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Article

Histological Evaluation of Gentamicin-Associated Myocardial Alterations in Adult Male Rats

Nawras Ali Hussein Al-Jubouri[1], Dhurgham Qasim Shaheed[2]

1. Al- Furat Al-Awsat Technical University, Najaf Technical Institute, Najaf, Iraq
2. College of Pharmacy, University of Al Kafeel, Najaf, Iraq

* Corresponding: nawres.ali.inj@atu.edu.iq

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Abstract: In order to understand how gentamicin affects the histology of the adult rat myocardium and its possible uses in clinical medicine, this study sought to investigate the effects of gentamicin administered over a period of time on adult rat myocardium by utilizing H and E stained sections evaluated by light microscopy. Gentamicin is widely used in human medicine as an aminoglycoside antibiotic which is effective against Gram-negative bacteria. Because aminoglycosides are potentially toxic to renal and auditory systems of mammals, many previous investigations have demonstrated that gentamicin, in particular, causes both nephrotoxicity and ototoxicity. However, despite the fact that gentamicin produces systemic toxicities to both kidneys and inner ears, very few studies have assessed whether gentamicin induces toxic changes to cardiac muscles of animals. Two sets of six adult male albino rats approximately 200-250 grams each were selected through random sampling. One group of rats (controls) received 14 consecutive intraperitoneal injections of normal saline throughout the duration of the experiment. The second group of rats (treated with gentamicin), received 14 consecutive intraperitoneal injections of gentamicin at a dosage of 31 mg/kg/day during the duration of the experiment. Once the experiment had concluded, both sets of rats were euthanized. Both sets of rats provided samples of ventricular myocardium (cardiac muscle). The samples were processed per standard laboratory procedure. Following sample processing the samples were sliced to produce thin cross-sections. Each cross-section was stained with H and E. All slices were observed with a microscope using a 40 X power lens. Once all samples were analyzed it was determined that the cross-sectional areas of cardiac muscle from control rats had well-defined elongated cardiac muscle fibers, large elongated nuclei, and regular spacing between individual cardiac muscle fibers. Conversely, cross-sectional areas of cardiac muscle from rats treated with gentamicin exhibited differences relative to those of control rats. Specifically, cross-sectional areas of cardiac muscle from rats treated with gentamicin included separation of individual cardiac myocytes; significantly larger than normal inter-cellular/inter-muscular spaces within the myocardium; smaller than normal coarseness of myocardial bundles; and slight disruption/distortion of the typical myocardial architectural design. These findings suggest that chronic exposure to gentamicin in male adult rats resulted in detectable histological changes to rat myocardium. Further biochemical/morphometric/immunohistochemical studies will be needed to evaluate the functional and molecular mechanisms that contribute to the previously identified changes in rat myocardium after gentamicin exposure.

Keywords: Cardiac Muscle, Gentamicin, Light Microscopy, Myocardium, Myocardial Changes, Rat Model.

Introduction

Cardiovascular function is achieved through the continuous contraction and relaxation cycles performed by the heart. A primary requirement for proper cardiovascular function is the organization of cardiac muscle fibers. An important characteristic of the myocardium is organization into long, branching cardiomyocytes (also referred to as cardiac muscle fibers) accompanied by supporting structures such as blood vessels and connective tissue. The organization of cardiac muscle fibers is important for preserving a normal architecture of the heart. Thus, it is essential that cardiac muscle fibers are aligned in a specific manner and separated by appropriate-sized inter-cellular spaces, have spaced-apart nuclei, and form groups of cardiomyocytes [1], [2]

Like some drugs that target specific tissues, certain medications may harm non-target tissues. For instance, gentamicin, which is generally administered as an antibiotic to humans suffering from severe infections caused primarily by gram-negative bacteria is capable of causing injury to both the kidneys and inner ear (hearing). More recently, studies have shown that additional essential tissues can suffer extensive damage as a result of multiple doses of gentamicin [3], [4], [5] .Many researchers suspect that these types of tissue injuries occur due to several different mechanisms such as reduced redox state, elevated oxidative stress, immune response activation and cellular membrane damage [6], [7], [8], [9].

