Effect of Intramuscular Injection of Ceftriaxone on the Cardiac Muscle of White Mice

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Abstract

The present study was designed to demonstrate the effect of Ceftriaxone administrated intramuscularly on the Cardiac Muscle Fiber of White Mice.

Cardiac Muscle Fibers consist of branched, striated muscle fibers, that appear cylindrical with one or two nuclei, presence of intercalated disks is seen as well.

In this study the experiment was designed using ten white mice from both sexes and randomly distributed into two main groups as following:

Group A: Control group was injected intramuscularly with 0.5ml D.W (once daily) for 6 days in a row.

Group B: Treated group was injected intramuscularly with 0.3g/kg of Ceftriaxone (once daily) for 6 days in a row.

After the end of the experimental period, the animals were dissected and the organs were processed for tissue examination under the microscope.

Histologically, cardiac muscle fibers alignment alteration in the mice cardiac fiber tissue was observed with the presence of atrophy of the cardiac muscle fibers and loss of cardiac muscle striation had been found.

These effects were all observed after only 6 days of ceftriaxone therapeutical dose and could lead to more extensive results if given for a long period of time, indicating the severe effect of this drug on the cardiac muscle and the necessity to prescribe and utilize this antibiotic with caution and medical awareness.

Key Words:- Ceftriaxone, Cardiac Muscle Fiber, Myocardium, histopathology

Introduction

Ceftriaxome is a broad spectrum cephalosporin resistant to different types of betalactomates. Is also shows defensive activity against gram-negative and gram=positive bacteria.

Ceftriaxone is a semisynthetic, sterile, third generation cephalosporin antibiotic that is used intravenous/intramuscularly. It is a drug used for various type of inflammation present in all type of organs such as the skeletal, cardiac and internal organ system.

The cardiovascular system is composed of the heart, arteries, capillaries and veins. The muscular wall (myocardium of the heart is composed of cardiac muscle with its four chambers. Layers of the heart wall: The three layers that constitute the heart wall are the endocardium, myocardium and epicardium. Myocardium is the thick middle layer of the heart composed of the cardiac muscle cells. (1)

Cardiac muscle is striated with the presence of actin and myosin arrangement in sarcomeres. These muscles usually have single (central) nucleus. Cells appear to be branched usually, and are tightly connected by specialized

junctions. Cardiac muscle fibers exhibit some of the same features that are seen in skeletal muscle fibers (as illustrated in pic.(1) showing a section of a cardiac muscle cut in both the longitudinal and the transverse planes).

Cardiac muscle fibers differ from skeletal muscles in that cardiac muscle fibers show branching without any change in their diameter. Also, each cardiac muscle fiber is shorter than a skeletal muscle fiber and contains a single central nucleus although bi-nucleate muscle fibers also may be seen. Myofibrils of individual cardiac muscle cells are also a component of cardiac muscle. (2)

Aim Of Study

1. To observe the histological and structural alterations present in the heart of white mice after they have been treated with Ceftriaxone injections

2. To show the presence of any side effects on the cardiac muscle after drug intake.

Material and Methods

Experimental Animals:

White healthy mice from both sexes were put into two cages (5 mice/cage)m and given pellet diet and water for a month and a half before the start of the experimental work.

All animals were kept in a controlled, well ventilated environment.

Experimental Design:

Mice were devided randomly into two main groups, with five mice in each group. Both groups were injected in the same manner daily at 6 PM.

Group A containing 5 mice were intramuscularly injected with 0.5 ml D.W daily

Group B: containing 5 mice also injected intramuscularly with 0.5 ml (0.3 g Ceftriaxone dissolved in 0.5 ml Distilled Water) daily. (3)

Histological Technique

Mice were killed using chloroform then dissected to undertake organ examination and tissue preparation for microscopy.

Fixation, using 10% neutral buffered formalin to stop autolysis and putrefaction for 24 hours, then washed to remove any traces of fixative agent used.

Dehydration & Clearing

Gradual alcohol baths were used to remove water in the tissue, beginning with 60%-100% tissue then is treated twice with xylene (60 min.)

