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Effects of X-ray Radiation on the Motor Neurons of the Corticospinal Tract of Albino Wistar Rats

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ABSTRACT

Introduction: the study focused on the effect of doses of radiation on the motor neurons of corticospinal tract of Albino Wister rats subjected to radiation of an x-ray.

Materials and Methods: the method involves twenty (20) adult male albino wistar rats grouped and exposed to doses of x-rays over a period of twenty one (21) days. Morris water maze test was performed to determine if radiation affected the cognitive functions of the rats. Each group was sacrificed after every seven days and the spinal cord being one of the target of the radiation induced damage was analyzed histologically.

Results: results indicated that there were increased rates of damage to the cytoarchitecture of the spinal cord proportional to increased time or duration to exposure to radiation. Arching of the back, sluggish motion, reduced time in completion of the Morris maze task were observed physically. Degenerative motor neurons, depleted cytoplasm, as well as vacuolation were also observed across the groups of rats histologically. This study strongly supports the hypothesis that exposure to radiation causes damage to the cells of living systems.

Conclusion: conclusively exposure to sustained or increased doses of radiation emitted from x-ray machines affects the cells of the spinal cord and also other parts of the central nervous system leading to muscle weakness and atrophy, paralysis of the limbs, loss of voluntary muscle control.

Keywords: Corticospinal tract; Irradiation; Spinal cord; Motor neuron, Cell vacuolation, X-ray radiation.

Introduction

An x-ray or x-radiation, is a penetrating form of high energy electromagnetic radiation. X-rays play a very important role in medicine today in the diagnosis or detection of disease or injury and also in the treatment of such medical conditions[1].

X-ray imaging creates pictures of the inside of your body. The images show the parts of your body in different shades of black and white. This is because different tissues absorb different amounts of radiation. Calcium in bones absorbs x-rays the most, so bones look white. Fat and other soft tissues absorb less and look gray. Air absorbs the least, so lungs look black[2]. The most familiar use of x-rays is checking for fractures but is also used in other ways; for example, chest x-rays can spot pneumonia, mammograms use x-rays to look for breast cancer.

Risks associated with the use of X-rays comes from the radiations that they produce called ionizing radiation which can cause harm to living cells and tissues of the body. Although these harmful effects of x-rays are not instantaneous, they manifest over a period of time following repeated exposures to the radiations. Ionizing radiation is a form of radiation that has enough energy to potentially cause damage to DNA[3]. Another risk of X-ray imaging is possible reactions associated with an intravenously injected contrast agent, or "dye", that is sometimes used to improve visualization. The risk of developing cancer from medical imaging radiation exposure is generally very small, and it depends on the radiation dose, patient's age, patient's sex (with higher risker at younger age and women), body region.

Due to the size and location of some tumors, it is very difficult or impossible to carry out a radiotherapy treatment without exposing the spinal cord to radiation.

Deficits in cognitive function occur in patients who receive radiation therapy to the spine.

Anatomy of the Corticospinal Tract and Motor Neurons

The primary origin of the corticospinal tract is from the front parietal cortices, including the primary motor cortex, secondary motor area, and somatosensory cortex[4].

The neural structure of the corticospinal tract takes up more surface area in the upper pons than in the lower pons as a result of this[5].

Motor neurons also called efferent neurons are the nerve cells responsible for carrying signals away from the central nervous system towards muscles to cause movement. They release neurotransmitters to trigger responses leading to muscle movement[6].

Motor neurons are responsible for integrating

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signals from the brain to the muscles, glands, and organs that intend to carry out the required motor function as they allow us to move, talk, eat, swallow, and breathe, therefore without these cells, we would not be able to complete many basic life functions[6].

Objectives of the study:

1. To determine the effects of x-ray radiation on the structure and functions of the motor neurons on learning, memory and behavior following the exposure of albino Wistar rats to x-ray radiation.
2. To determine the histological changes in the spinal cord and cell death rate of the motor neurons due to exposure to x-radiation.
3. To determine the significant difference if any across the groups of radiated rats using statistical analysis.