Because cardiac muscle fibers have extremely high energy requirements, they are susceptible to adverse impacts resulting from toxic or metabolic disturbances. As such, cardiomyocytes must maintain healthy mitochondria, intact cellular membranes and coordinate signals with neighboring cardiomyocytes [10], [11], [12].Potential histological changes in the myocardium can include separation of cardiomyocytes, larger than usual sizes between cardiomyocytes and intermuscular spaces in the myocardium, poorly formed myocardial bundles, disruptions to tissue integrity or disrupted organizational patterns in cardiac muscle [1], [2].

Routine hematoxylin and eosin staining combined with light microscopic analysis enables examination of general histological properties of heart tissue. This type of analysis enables investigators to determine if cardiac muscle fibers are arranged in an ordered fashion, where nuclei are located/distributed, what size is present between individual cardiomyocytes/myocytes, how dense are myocardial bundles and if there exists any localized or widespread abnormalities within individual myocardial sections [2].Additionally, H&E staining employed for histopathological assessments provides yet another useful basis for determining morphologically the impact of drugs on cardiac muscle [13].

Based on the above premises we chose to conduct this study to compare the histological changes produced by repeated administration of gentamicin in adult male rat cardiac muscle. Our objective was to make comparisons concerning similarities in orientation/direction of myocardial fibers, distances between individual cardiomyocytes or other cells and compactness vs. dispersion of cardiac muscle tissue as well as extent of preservation or distortion of cardiac muscle architecture by utilizing light microscopy on H&E stained sections [14], [15], [6].

Materials and Methods

Study design and animal handling

The goal of this study was to describe histologically what happens to heart muscles after long-duration genta mycin use. Twelve healthy male albino rats were utilized as test subjects, each weighing between 200-250g. The acclimatization process lasted two weeks prior to starting treatment in order to keep stress-related variables to a minimum [16].

The cages contained clean bedding material and were kept in an environment with an average temperature range of 24 ± 4 degrees Celsius to provide adequate living conditions. Additionally, the cages used to house the animals were subjected to a 12 hour light / 12 hour dark cycle. During the course of this study the animals had access to the standard laboratory pellet food, and water from a hydration supply at all times.

Grouping and treatment schedule

The experimental rats were acclimatized for a minimum of (10) days prior to the actual experiment. A total of (12) rats were housed in two separate cages for the purpose of this experiment. The experimental control rats were administered an intraperitoneal injection of normal saline once daily for (14) consecutive days. The experimental treatment rats were administered of Gentamicin intraperitoneally once daily for the same (14) days. The injection volumes of both gentamicin and normal saline were adjusted according to body weight and ranged approximately from 0.155 to 0.194 mL/day.

The amount of saline administered to control animals was equal to the volume given to the gentamicin treated group, so that both groups would receive drugs via the same routes / at the same intervals / and according to the same schedules. Commercially manufactured gentamicin injectable ampoules (DEVA Holding, Turkey) were used for the gentamicin administration. Every ampoule contained 2 ml of solution containing 80 mg of gentamicin (40 mg/ml).

A dose of 31 mg/kg/day was chosen for the rat model of exposure. This value corresponds to the adult human dose from which it was derived; therefore, it was calculated by applying a body surface area (BSA) based conversion (Km of 37 for adult humans and 6 for rats) [17]. The formula used to calculate the doses is outlined below:

$$\text{Rat dose (mg/kg)} = \text{Human dose (mg/kg)} \times \text{Human Km} / \text{Rat Km}$$

$$\text{Rat dose} = 5 \times 37 / 6 = 30.83 \text{ mg/kg/day}$$

Using a gentamicin solution's respective concentration as well as the individual rat's body weight, a daily dose value can be calculated in mg/kg/day. The daily volume for animals within the weight range of 200 to 250 g will likely be between 0.155 and 0.194 mL per day depending on the calculated value before each injection.

Intraperitoneal administration

Sterile single use syringes were used to administer all injections via the peritoneum. Gentamicin and/or saline were injected into lower abdominal quadrants. The injection sites and the handling methods were kept uniform throughout the treatment process to control for variability in the procedures.

During the 14-day experimental period, the animals were monitored daily for general activity, feeding behavior, and obvious signs of discomfort or distress.

Anesthesia and cardiac tissue collection

At the end of the treatment period, rats were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). These two medications were commonly given together in laboratory rodents, and both were also used for short surgical procedures or in situations where scientists collect tissues [18], [16].Once the researcher confirmed the animal had reached deep levels of unconsciousness, the animal was euthanized.

