

Histoarchitecture of the Germinal Epithelium of Cyanide Treated Rats

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Abstract

The Germinal epithelium is the innermost layer of the seminiferous tubule is also known as the wall of the seminiferous tubule within the testes. The histoarchitecture of the germinal epithelium is characterized by the presence of spermatogonia, primary spermatocytes, secondary spermatocytes, sertoli cells, spermatozoa, myocytes and the lumen of the seminiferous tubule. They secrete testosterone. The cells of the germinal epithelium are simple cuboidal. 8 F₁ Male Adult Albino Wistar Rats were used for this research, they were divided into two groups, control and treatment group. The treatment group were given 2mls KCN via oral administration for 7 days. Histological studies revealed that KCN treatment caused severe disorganization and denudation of germinal epithelial cells of seminiferous tubules with distortion and degeneration of the lumen with formation of abnormal tails of spermatozoa. This research further showed decline in spermatogenesis, decrease in sperm count and increase in sperm abnormality on exposure to a low dose of Cyanide.

Keywords: KCN; Germinal Epithelium; Seminiferous Tubule; Spermatogonia

Abbreviation

KCN: Potassium Cyanide

Introduction

The testes (singular, testis) are located in the scrotum (a sac of skin between the upper thighs). In the male fetus, the testes develop near the kidneys and then descend into the scrotum just before birth. Each testis is about 1¹/₂ inches long by 1 inch wide. Testosterone is produced in the testes which stimulates the production of sperm as well as give secondary sex characteristics beginning at puberty. The seminiferous tubules are located inside the testes; each testicle contains about 900 coiled seminiferous tubules. Around 90% of the weight of each testis consists of seminiferous tubules. The seminiferous tubules are the functional units of the testis, where spermatogenesis takes place [1]. Once the sperm are produced, they are moved from the seminiferous tubules into the rete testis for further maturation. The seminiferous tubules are responsible for the formation of sperm (Alevi., *et al.* 2013). The germinal epithelium of the seminiferous tubule is composed of cells which are responsible for spermatogenesis. Cyanide toxicity leads to a distorted lumen and formation of abnormal tails of sperm in the seminiferous tubules. This situation may lead to reduced motility of sperm thereby causing infertility in males. This research reveals decline in spermatogenesis, decrease in sperm count and increase in sperm abnormality on exposure to a low dose of Cyanide. Similar result was reported on exposure of Adult Wistar rats to Diazinon, an organophosphate pesticide [2].

Materials and Methods

Animal preparation

The animals were housed at the animal holding facility of Afe Babalola University, Nigeria. The animals were allowed to acclimatize to the new environment for 1 week. 8 male animals were used for this research. The animals were kept under alternating light and darkness with rat chow and water being given to the animals daily. The albino Wistar rats were kept in separate cages, 2 animals per cage in 4 cages for 7 days. All protocols were in accordance with the IACUC animal use guidelines and were approved by the Animal Use Ethical Committee of the Afe Babalola University, Nigeria.

Tissue processing

Testes of cyanide treated rats and non-cyanide treated rats were obtained via abdominopelvic dissection after sacrificing. The specimens were fixed 10% formal saline and processed histologically to obtain paraffin wax embedded sections at the histology laboratory of Afe Babalola University, Ado-Ekiti.

Histology

Tissue sections were processed for routine Haematoxylin and Eosin following the methods of Zhang, *et al.* [3] to demonstrate the general morphology of the tissues and vessels in the tissue.

Histomorphometry

Cell cycle markers (*p53*, *Cathepsin D* and *Bax*): These were immunolabelled in the glandular and muscular prostate tissue, using anti Human-*p53* (polyclonal), Rat anti Human-*Bax* and anti-*Cathepsin D* (Monoclonal) to demonstrate cell cycle dysregulation, cell death and onset tumorigenesis. This was then mapped against regions of α 1A over expression. [Dilutions; *p53* (1:100 in PBS), *Cathepsin D* (1:350 in TBS) and *Bax* (1:1,000 in PBS)].

