm, which begot moderate heart failure. The animals were then paced at 205-215 bpm for 42 days (severe heart failure) and for 28 days (advanced heart failure). Data collected from echocardiography showed an increased left ventricular area, mitral valve regurgitation, and left ventricular end-diastolic pressure and decreased left ventricular wall thickness and left ventricular ejection fraction. This ovine heart failure model allows an examination of both structural changes and hemodynamic parameters of heart failure.

Peter Feindt et al. induced dilated cardiomyopathy in pigs with rapid ventricular pacing (220 bpm) for at least 4 weeks.[8]

Masami Takagaki et al. created a dog model of dilated cardiomyopathy by rapid ventricular pacing (230 bpm) for 4 weeks and maintained it by reducing the rate (190 bpm) for another 4 weeks.[9]

Mikhail Vaynblat et al. induced dilated cardiomyopathy by an intracoronary administration of Doxorubicin weekly for 4 weeks in 10 dogs.[10] Left ventricular end-diastolic pressure and diameter, as well as right ventricular end-diastolic diameter increased, and ejection fraction fell from 0.60±0.10 to 0.40±0.04 ($p = 0.0009$).

Valery chekanov et al. created a dog model of dilated cardiomyopathy by a carotidojugular shunt and Doxorubicin injection (2.5 mg/kg IV) weekly for six weeks.[11] After that, the mean ejection fraction of the dogs decreased about 30 percent.

We conclude that the sheep is sensitive species to Doxorubicin and that the dosage that is enough for creating dilated cardiomyopathy in dogs is very toxic for the sheep.

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