The thoracic cavity was opened with care, and the heart was swiftly extracted. Cardiac samples were obtained from the ventricular myocardium (ventricular wall). The specimens were gently rinsed in saline to remove residual blood, and the specimen was individually placed in a fixative without distortion from excessive pressure or pulling to preserve the myocardial architecture.

Histological processing and hematoxylin-eosin staining

Cardiac tissue was fixed in a 10% formalin solution for about 24 hours. Following fixation, the tissues were processed using typical paraffin histology techniques. The tissues were dehydrated

progressively with increasing percentages of ethanol, cleared and then embedded in paraffin wax before sectioning into thin slices for light microscopic examination [19], [13].

Sections that had been prepared underwent hematoxylin and eosin staining. The purpose of using hematoxylin was to highlight details of the nucleus, whereas eosin would stain both cytoplasm and muscle tissue of the myocardium. Prepared specimens were later examined under a microscope following staining.

Light microscopic evaluation

Histological sections (3-5 micrometers thick) of control and gentamicin-treated animal groups were examined with a light microscope at a 40X magnification. Parameters evaluated for all histological sections included: myocardial fiber arrangement; cardiac muscle tissue compactness; alignment of cardiomyocytes; nuclear distribution; intermuscular spacing; separation of myocardial bundles; myocardial architecture restoration/altersation.

Findings were recorded descriptively by comparing representative cardiac sections from the control group with those from the gentamicin-treated group.

Results and Discussion

(Two Weeks into Treatment) Thin sections of cardiac tissue from control and gentamicin-treated rats were prepared by hematoxylin/eosin stains and evaluated by light microscopy. Areas evaluated included; architecture (arrangement) of cardiac muscle fibers, compactness (density) of myocardial tissue, morphology of elongated nuclei, considerations of intermuscular space, and evaluative of general cardiac muscle architecture for signs of preservation or alteration.

The cardiac tissue in the control group displayed a relatively intact myocardial architecture. Most of the muscle fibres of the heart were well-assembled into either parallel or branching formations. Nuclei could be seen forming lines along the muscle fibres; and there was no great change in width of the intermuscular spaces. Overall, the control myocardium displayed evidence of normal histological organization of cardiac muscle tissue based on the experimental procedures. These findings are presented in the accompanying control images of Figure 1 A-D.

Unlike those of the control animals, the cardiac tissue samples obtained from gentamicin-treated rats had marked differences from each other in terms of histology. In several fields examined, myocardial fibers were visibly less dense with evidence of partial separation and widening of intermuscular areas between some adjacent fibers. Additionally, there was visible evidence of mild irregularity or fiber alignment as well as a reduction in continuity of myocardial bundles in some areas. The representative images for the gentamicin-treated group are shown in Figure 2A-D.

In the gentamicin treated group, a consistent histological finding of myocardia was the alteration in the compactness of the heart. Upon examination of the treated myocardium versus the myocardium of the controls, there were wider than normal avascular spaces between individual muscle fibres as well as a less uniform pattern of organisation of the treated myocardial tissue. The above finding may suggest that there were areas of interstitial dilation; areas of separation of the myocardial tissues; or possibly early structural alteration of the myocardial framework. Further, while the changes from the gentamicin treatment were not of sufficient severity to be classified as severe myocardial injury the consistent finding of detectable histological responses that occurred from repeated exposure to gentamicin was evident.

The maintenance of close fiber organization in the control group indicates that the experimental conditions and handling methods were appropriate. Conversely, the separation of myocardial fibers and loss of tissue density in the gentamicin -treated group suggest that repeated administration of gentamicin may affect cardiac muscle tissue. These changes have significance since myocardial function relies on the structural integrity and spatial coordination of the cardiac muscle fibers. Thus, disruption of the normal order of organization may serve as an early morphological sign of tissue traumatisation.

The aminoglycoside antibiotic gentamicin is known to cause damage to the Kidneys & Ears yet studies have shown other tissues may also suffer from toxic effect from Gentamicin [5], [15], [4].The Myocardium depends significantly upon Mitochondrial Energy Production, Membrane Integrity and Adequate Cell Organization [11], [12].There are multiple Mechanisms which Could Have contributed to the observed Myocardial Abnormalities in the current study: Oxidative Stress, Mitochondrial Dysfunction, Inflammatory Response, Membrane Injury [15], [7], [6].These Mechanisms were not quantitatively measured as part of the current study but are put forth as likely possibilities based on the previous literature.