The paraffin wax embedded sections were mounted on a glass slide in preparation for antigen retrieval where the slides were immersed in urea overnight and then placed in a microwave for 45 minutes to re-activate the antigens and proteins in the tissue sections. Primary antibody treatment involved treating the sections with biotinylated goat serum for one hour following which the sections were transferred to 1% bovine serum albumin (BSA) to block non-specific protein reactions. Secondary treatment involved the use of diluted anti-*p53*, anti-*Cath D*, anti-*Bax* and anti-*CD45* on the pre-treated sections for one hour. The immunopositive reactions were developed using a polymer 3'3' Diaminobenzidine Tetrachloride (DAB) with colour intensification involving the use of mathenamine silver kit. The sections were counterstained in Hematoxylin and treated in 1% acid alcohol (freshly prepared).

Results

Histology

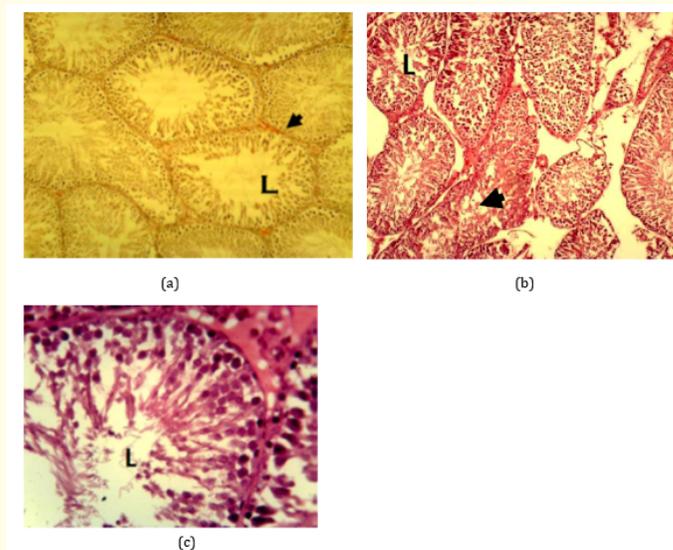


Figure 1: (a) General morphology of the testes (Control) stained with Hematoxylin and Eosin. The lumen is observable with the protruding tail of the matured sperm cells. The general outline is demonstrated as germinal epithelium within the seminiferous tubule (lumen; L); arrow head indicates the location of the blood vessels adjacent to the basement membrane (Magnification X100). (b) General morphology of the testes of adult Wistar Rats treated with 20 mg/Kg B.W of orally administered KCN for 7 days. The testes shows general distortion of the lumen and the tail of sperm cells are not prominent when compared to the control group. The tissue section shows signs of extreme degeneration (Magnification X100). (c) General morphology of cyanide treated rat testes at X400. The distortion in the cytoarchitecture of the basement membrane can be seen at this magnification. The cell diameter is greater reduced compared to the control group at the same magnification (X400).