Infiltration & Embedding

Paraffin is used to prepare tissue for microscopic examination. Infiltrating the tissue in two changes for an hour and a half.

Then the block is allowed to harden.

Sectioning

Rotary Microtome (LKB-U.K.) Used to trim the block at 5 Micrometer. (Mm) and carefully put in a water bath of 370C.

Tissue Attachment

After ribbons are removed from the water bath, they are placed onto marked slides using Mayer's Egg Albumin Mixture. Finally stained with the usual Hematoxylin & Eosin Stain.

De-waxing & Hydration

Dehydration in ascending alcohol starting from 100-60% and later in xylene to insure the removal of the paraffin.

Staining

Slides were put in heamstoxylin for 10 min., washed in tab water then stained with Alcoholic Eosin for 6 minutes.

Mounting

Using D.P.X. and cover slips and examined using Novel/SN Italy Microscope. (1)(4)

Results

- Control Heart

Normal cardiac fibers are seen with branching, central nucleus presented as well with normal alignment of tissue.

- Treated Heart
- Segmentation present in the myocardium is seen in Fig. (4), this segmentation into masses of sarcoplasm of eosinophilic stain with the presence of poorly recognized nuclei is seen, the delicate connective tissue in-between muscle fibers are distributed in a long-comb like shape. Macrophages are demonstrated in the connective tissue.

The section reveals the presence of muscle fibers extended along the field and these fibers lost their striation, the nuclei is difficult to detect, otherwise, there are muscle fibers with severe atrophy and appear to be segments and branched. We also can see in the picture the presence of the delicate C.T in-between muscle fibers that are wide and stained lightly by H&E.

The cardiac muscle fibers seen in Fig. (6) are present in parallel arrangement, some of them were atrophied with the breaking down of others. Striation of actin and myosin in the sarcoplasm and the nuclei is poorly recognized.

We can observe the myocardium appearance as a great mass extended and widespread in the field, while the connective tissue was present as narrow bands in-between. The nuclei of the sarcoplasm were difficult to demonstrate due to extensive eosinophilic stain of sarcoplasm.

Atrophy of the myocardial muscle fibers was seen and certain number of them were broken down. Myocardial muscle fibers had lost its striation while the connective tissue in-between muscle fibers were highly extensive due to atrophy of muscle fibers.

As seen the myocardial cells are mostly segmented into masses with the loss of striation from its sarcoplasm, furthermore, the nuclei were not well recognized.

The connective tissue in-between muscle fibers were extensive and delicate.

Discussion

Over the last decade, cardiovascular dysfunction and diseases are the first cause of death in the world, hence many drugs are taken to cure such diseases while neglecting the side effects these drugs could cause on the cardiac muscle fibers and myocardium on the long term.

Ceftriaxone is a white/yellowish crystalline powder that is soluble in water, slightly soluble in methanol while showing very slight solubility in ethanol. It is stored at room temperature.

Ceftriaxone is useful and used in a lot of medical fields due to its broad spectrum and long elimination half-life (5).

It is used as an antibiotic and should not be used if any allergy reaction to certain antibiotics similar to ceftriaxone such as Ceftins, Keflex, Omnifex or Penicillin.

This Cephalosporin antibiotic is used for the treatment of many infections alternating from

severe such as life threatening ones (Meningitis) to mild infections such as Skin and Urinary Infections.

After treatment with therapeutical dose and examining the specimens under the microscope, the following data was illustrated and many observations were taken in consideration regarding the effect of ceftriaxone on cardiac muscle fibers. Atrophy of the cardiac muscle fibers and decrease in muscle and tissue mass was observed, myocardium segmentation and breakage.

Many studied had views on the effect on ceftriaxone on many organs. However, some studies focused on its effect on certain muscles such as cardiac and smooth muscle, one study showed the effect of graded increased concentrations of ceftriaxone on isolated rabbits heart and guinea pig's auricles produced a dose dependent negative inotropic effect. It was conducted that ceftriaxone directly stimulated the smooth muscles of gastrointestinal tract and depressed those of the uterus as well as cardiac muscles. (6)

The present study was in agreement with (6) showing ceftriaxone effect on isolated white mice muscle that observed the negative effect of ceftriaxone on the mice heart causing depression of the myocardium.

