AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 34 NUMBER 2 JUNE 2005

ISSN 1116—4077

Vol. 1 Med med Sci (2005) 34, 161-165

The effect of dexamethasone, metronidazole and ascorbic acid on the morphological changes induced by gamma rays on the spinal cord of Wistar rats

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Summary

We studied the effects of dexamethasone, ascorbic acid, and metronidazole on the irradiated spinal cord of Wistar rats. Thirty adult Wistar rats were randomly assigned into 3 groups. Five rats served as the control group. Another group of 5 rats were irradiated in the neural axis with 2.5 Gy of gamma rays. The last group of 20 rats were irradiated and then divided into four subgroups of 5 rats each: one subgroup was administered dexamethasone alone, a second subgroup had metronidazole alone, a third subgroup was treated with dexamethasone and metronidazole combined, and a fourth subgroup had ascorbic acid alone, given intraperitoneally for 7 days before exposure to radiation, and also for 5 days after-irradiation. All irradiated animals demonstrated similar vascular changes in form of splitting of the smooth muscle layers of the arterioles of the anterior spinal arteries. Similarly, all the irradiated spinal cord demonstrated shrinkages as noted in the diminution of the neuronal sizes measured by a microscope with a micrometer embedded in the eye-piece objective. The drugs did not individually protect neurons from damage at the level of our investigation. However, the combination of dexamethasone and metronidazole produced a reduction of the degenerative effect of radiation on the neurons when the post-irradiation diameters of the neurons were compared with the control and those of the other experimental groups. We conclude that gamma ray induced damage in the spinal cord may be ameliorated by combining dexamethasone with metronidazole but not by individual treatment with any of the three drugs.

Keywords: *Dexamethasone, metronidazole, ascorbic acid, radiation, gamma rays, spinal cord, Wistar rat.*

Résumé

Nous avons étudié les effects du dexaméthasone, l'acide ascorbique et matronidazole sur les irradiations de la moélle épiniére des rats. Trente rats adulte étaient groupés au hazard dans 3 groupes et une groupe de controle de 5 rats chacun. Un autre groupe de 5 rats étaient irradiés dans l'axe neurale avec 2.5Gy des rayons Gamma. Le dernier groupe de 20 rats étaient divisés en 4 groupes de 4 rats chacun, exposés aux radiations et traités au dexamethasone, metronidazole, une combination de metha et metronidazole et l' acide ascorbique donne intrapéritoneallement pendant 7 jours et 5 jours après l'irradiation. Les changements vasculaire et la dimmunition en diamétre neuronale des muscles striés et artérioles des artéres antérieures de la moélle épiniére étaient observés. Les médicaments individual ne protegaient pas la destruction des neurones dans cette étude. Cependant , la combination du dexa et du metro producait une réduction de l'effect dégenerative de radiation sur les neurones en comparant les diamétres des neurones pré et post- irradiation aux different groupes et au controle. Nous avons conclu que la destruction de la moélle épiniére induite par les rayons Gamma peut être ameliorée par la combination du métro plus dexa, et non par monotherapie.

Introduction

Although the spinal cord constitutes only 2% of the entire central nervous system [1], its functions are vital and indispensable. These include mediation of voluntary motor function, modification of muscle tone, conduction of afferent impulses from most parts of the body, mediation of segmental reflexes, and providing autonomic innervation from its thoracolumbar and sacral regions. Severe spinal cord injury below the cervical enlargement results in paraplegia, whereas injury above the cervical enlargement may produce quadriplegia, imposing a great burden on the individual afflicted as well as family, friends, and society at large as noted by Kamencic [2].

Since the important discovery by Wilhelm Conrad Roentgen of the 'X' rays in 1895 [3, 4], the field of radiation technology has had wide applications including medical diagnosis and therapeutics. However, the exposure of biological material to radiation may impair the health of the tissue or cause cell death [4]. As part of treatment modalities, radiotherapy of tumours close to or within the normal central nervous system is limited by the tolerance level of the normal tissue [5]. Despite this however, irradiation of neural tissue often accompanies radiotherapy to other tissues [6]. For example, irradiation of some neoplastic lesions of the nasopharynx, hypopharynx, and cervical lymph nodes, could have adverse effects on nearby normal tissues, one of which is the occurrence of radiation

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myelopathy [6], when the tolerance dose which is 40 Gy in man is exceeded. Similar lesions to those seen in man have been demonstrated in laboratory animals irradiated experimentally [7,8,9,10,11].