Materials and Methods

The study carried out at the animal house of the Department of Pharmacology, University of Port Harcourt, Nigeria, focusses on the effects of x-ray radiation on the motor neurons of the corticospinal tract of Albino Wister rats. Twenty Adult male Albino Wister rats weighing 150 to 200 grams were drawn randomly from a population of healthy breeding Albino Wister rat colonies. Out of the four weeks of this experimental procedure, the initial one week was for acclimatization of the rats, while the remaining three weeks was for grouping and experimentation. At the end of every week, the rats in the groups were sacrificed, dissected and the spinal cord extracted.

Grouping of experimental animals

The animals were placed into 4 groups (A - D), each group had a total number of 5 rats. Group A was the control group and was fed with feed and distilled water only with no exposure to x-ray radiation. Groups B, C and D were exposed to x-ray radiation for 7 days, 14 days and 21 days respectively.

Irradiation techniques

The duration of exposure of the test animals was four weeks. The amounts of irradiation dose was constant across all the groups except for the control group which was not subjected to radiation.

The difference among the groups was the total number of days used for exposure.

The X-ray machine used in the study was "The Sovereign II".

The anesthetized animal was placed centrally on a bench the distance between the animal and x-ray machine was 20cm.

The major parts of the body that were focused on were the head region and Spinal cord. Personal protective equipment was worn during exposure time.

Group A - was the control group and no radiation was administered to this group.

Group B - was irradiated with 3 exposures of 3.5Gy/min for seven days

Group C - was irradiated with 3 exposures of 3.5Gy/min for fourteen days

Group D - was irradiated with 3 exposures of 3.5Gy/min for twenty-one days

Behavioural studies: Morris Water Maze test

In this study, it was aimed at determining if the radiation affected the learning, memory and swimming patterns of the test subjects and also it there was any changes in way the animal walked. The pool was filled with water and contained a small platform, visible above the water level. This small platform allows the animals to escape the water and allows them to stand without the stress of swimming.

Apparatus used for this procedure include, a circular water tank with 6ft diameter and 2ft depth.

Histological procedures were followed after which slides were viewed using a light microscope and the cyto-architecture of the motor neurons will be observed and recorded.

Cell count using Image J

The cells of the motor neurons from the micrographs of tissues of the spinal cord were counted. ImageJ software was used for the cell count.

Results

Histological results of the spinal cord from various x-ray exposure periods

The following are photomicrographs of the histological findings gotten after radiation at the end of the experiment.

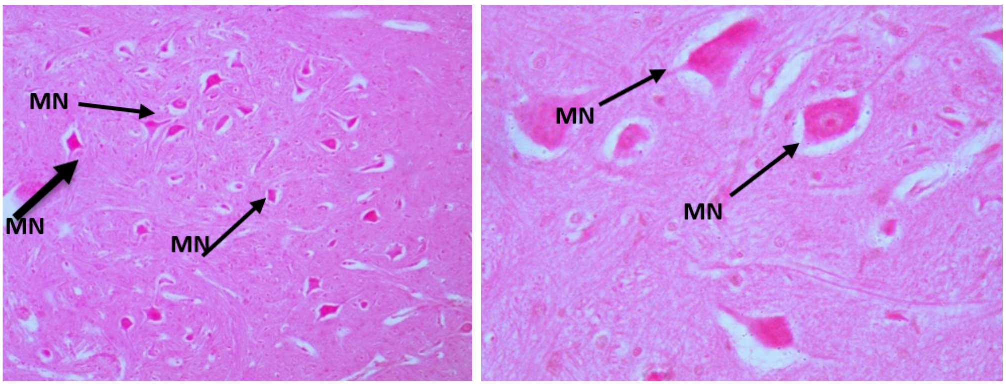

Figure 1. SPC D1, Group A: Photomicrograph section showing normal Motor neurons (MN) in the anterior horn of the spinal cord. (H&E x100 and x400).