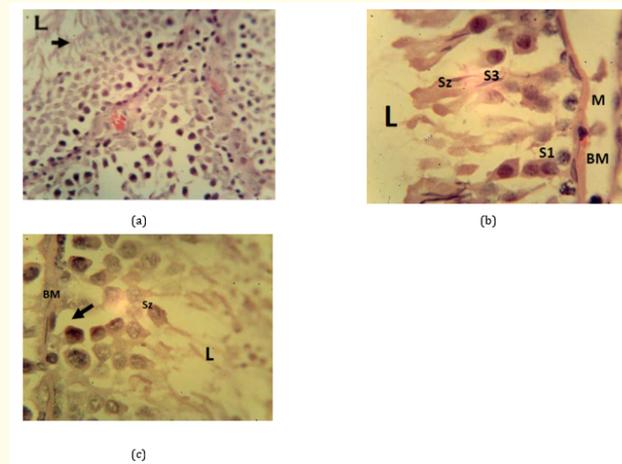


Figure 2: (a) General morphology of the testes at X400, note the long tail of the sperm cells (arrow head), also observe the structure of the basement membrane and location of blood vessels close to the base of the Sertoli cells (Magnification X400). (b) Histological demonstration of the germinal epithelium using the oil immersion X100 objective. This is to clearly demonstrate and characterize the cell types in the testes. S0 (spermatogonia), S1 (primary spermatocytes), S2 (secondary spermatocytes), S3 (Spermatids), Sz (spermatozoa), L (lumen), BM (basement membrane), M (myocyte), Sc (Sertoli cells) and V (blood vessels). Observe the chromatin condensation in the spermatocyte. (c) Histoarchitectural demonstration of the germinal epithelium for cyanide treated rats. Arrow heads indicates sites of tissue and cell degeneration due to cyanide induced stress. S0 (spermatogonia), S1 (primary spermatocytes), S2 (secondary spermatocytes), S3 (Spermatids), Sz (spermatozoa), L (lumen), BM (basement membrane), M (myocyte), Sc (Sertoli cells) and V blood vessels). Observe the chromatin condensation in the spermatocytes.

Immunohistochemistry

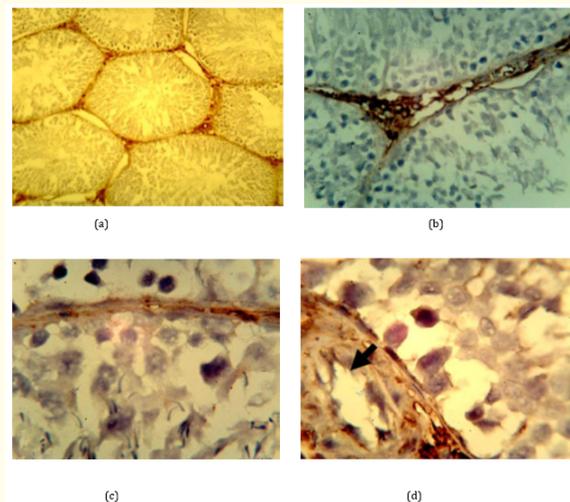


Figure 3: (a) Immunohistochemical localization of cell proliferation protein Ki-67 localized using polyclonal Goat anti-Rat Ki-67 antibody developed using 3’3’ Diaminobenzidine tetrachloride (DAB). Counter stain was done using Hematoxylin. The location of the rapidly proliferating cells was mapped around the basement membrane (Magnification X100). (b) Immunohistochemical localization of cell proliferation protein Ki-67 localized using polyclonal Goat anti-Rat Ki-67 antibody developed using 3’3’ Diaminobenzidine tetrachloride (DAB) showing highly immunopositive spermatogonia close to the basement membrane (Magnification X400). (c) Histological demonstration of germinal epithelium, labelled with cell proliferation antibody Ki-67. The immunopositive cells were localized in the base of the epithelium, general outline shows degeneration and reduced cell population per unit area (Magnification X1, 000). (d) Immunohistochemical demonstration of the germinal epithelium cell proliferation profile. Arrow head indicates rapid cell proliferation in the basement membrane and around the blood vessels (Magnification X 1,000).

Morphometry

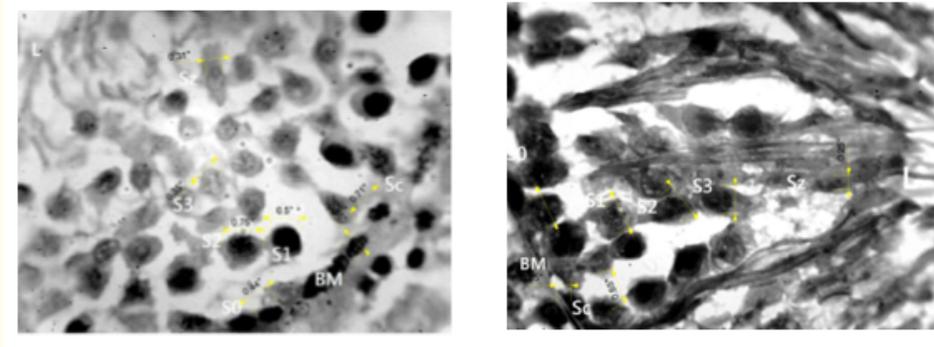


Figure 4: (a) Histomorphometry of germinal epithelium cells of control Wistar Rat. (b) Histomorphometry of germinal epithelium cells of Cyanide treated Wistar Rat.