Another study (7) also illustrated the effect of Ceftriaxone on the mouse heart and was in correlation to the results obtained showing the direct and negative depression in contractility of rabbit's heart in a dose dependent manner.

This study was also in agreement with results obtained by (8) showing the drugs effect on the hearts function and mechanism, the study showed pharmacological effects of Cephamycin at doses above 800 mg/kg i.v. it illustrated it slightly raised blood pressure levels in dogs with slight increase in blood flow and heart rate when given above 400 mg/kg intravenously.

This effect could be led due to the fact that contraction of cardiac cells is dependent on the calcium ion concentration of the contractile apparatus to form what is known as "cardiac action potential" which is a short change in the voltage that passes through the cell membrane of heart cells, this action potential passes through the cell membrane causing the cell to contract.

The cardiac cell dependence on the calcium ion lead to the direct depression of the myocardia due to ceftriaxone in this study that effected it and could be attributed to a modification of calcium function. (9)(7)

Conclusion

In conclusion, we can confirm that Ceftriaxone has a negative impact on the histological and structural alignment of cardiac muscle fibers that caused alteration in the mice cardiac tissue with the presence of atrophy of the cardiac muscle fibers, and loss of its striation.

Permanent loss of coordination/alignment in between the fibers was seen. These results show the capability of such drugs to effect the structure of cardiac muscle leading to alterations in its function hence these findings indicate that ceftriaxone had scarcely effected cardiac muscle leading to adverse histological effects that should spread precaution and clinical awareness before drug utilization without medical knowledge.

References

1- P. Gartner, L.Hiatt. (2001). Color Textbook Of Histology (2nd Edition). W.B Saunders Company, USA.

2- P. Eroschenko. (2005). Atlas Of Histology With Functional Corrections (10th Edition). Lippincott Williams & Wilkins, USA.

3- Riviere J., Papich M.&Adams H., (2011). Veterinary Pharmacology and Therapeutics. (9th ed.) . WILEY-BLACKWELL, A John Wiley & Sons, Inc., Publication. USA.

4- Histology Processing Workshop, Collage Of Veterinary, 2017. University Of Tikrit.

5- AC.Cremieux, B. Maziere, JM. Vallois, *et al*. (1991). Ceftriaxone Diffusion into Cardiac Fibrin Vegetation, Qualitative and Quantitative Evaluation by Autoradiography. Fundamental & Clinical Pharmacology. Volume 5, Issue 1, Pages 53-60.

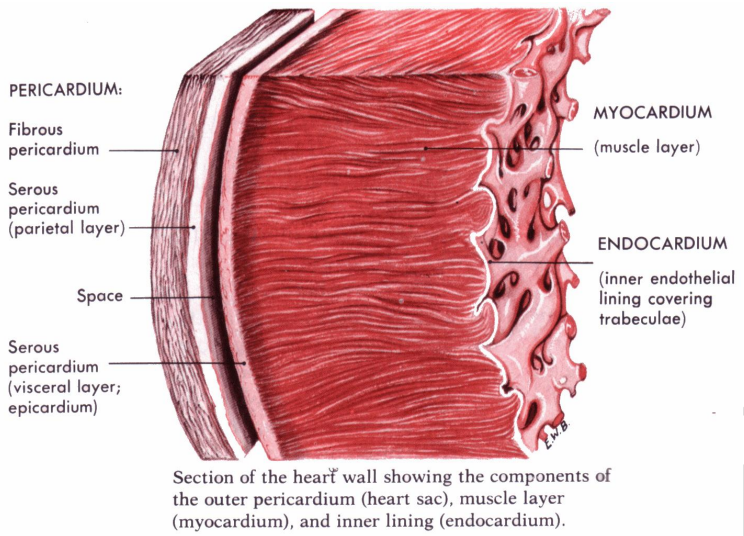
6- M.G.Alasyed, A.A.Elkomy and M.H.Aboubaker. (2011). Effect of Ceftriaxone on Isolated Smooth, Cardiac Muscles and Neuromuscular Junctions. Department of Pathology, Benha University, Egypt. IJAVM'S, Vol.5, Issue 20:138-151.