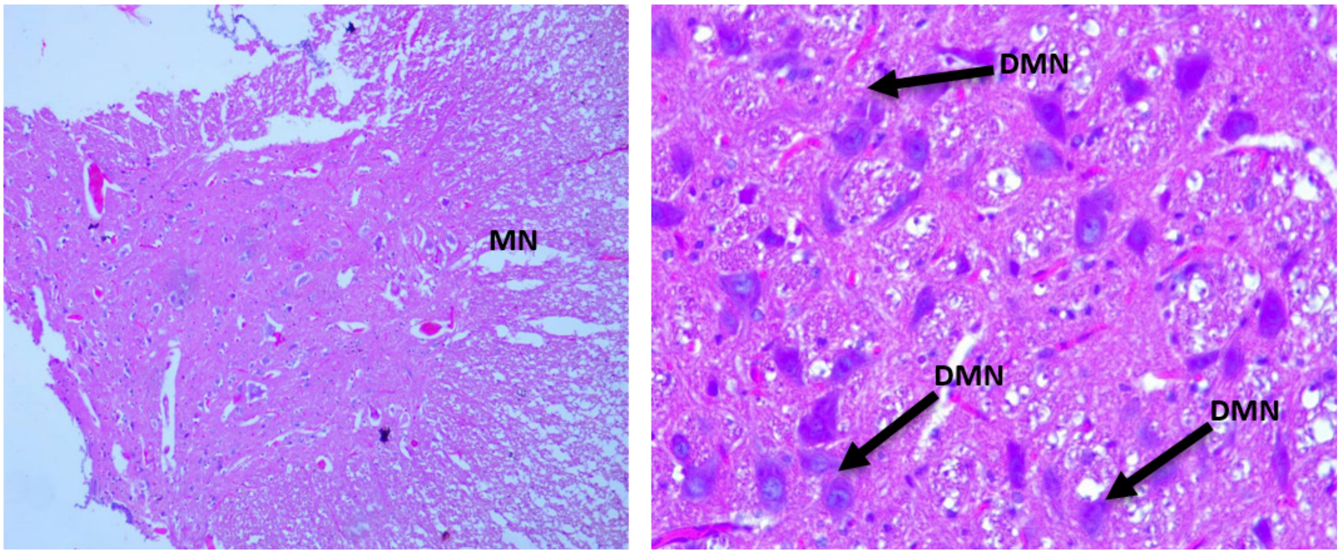

Figure 2. SPC D5, Group B: Photomicrograph section showing distorted, damaged motor neurons (DMN) in the spinal cord grey matter of wistar exposed with radiation for 7 days. (Spinal cord, H&E x100 and x400).