Free radicals are formed when weak chemical bonds split leaving a molecule with an odd, unpaired electron. Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. They have been implicated in the pathogenesis of radiation myelopathy according to Meschan [3], and Ilhan [12]. Lysosomes are the most sensitive organelles to radiation [13], and being stores of various lytic enzymes, damage to the single membrane of lysosomes has serious consequences on other cellular components (of the spinal cord tissues).

Dexamethasone (9α-fluoro-16α-methyl-prednisolone), is a synthetic glucocorticoid with potent anti-inflammatory activity and the ability to stabilize lysosomal membranes [14]. Ascorbic acid (2, 3-enediol-L-gulonic acid), is a reducing agent with strong redox potentials and an antioxidant with the ability to scavenge water soluble free radicals [12]. Metronidazole (1-β-hydroxyethyl-2-methyl-5-nitroimidazole), is an effective anti-parasitic and antimicrobial drug, which also has the ability to sensitize hypoxic cells to radiation [15]. Since any, or all of these three drugs, may be indicated in patients simultaneously requiring, or undergoing irradiation, the effect of the interaction between these two parameters on irradiated tissue is worth being investigated.

Acute injury to the spinal cord usually initiates a sequence of biochemical, cellular, inflammatory, and vascular changes that result in the development of secondary tissue damage as noted by Olby [16]. The objective of this study is to assess the usefulness of these drugs in protecting the cells and vasculature of the spinal cord in irradiated Wistar rats.

Materials and methods

Thirty adult Wistar rats of both sexes, weighing between 160g to 200g were obtained from the breeding colony of the animal house of the Physiology department, University of Ibadan. After acclimatization, they were assigned by simple random sampling into the experimental and control groups. Group A rats (N = 5) served as control. Group B rats (N = 5) were irradiated in the neural axis with 2.5 Gy of gamma rays only. Group C rats (N = 20) were subdivided into four subgroups and treated with irradiation, in addition to these drugs: dexamethasone at 1 mg/kg/day (N = 5), metronidazole at 50 mg/kg/day (N = 5), dexamethasone (1mg/kg/day) and metronidazole (50mg/kg/day) combined (N = 5), and ascorbic acid at 8.6 mg/kg/day (N = 5). The drugs were given intraperitoneally for seven days before exposing the animals to gamma radiation, and for five days post irradiation.

Each of the animals in both the control and experimental groups were weighed before and after irradiation, recorded as day 1, and thereafter on the 5th, 10th, and 14th day after irradiation

Each experimental rat was injected with 0.6ml / 100g of urethane, an anaesthetic agent, intraperitoneally, prior to irradiation. Each rat was then strapped in a prone position on a specially prepared plank. A dose of 2.5 Gy of gamma rays obtained from a Cobalt-60 source, was then delivered to the neural axis by an AECL 'Theratron 780-C' machine, at a depth of 5cm, and a field size of 10cm by 10 cm. Observations and weight measurements were taken until the 14th day post irradiation, when the animals were killed and the extracted spinal cord fixed in 10% formaldehyde. A Swiss Microwa balance type 7720 was used in weight measurements. The cervical segments of the spinal cord, at the area of maximum enlargement were then processed routinely for histological assessment.

We measured the widest diameter of the anterior horn neurons of the spinal cord using a microscope with a micrometer embedded in the eye piece objective at X 400 magnification. The micrometer was calibrated using a slide with a customised 2mm ruler engraved on a cover slip (Zeiss). Measurements were made on each section from all the experimental and control groups. For each section, twenty observations were made from twenty adjacent different high power fields. The mean diameter in micrometres and standard deviations were then calculated. The weight of each of the 5 animals in each of the groups were measured on the day of irradiation administration, also on 5th, and 14th day post – irradiation. The mean of the 5 determinations was then calculated, and the values for the 5th day evaluated, being the period of onset of recovery from the effect of copious diarrhoea following the exposure to the irradiation.

The data was analysed using the student's 't' test, and confidence interval was calculated at 95% level. The level of significance was fixed at less than 5% probability for the null hypothesis being true by chance.

Results

All animals that received gamma rays developed copious diarrhoea, diminished neuromuscular activity as evidenced by lethargy and inability to move away from touch or noxious stimuli, skin epilation and alopecia. One of the irradiated animals in the dexamethasone group, developed corneal opacity and thickening.