7- MG. El-Sayed, MR. Hassanin, MH.Hafez, *et al*. (1997). Some Pharmacodynamic and Biochemical Aspect of Cefamondole. Dtsch Trerarztl Wochenschr; 104:481-487.

8- M. Kurebe, H. Asaoka, Y.Yamak, *et al*. (1984). Pharmacological Studies on New Cephamycin, It's Effect on Central Nervous System, Respiration and Cardiovascular System. The Japanese Journal of Antibiotic, 37 (7): 1428-1439.

9- AM. Katz, DI. Repke. (1973) Calcium-Membrane Interaction in the Myocardium; Effect of Ouabain, epinephrine and 3',5'-cyclic adenosine monophosphate. AM.J.Cardiol. Issue 32:1356-1371.

10- Rudy, Y. (2008). Molecular basis of cardiac action potential repolarization', Annals of the New York Academy of Sciences., 1123, pp. 113–8.



Section of the heart wall showing the components of the outer pericardium (heart sac), muscle layer (myocardium), and inner lining (endocardium).

Fig.(1)- Section Of The Heart Wall Tissue (10)

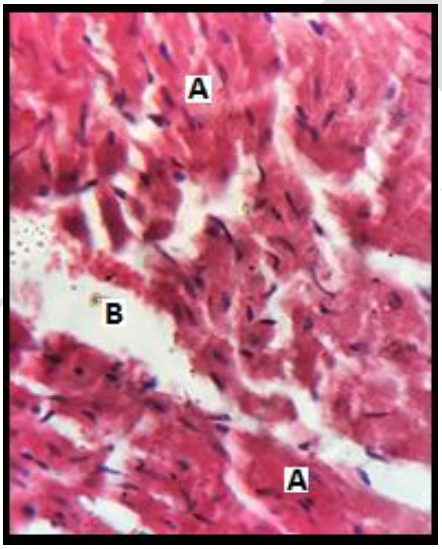


Fig.(2): Control Heart. Myocardium (A), Sarcoplasm (B). (H&M Stain, 400x)

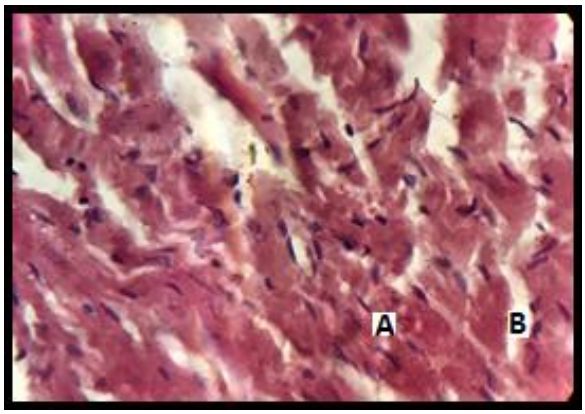


Fig.(3): Control Heart. Myocardium (A), Connective Tissue (B). (H&M Stain, 400x)

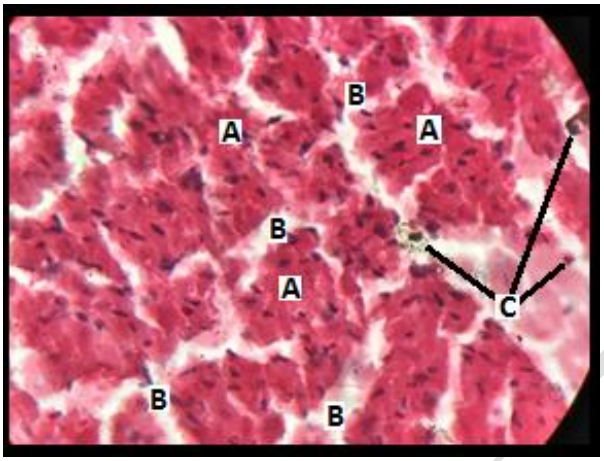


Fig.(4): Treated Heart. Myocardium Segmentation (A), Connective Tissue (B) Macrophages (C). (H&M Stain, 400x)