Current histology findings will therefore be interpreted as the morphological showing of cardia tissue that have undergone weight loss as a result an administration of gentamicin (H&E) [2]. H&E staining allows to view the overall shape and structure of cardiac muscle tissue in both control and treated groups. The results of this experiment show that there was a difference between both groups; especially in fiber structure, space between muscles, and compactness within the heart [2].However, additional studies utilizing oxidative stress (biochemical markers), cardiac enzyme evaluation, immunohistochemical staining, and morphometrics would provide even more clarity in understanding both functional & molecular significance of these histological findings [14], [15], [6].

The results demonstrate that administering gentamicin daily for a total of 14 days results in causing mild to moderately affected cardiac muscle histology in adult rats. The treated group exhibited a partial separation of myocardial fibers, increased intermuscular area, lesser tissue compactness, and moderate architectural disorganization. This indicates that the myocardium should also be considered as being affected by chronic gentamicin use and warrants additional investigation regarding potential cardiac toxicity during animal studies of tissues affected by repeated use of aminoglycoside-type antibiotics [14], [15], [6].

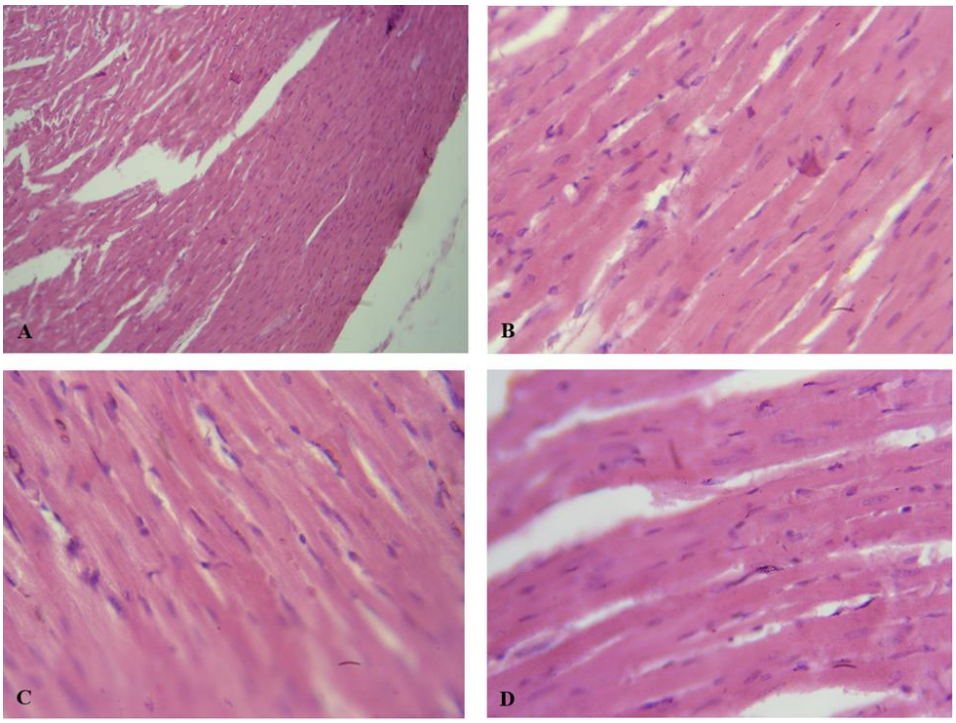

Figure 1. Light micrographs of cardiac muscle tissue from the control group after 2 weeks of administration of normal saline. Hematoxylin and eosin sections show that cardiac muscle architecture is preserved. There are many closely compacted parallel cardiac muscle fibres with elongated and identifiable nuclei. Moreover, the intercellular spaces between cardiac myocytes appear intact with no evidence of separation of myocardial fibres and there is not an obvious widening of intermuscular spaces. Photographic panels A-D show the controls examined at 40x magnification.