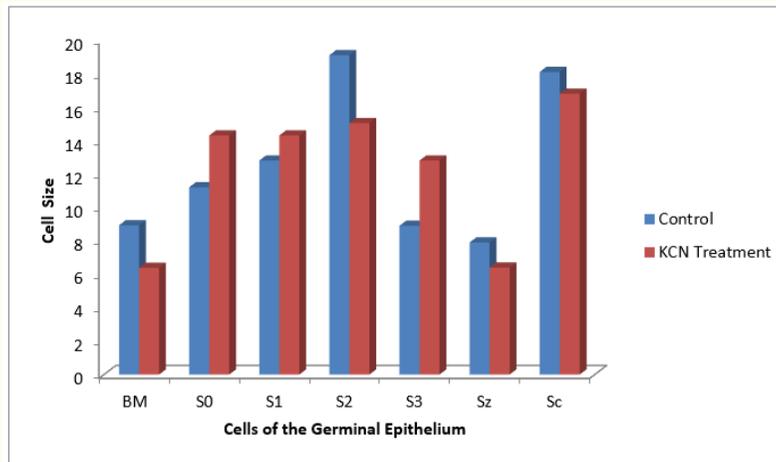


Figure 5: Bar chart representing the cell diameters in micrometres for the treatment and control groups, The graph shows a reduction in size of the cells basement membrane, secondary spermatocytes, spermatozoa and the sertoli cells of cyanide treated rats (KCN) when compared with the control.

Cell type	Measurement (Inches) at X1,000	Measurement (mm) at X1,000	Measurement (µm) at X1,000	Measurement (µm) (Actual)
Basement membrane (Bm)	0.35	8.93 x10 ³	8.93	8.93
Spermatogonia (S0)	0.44	11.2 x10 ³	11.2	11.2
Primary Spermatocytes (S1)	0.50	12.8 x10 ³	12.8	12.8
Secondary Spermatocytes (S2)	0.75	19.1 x10 ³	19.1	19.1
Spermatids (S3)	0.35	8.9 x10 ³	8.9	8.9
Spermatozoa (Sz)	0.31	7.9 x10 ³	7.9	7.9
Sertoli cell (Sc)	0.71	18.1 x10 ³	18.1	18.1

Table 1: Table showing measured values of germinal epithelium cells of Control Wistar Rat. Converting 1 inch is 25.5 mm, thus each of the values in inches is converted to mm at X1,000. This was then divided by 1,000 to get the value for the actual measurement and values in µm.

Cell type	Measurement (Inches) at X1,000	Measurement (mm) at X1,000	Measurement (µm) at X1,000	Measurement (µm) (Actual)
Basement membrane (Bm)	0.25	6.38 x10 ³	6.38	6.38
Spermatogonia (S0)	0.56	14.3 x10 ³	14.3	14.3
Primary Spermatocytes (S1)	0.56	14.3 x10 ³	14.3	14.3
Secondary Spermatocytes (S2)	0.59	15.04 x10 ³	15.04	15.04
Spermatids (S3)	0.50	12.8 x10 ³	12.8	12.8
Spermatozoa (Sz)	0.25	6.4 x10 ³	6.4	6.4
Sertoli cells (Sc)	0.65	16.6 x10 ³	16.6	16.6

Table 2: Table showing measured values of germinal epithelium cells of Cyanide Treated Wistar Rat. Converting 1 inch is 25.5 mm, thus each of the values in inches is converted to mm at X1,000. This was then divided by 1,000 to get the value for the actual measurement and values in µm.