On day 5 post-irradiation, the average body weight for the control group (184.4 ± 7.27g) was greater than for the group treated with irradiation only (170.4 ± 6.45g), and this was statistically significant ($p = 0.041$). The weight also decreased in the remaining groups but was not statistically significant (please see Table 1).

Table 1: Weight of animals: controls, irradiation only, irradiation + drug treatment, on day 5, post-irradiation in grams

Group of animals	Mean	S.D	p-value
Control	184.4	7.27	0.041
Irradiation Only	170.4	6.54	
Irradiation Only	170.4	6.54	0.061
Irrad. + Dexamethasone	181.2	10.71	
Irradiation Only	170.4	6.54	0.405
Irrad. + Metronidazole	167.2	5.76	
Irradiation Only	170.4	6.54	0.823
Irrad. + Dexam + Metro	168.4	13.97	
Irradiation Only	170.4	6.54	0.048
Irrad. + Ascorbic Acid	163.60	5.18	

Irrad = Irradiation
Metro = Metronidazole

Microscopic examination showed vascular and parenchymal changes in the cervical segments of the spinal cord of all irradiated animals irrespective of drug(s) given. These changes included the splitting of the smooth muscles of the tunica media of arterioles of the anterior spinal artery (Figs. 1a and 1b), and neuronal shrinkages in the anterior grey matter of the spinal cord (Figs. 2a and 2b).

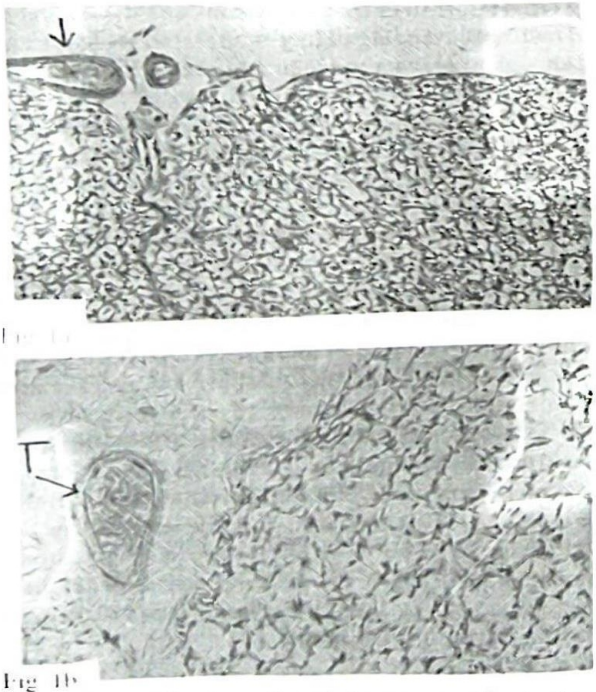

Fig. 1b:
Photomicrographs of spinal cords from a control animal (upper) showing normal arteriole (arrow) and an irradiated (lower showing splitting of the tunica media (TM) indicated by an arrow (Haematoxylin and eosin, X 250)

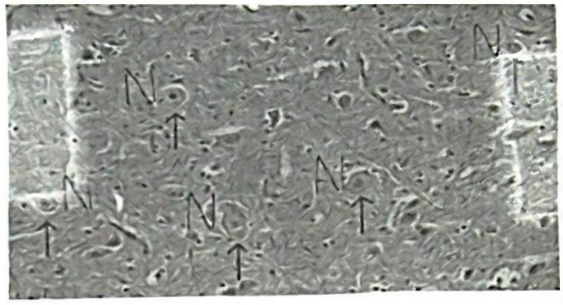

Fig. 2a:

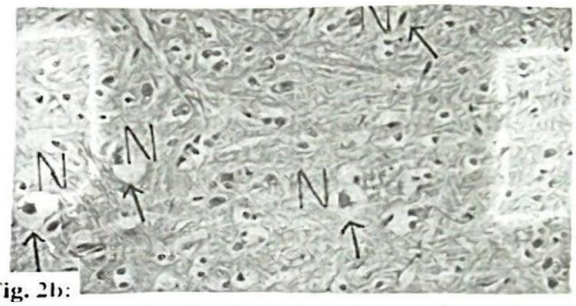

Fig. 2b:
Photomicrograph of the anterior horn of a control animal (upper) showing normal cell bodies (N) indicated by arrows. The section at the bottom shows neuronal shrinkage (N) in the anterior horn of an irradiated animal (Haematoxylin and eosin, X 160)

Histomorphometry showed that the average diameter of the neurons of the anterior horn of the spinal cord in the control group (27.2 ± 4.23μm) was significantly greater (p=0.000), than that of the neurons of the anterior horn of the spinal cord of the rats that received irradiation only (12.9 ± 2.20μm). This was also the case in each of the drug - treated groups (Table 2). The group treated with a combination of dexamethasone and metronidazole (15.2 ± 2.83μm) showed a greater mean value of the widest diameter of neurons when compared with the group that had irradiation only (12.9 ± 2.20μm), as shown in Table 2.