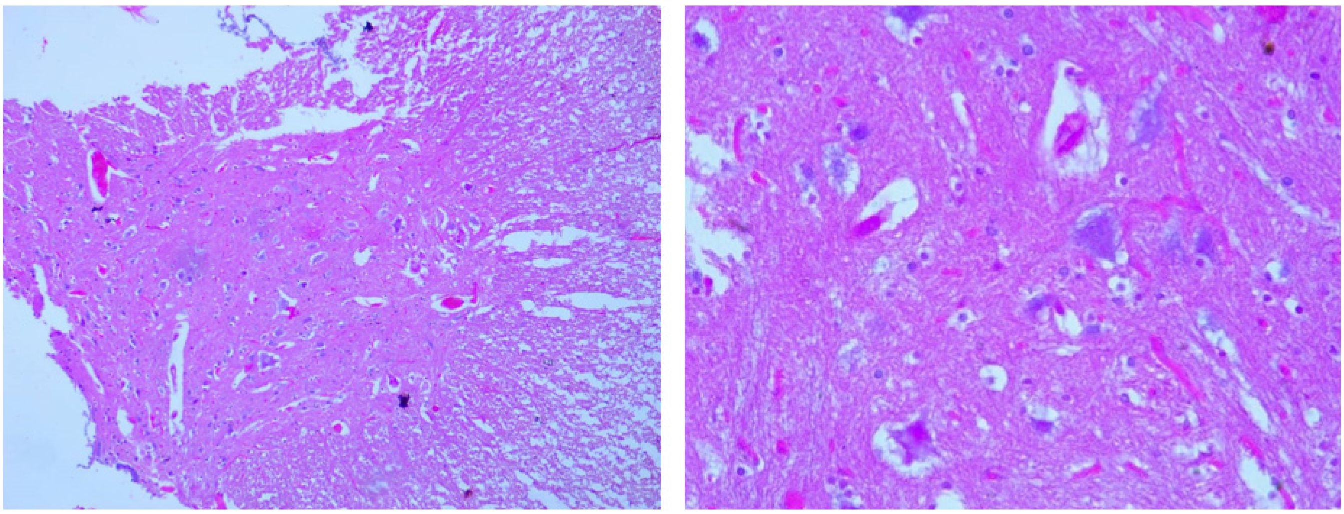

Figure 3. SPC D10, (Group C: Photomicrograph section of spinal cord showing severe motor neurons damage (SMND) in the anterior and lateral horns grey matter of the spinal cord. (H&E x100 and x400).

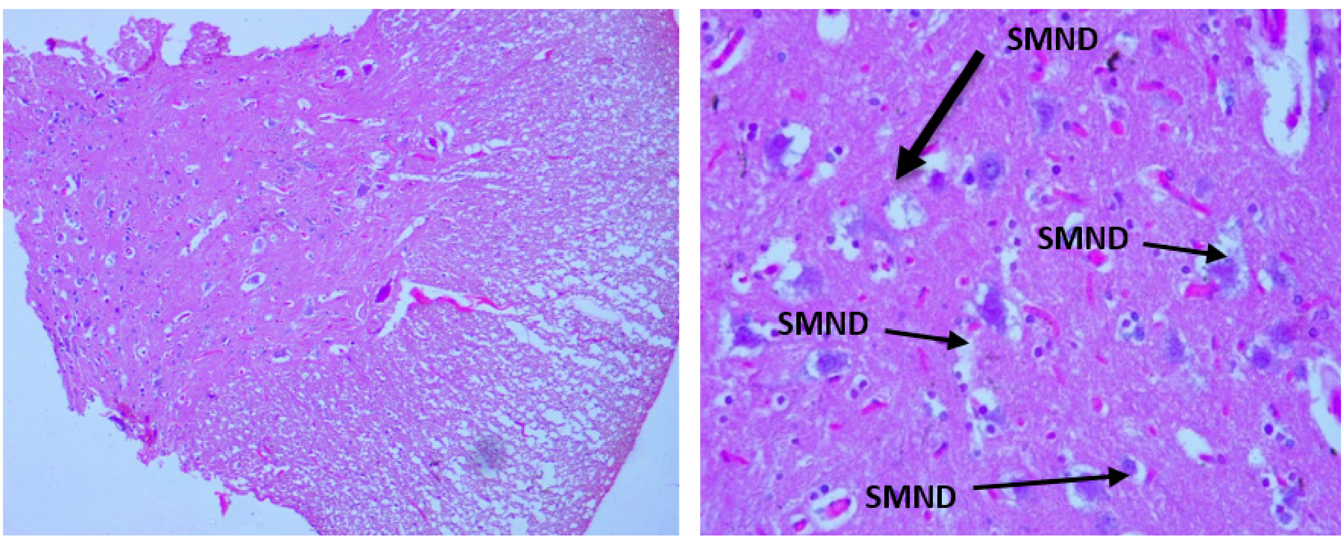

Figure 4. SPC D15 (Group D). Photomicrograph section of spinal cord showing severe motor neurons (SMND) in the anterior and lateral horns grey matter of the spinal cord. (H&E x100 and x400).

Result Analysis

Below are the results of the statistical analysis carried out using one way Anova.

Table 1. Analysis of motor neurons in the anterior and lateral horns

GROUP	ATH	LTH
A	20.75±1.71	14.50±0.57
B	10.03±0.82[b]	9.83±0.35[b]
C	12.35±0.72[a]	12.40±0.44[a]
D	13.63±0.54[a]	12.73±0.28[a]

A = Control; B = 7 days X-ray exposure; C = 14 days X-ray exposure; D = 21 days X-ray exposure; n=5; Data expressed as mean±SEM, [a] p<0.05 when compared to the control; b p<0.01 when compared to control.