Discussion

Cell cycle describes the cellular control mechanism in place to check and control all the different phases involved in cell reproduction, activities and cell death. Each of the different stages of the cell cycle is said to be controlled by several cell switch systems involving the cyclins and cyclin dependent kinases (*Cdks*). During the process of rapid cell division, the cell cycle puts in place resting phases (Gap phases) in between the important phases (Synthetic or S-Phase, Mitosis or M-Phase), the cell cycle is characterized a specific resting duration during which the cell proofreads its genome for errors. If such errors are repairable, the cell amends such errors via molecular control mechanisms by literally stitching the broken DNA material into the rest of the genome. Although the cell is equipped with the metabolic machinery to stitch the broken genetic fragments during replication, it is however not endowed with tools to recognize the actual sites. In certain circumstances, the fragmented gene is stitched to a wrong site which might alter gene regulatory region that will prone a cell to over expression of certain proteins that can lead to cancers. In a second mechanism, if the DNA breakage is vast and cannot be repaired easily, the cell quickly sends itself to a permanent resting phase or G0 (apoptosis). This state is achieved via the increase in the transcription of the *p53* gene in response to such genetic errors. The nuclease digests the DNA thus; such cells are believed to be in a state of self-termination (apoptosis). *P53* gene increases the fidelity of *PCa* due to mismatch repair as described by Bansal., *et al.* 2012. Several studies show that the expression of *p53* corresponds with the progression of the *PCa* and has thus been regarded as a prognostic marker and predictor of endocrine therapeutic effect for prostate cancer. The *p53* signaling pathway activates *Bax* as a form of pre apoptotic signal, this is accountable for the over expression of *p53* and *Bax* in the *PCa* biopsies (Figure 2 and Table 1).

The role of lysosomal proteases has been suspected to be involved in malignancy of tumors in the prostate. The enzyme is a protease that caused degeneration of the intercellular matrix, thus facilitating the escape of neoplastic cells. This is not a wide spread occurrence in *PCa* as it is restricted to specific tissue sites (Figure 4 and Table 1). The study uses anti-Human *Cath D* (Mab) to map the location of *Cath D* in *BPH* and *PCa* tissues. The *BPH* biopsies showed moderate Cathepsin D expression which is characteristic of cells found to be undergoing cell proliferation and migration in the basal region of the control testicular tissue (S38), although the expression of *CathD* is higher in the *PCa* biopsies, it does not necessarily imply malignancy rather it might be an implication of early onset malignancy. Certain invasive cancer cells also showed *CathD* over expression in isolated cell populations within the glandular tissue. The study of Kuczyk., *et al.* 1994 reports that *CathD* expression was observed in the cytoplasm and the surface of tumor cells invading glandular tissue and in single cells involving prostatic stoma. This is also important in the determination of biological aggressiveness of prostate cancer, the importance of *CathD* over expression is an important prognostic tool for distinguishing *PCa* from *BPH* in epitheliomas but cannot be isolated except analyzed with other cell cycle markers like *Bax* and *p53* to determine the holistic role of cell cycle regulation in *BPH* and *PCa*. The reduced *p53* in *BPH* is understandable, especially when co-analyzed with *Bax* as it implies increased senescence not due to tumor genesis. This scenario describes a well-organized cell cycle but short timed to give numerous cells over a short period of time. Other studies involving the detection of epithelium protein *E Cadherin* shows intact epithelium with orderly arranged lamellae that is restricted to the fibromuscular epithelium. In *PCa*, the increased levels of *P53* and *Bax* signals pre-apoptotic tendencies for rapidly proliferating un-coordinated cells which can be located at random locations due to loss of matrix and adhesion molecules described in high *CathD* levels. In conclusion, *p53*, *CathD* and *Bax* co-localization gives a holistic approach to understanding the cell cycle in *BPH* and *PCa* and in distinguishing the pattern of cell proliferation in both conditions.

Conclusion

In a nutshell it could be concluded that the degeneration of lumen, abnormal shape of sperm tails and a general disorganization of the histoarchitecture of the germinal epithelium may be as a result of the toxicity of cyanide. Malformation of the histoarchitecture of the germinal epithelium of the seminiferous tubule would lead to the formation of abnormal spermatozoa or even absence of spermatozoa, resulting into infertility in males.

Conflict of Interest

The Authors hereby declare there is no conflict of interest associated with this study or any of the procedures and materials used for the purpose of the study.

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We will like to acknowledge Dr. Olalekan Ogundele of LSU for his tutelage and advice during the process of this research. We will also like to appreciate the Department of Anatomy, Afe Babalola University for allowing us to make use of some their equipment for this research.

Ethical Approval

All animal handling protocols were in accordance with the Institutional Animal Care and Use Committee (IACUC) animal use guidelines and were approved by the Animal Use Ethical Committee of the Afe Babalola University, Nigeria.

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