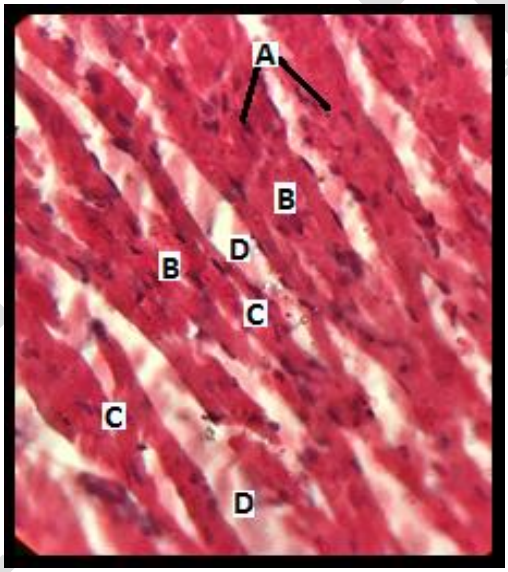


Fig.(5): Treated Heart. Muscle Fibers (A), Unclear Nuclie (B) Muscle Fiber Atrophy (C), Delacate Connective Tissue (D). (H&M Stain, 400x)

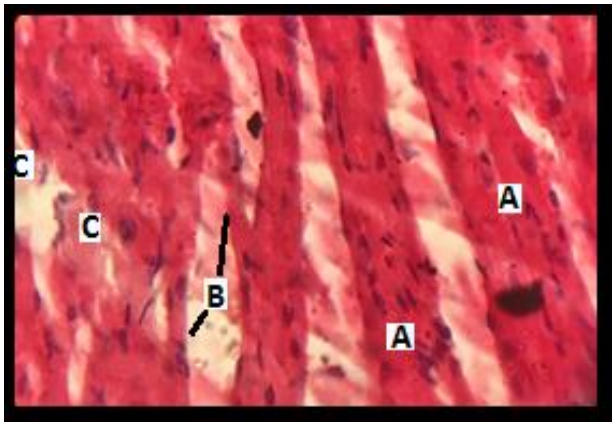


Fig.(6): Treated Heart. Cardiac Muscle Fibers (A), Atrophied Cardiac Muscle (B) Cardiac Muscle Breakage (C). (H&M Stain, 400x)

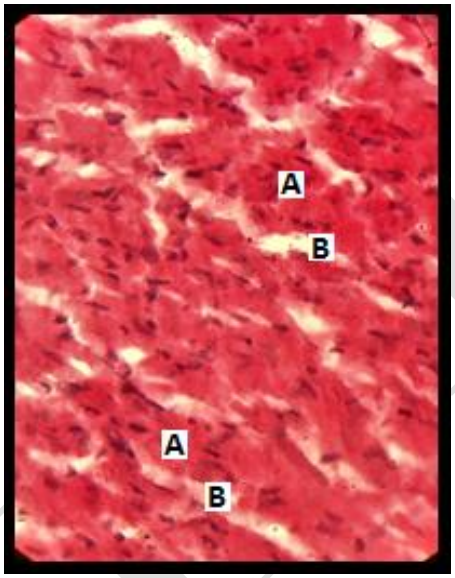


Fig.(7): Treated Heart. Myocardium (A), Connective Tissue (B). (H&M Stain, 400x)

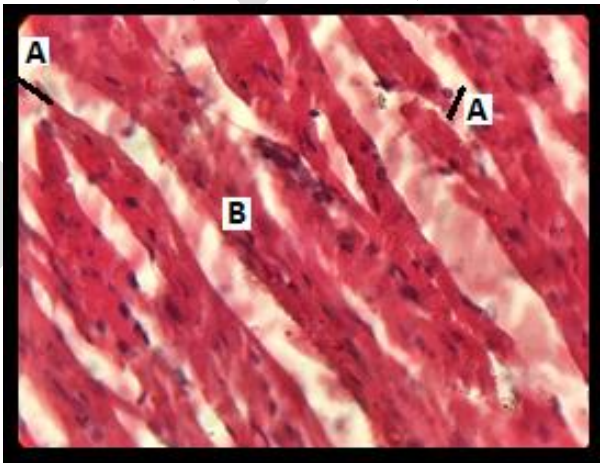


Fig.(8): Treated Heart. Myocardial Muscle Fibers (A), Myocardial Striation (B). (H&M Stain, 400x)