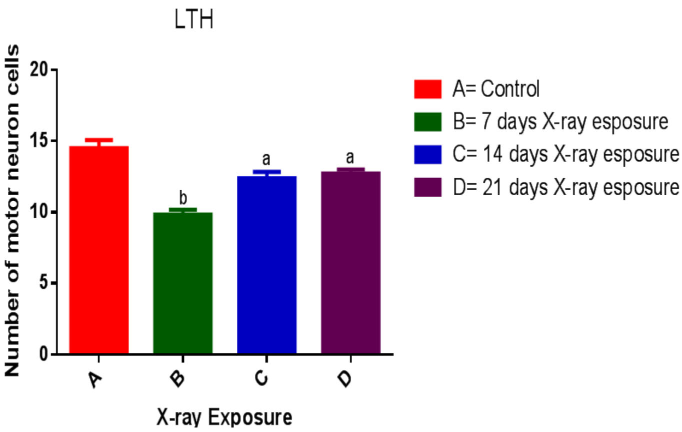

Figure 5. Comparison of the number of motor neurons of the lateral horn across the various groups.

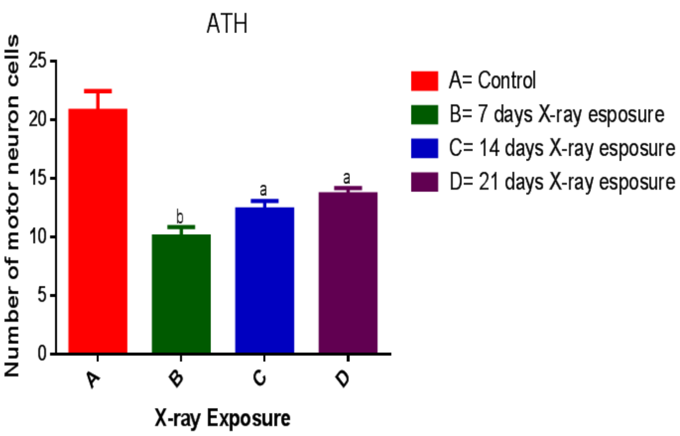

Figure 6. Comparison of the number of motor neurons of the lateral horn across the various groups.

Table 2. Paired sample statistics of anterior and lateral horns

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	ATH	14.1900	20	4.62019	2.31009
	LTH	12.3650	20	1.92518	.96259

Table 3. Paired sample tests

Paired Samples Test						
		Paired Differences				
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference	
					Lower	Upper
Pair 1	ATH LTH	1.82500	2.97728	1.48864	-2.91251	6.56251

Table 4

Paired Samples Test				
		T	Df	Sig. (2-tailed)
Pair 1	ATH – LTH	1.226	3	.308

Discussions

After seven days of exposure to x-ray radiation, there was damage and distortion of the motor neurons in the grey matter (fig. 2), across the members of group B. This damage amounted to a reduction in the number of motor neurons of both the lateral and the anterior horns of the spinal cord. In comparison to the un-radiated control group A, there was about 33% reduction in the number of motor neurons in the lateral horn (fig 5) and about 50% reduction of motor neurons in the anterior horn (fig 6) within members of group B which were exposed to radiation for seven days.

Members of group C were radiated for fourteen days and the histological findings were presented as follows. There was severe motor neuron damage (SMND) in both the anterior and lateral horn grey matter of the spinal cord (fig 3). Degeneration of pyramidal cells was observed in fourteen days x-ray exposed rats. Degenerative motor neurons, depleted cytoplasm, as well as vaculations were also observed.

In comparison to the control group, there was a 13% reduction in the number of motor neurons in the lateral horn. However, there was a 20% increase when compared to group B. In the anterior horn, there was also an increase in the number of motor neurons in comparison to group B, In group D, there was severe motor neuron damage. Degeneration of pyramidal cells was observed in 21 days, degenerative motor neurons (DNs) likely chromatolysis, atrophy, neuronophagia as well as vacuolation were also observed in members of the group D.

Statistically, from the results, we saw that for the anterior horn, there was a reduction in the number of motor neurons in Group B. The superscript B means that the reduction was half the P value of 0.01 in comparison to the control group.

After 14 days of exposure, the reduction reduced, that is there was an increase in the number of motor neurons. This reduction was significant at a P value of < 0.05, denoted by the superscript A.