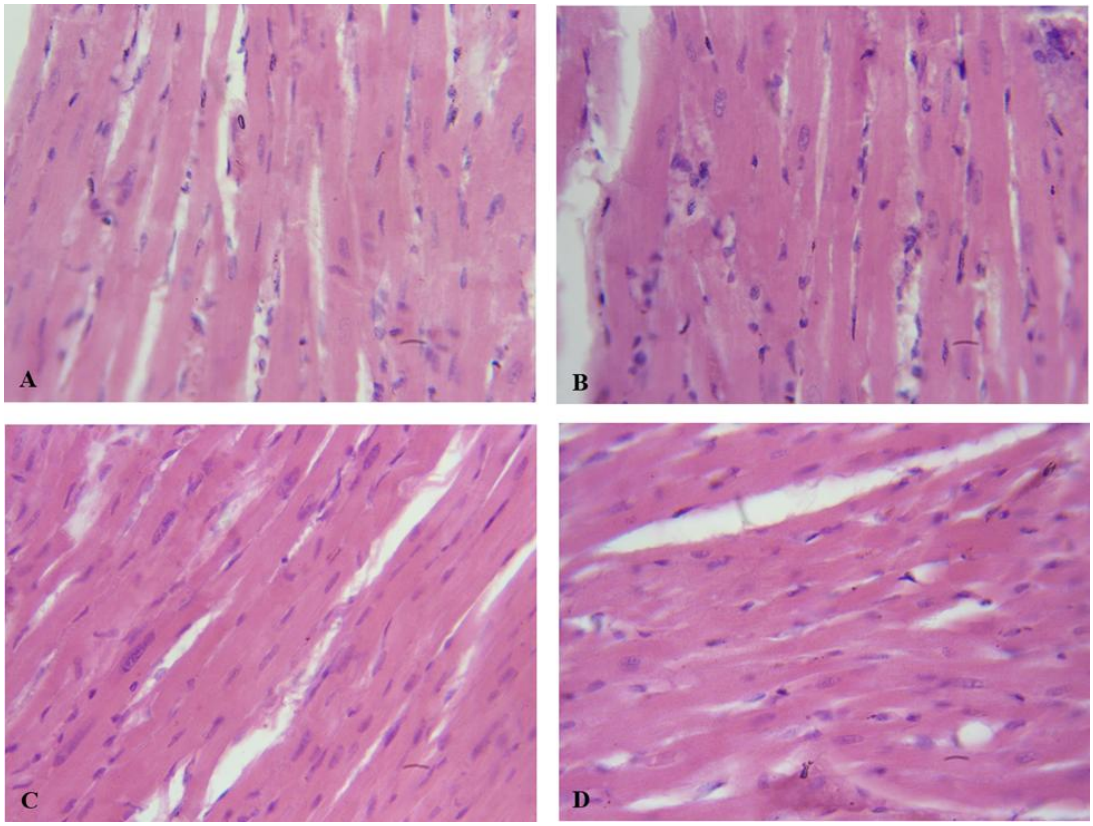

Figure 2. Light micrographs of cardiac muscle tissue from gentamicin-treated rats after 2 weeks of treatment show histological changes in the myocardial tissue following treatment with gentamicin as seen in the sections stained with hematoxylin and eosin. The gentamicin-treated sections of myocardial tissue have partially separated myocardial fibres, enlarged spaces between the myocardial fibres (intermuscular spaces), decreased density of cardiac muscle bundles and mild disorganization of the architecture when compared to the control group. The panels represent gentamicin-treated myocardial field at 40x magnification.

Conclusion

The results of this study indicate that repeated dosing of gentamicin for 14 days in adult rats resulted in observable histological alterations to their cardiac heart muscle tissue when compared to an untreated control group. The histological examination of myocardial specimens from gentamicin treated rats had partial separation between muscle fiber cells, increased distance between muscle fibers, decreased density of myocyte bundles, and a mild disturbance of normal myocardial architecture.

These results suggest that exposure to gentamicin may impact the organization of the structural makeup of cardiac muscle, especially at the level of intercellular spacing and alignment of individual myocardial muscle fibers. The control group's compact myocardial architecture supports that there are gentamicin-related histological changes to myocardium.

The present study uses routine hematoxylin/eosin stained sections to provide data on morphological changes indicative of gentamicin-associated myocardial alterations; therefore additional studies using biochemical markers of cardiac function, oxidative stress assays, immunohistochemical techniques, and morphometric analyses should be performed to further elucidate how these alterations can alter or how they are correlated with alterations at the functional level and/or at a molecular level.

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