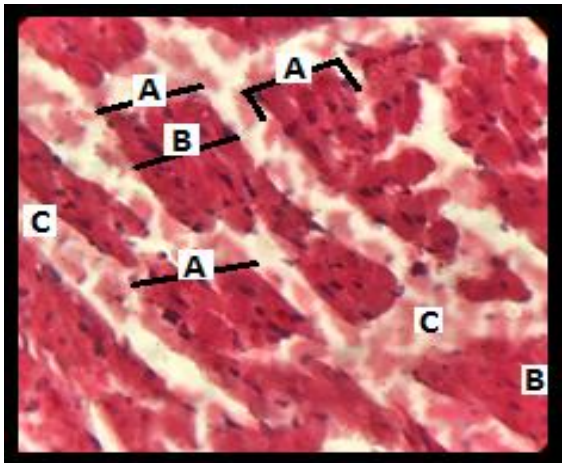


Fig.(9): Treated Heart. Myocardial Cells (A), Sarcoplasm (B), Connective Tissue (C). (H&M Stain. 400x)

Table (1): A list of materials and instruments used in the present study.

Material	Instruments
Absolute Alcohol	Beaker
Chloroform	Glasses
Ceftriaxone	Rotary-Microtome
Crystal Wax	Insulin Needle
Distilled Water (D.W)	Oven
Distrene Plasticizer Xylene (D.P.X)	Water Bath
Heamatoxyline & Eosin (H&M)	Glass Slide &Glass cones
Mayer's Egg Albumin	Light Microscope
Paraffin Wax	Dissecting Kit
Xylene	Blocks

Group	Dose	Duration	No. of Mice
A	0.5ml D.W	6 days	Five
B	0.3g Ceftriaxone	6 days	Five

الخلاصـــة

صممت هذه الدراسة لمعرفة تأثير استخدام دواء السيفترياكزون المحقون في العضلة على نسيج عضلة القلب في الفأر. تتكون نسيج عضلة القلب من تفرعات وشعيبات للألياف العضلية المخططة، والتي تظهر كشكل اسطواني مع وجود نواة أو اثنتين، ومن الممكن أن نرصد وجود الأقراص البينية أيضا.

لقد صممت تجربة تحوي على عشرة فئران تجارب من كلا الجنسين حيث تم تصنيفهم عشوائياً لمجموعتين رئيسيتين كالآتي:

1-**مجموعة** أ : مجموعة السيطرة، حقنت في داخل العضلة بجرعة 0،5 مل ماء (مرة واحدة يومياً) لمدة 6 أيام متواصلة

2-**مجموعة ب** : المجموعة العلاجية، حيث حقنت في داخل العضلة بجرعة 0،3 غرام\كيلوغرام من عقار السيفترياكزون لمدة 6 أيام متواصلة.

تم تشريح الحيوانات في نهاية التجربة ودراسة الأنسجة تحت المجهر لكي يتم فحص العينات وتحليلها نسيجيا.

تم العثور على تغيير وخلل في الترتيب الطبيعي للألياف العضلية مع وجود ضمور في هذه الألياف وفقدان عضلات القلب تخطيطها.

تم العثور على هذه النتائج بعد 6أيام فقط من جرعة المعالجة بالسيفترياكزون، فمن الممكن أن يؤدي إلى نتائج ومضاعفات أخرى أخطر وأكثر ضرراً على العضلات القلبية إن تم الاستمرار بأخذها لفترة أطول، مما يظهر لنا التأثير القوي لهذا العقار على عضلات القلب، فيظهر لنا ضرورة النظر وأخذ الحذر عند تعاطي ووصف هذا الدواء من غير تصريح طبي وبالأخص لمن لديه مضاعفات في القلب.