At the lateral horn, the results were similar. The reduction occurred at P < 0.01 for Group B while for Groups C and Group D, it occurred at P < 0.05.

T- Test was used to analyze the mean of the two groups if they are common or not and to find out if there is any significant difference between the two variables. When P < 0.05, then that means that there is a significant difference between the two groups but when P > 0.05 then it means that there is no significant difference between the groups. Results of the T-Test done for significant difference between anterior and lateral horns showed that P = 0.308 (Tab 4). Since P > 0.05, it means that there is no significant difference between the anterior and posterior horns.

From the results obtained from this experiment, the following observations were made.

This included:

There was generation and reduction in number of the motor neurons. There was also depletion of cell cytoplasm. Vacuolations were also observed in this rats belonging to group B. Less motor neurons was seen.

Previous discussions on the effects of radiation on the nervous system have been divided into four sections: electrophysiological changes, higher nervous functions and behavioral studies, changes in developing embryo, and biochemical and histological changes.

It can be deduced as also agreed[7] reported that, the tolerance of the spinal cord to radiation is dependent upon the volume of the spinal cord irradiated, the total dose, the dose per fractional, the elapsed time between the treatment and the region of the spinal cord involved[8], in a work similar to this established that with single exposures of 100 to 300 R to prenatal and early postnatal whole body radiation depending on the dose and stage of life, yielded a high series of malformations, involving cortex, cerebellum and other structures[9], found that low doses primarily altered expression of genes regulation ion channels, Synaptic plasticity, and vascular damage, while high dose responses affected oxidative stress,[10] reported that after 14 days x-ray exposure in the rats, it was observed that there was Degeneration of pyramidal cells. From this work it can be noted that further degenerative motor neurons (DNs), depleted cytoplasm as well as vacuolation were also observed in this rats. From these finding it can be concluded that exposure to x-ray radiation will indeed had a negative effect on the spinal cord, thus leading to loss of motor functions and paralysis[11,12], demonstrated through a work on neuronal morphometry investigation using Golgi Silver Stain in mice and rats and fluorescence microscope of transgenic mice expressing enhanced green fluoresce protein (EGFP) in neuron that x-rays, protons, and 56Fe radiation cause reductions in hippocampal neuron arborization (>50% at 30days) as well as loss of dendritic spines, each of which would limit the complexity of signal processing[13].

While studying the radiation damage to the rat's spinal cord found at that paralysis occurred due to high amount of radiation after a period of one year. This study is in line with[14] but the later noted the paralysis on the hind legs which develops from about three months to one year after radiation[15].

While studying the therapeutic target for external beam x-irradiation in experimental spinal cord injury noticed that z-irradiation of the spinal cord with beam centered at the contusion epicenter resulted in significantly greater increase in locomotors recovery compared to un-radiated rats.

Conclusion

From the findings of this study, it is observed that there were histological changes and damage to the motor neurons of the spinal cord. The effects were prominent in the first week of exposure causing a significant reduction in the number of motor neurons. Following the second and third weeks of exposure, there was in increase in the number of motor neurons which may be as a result of cell regeneration and tissue repair hence radiation-induced cognitive deficits are observed following continuous exposure to radiation.

The exposure of spinal cord to x-ray radiation was detrimental to the motor neurons of the corticospinal pathway where it caused cell vacuolation and degeneration leading to cell death and the death of the motor neurons would mean in impairment in the locomotive functions of the animal.

This study will help to determine how motor neurons of the spinal cord are affected by x-ray radiation. This will aid medical practitioners better understand the effects of x-ray radiation on the cells of the patients whom they diagnose and treat as well as help to know the latent period if any within which the cells regenerate after exposure to x-ray

Recommendation

Recognizing spinal cord dose limits in various fractionations is essential to ensure adequate dose for radiotherapy while minimizing the chance of radiation-induced myelopathy.

It is also recommended that the prescription rate of x-ray related procedures by doctors to patients be reduced except when it is needed in life threat

It is essential for there to be a break within periods of exposure to radiation. This would give the cells so vital time to regenerate and repair themselves.

There should be a universal x-ray bank where patients x-ray can be accessible anywhere from the hospital and this will avoid the unnecessary repeating of x-ray procedures.

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