Table 2: Maximal diameter in micrometres of anterior horn neurons of animals on day 14 post-irradiation and in control cases.

Group of animals	Mean	S.D.	p-value
Control	27.2	4.23	0.000
Irradiation Only	12.9	2.20	
Irradiation Only	12.9	2.20	0.000
Irrad + Dexamethasone	9.5	1.76	
Irradiation Only	12.9	2.20	0.000
Irrad + Metronidazole	9.6	2.50	
Irradiation Only	12.9	2.20	0.007
Irrad + Dexameth + Metro	15.2	2.83	
Irradiation Only	12.9	2.20	0.000
Irrad + Ascorbic Acid	9.5	1.76	

Irrad = *Irradiation*
Metro = Metronidazole

Discussion

The principal injurious effect of radiation at the lowest exposure level is damage to the cell nucleus particularly the chromosomes. According to Henry [3], slight damage or breaks in the membrane might lead to disturbances in such factors as cellular water balance, permeability to various chemicals, and enzyme formation and action. At the organelle level, lysosomes are the most sensitive to radiation, hence the consequences of damage to its single membrane on other cellular components [13].

The clinical signs observed in this study were copious diarrhoea, diminished neuromuscular agility, skin epilation, and alopecia, all of which confirmed previous observations of Henry [3], and Meschan [4]. The copious diarrhoea exhibited by all the irradiated animals was due to acute radiation injury, inflammatory responses, decreased absorption of water and nutrients by the rapidly dividing cells of the intestinal epithelium. The fluid imbalance due to loss of body water must have contributed greatly to the weight loss observed in all the irradiated animals.

Earlier workers have demonstrated that skin epilation and alopecia following radiation injury occurred due to the damage done to the mitotically active cells of the basal layer of the epidermis, as well as to the hair follicles [16, 17]. Hairs, sebaceous gland, and skin appendages in mammals are radiosensitive being subject to reversible and irreversible damages due to suppression of deoxyribonucleic acid [DNA] synthesis [13]. Diminished neuromuscular activity could have been precipitated by the acute radiation injury to both the neurons, and the myelin of the neurites thus causing a reduction in the conductance of nerve impulses, inflammation in the spinal cord as well as water and electrolyte imbalance.

The splitting of the smooth muscles of the tunica media of the arteriolar branches of the anterior spinal artery confirms the previous observations of Bowen and Hopewell [6,9], who reported the loss of smooth muscle cells of arterioles as a response of brain tissue to irradiation. This we also noted in the spinal cord tissues. Also, since radiation damages the endothelial cell barrier of capillaries and arterioles [7], dysfunction of the endothelium lining the arterioles would lead to oedema of the arteriolar muscle wall causing the separation of the smooth muscles of the tunica media of the arterioles. However, oedema of the cord itself was not noticed as would have been expected if capillaries were damaged. The splitting observed here may be due to the loss of smooth muscle cells as explained earlier.

Generally, the neurons of the anterior horn of the spinal cord have large, round or ovoid nuclei within their perikarya [17,18]. Acute irradiation injury to the neuronal DNA as well as to the membranes of the lysosomes and other organelles could have caused cytosolic fluid loss hence the shrinking of the anterior horn cells observed in all the irradiated spinal cord tissue which is consistent with the findings of other workers [7], as reflected in Table 2, and Figs. 2a and 2b

Individual drug treatment, appear not to be neuroprotective for the spinal cord by the technique and at the levels of our investigations in this study. Verma [19] had noted the role of steroids in traumatic spinal cord injury, especially the ability to inhibit free radical-induced lipid peroxidation, but remarked that protective effects have not been consistently demonstrated with other corticosteroids, such as dexamethasone. Tada *et al.* [20] in their experiment concluded that dexamethasone treatment may have an anti-oedema effect and also modify subsequent development of vascular and inflammatory changes, but may have no effect on preventing radiation-induced necrosis. We had anticipated that the anti-inflammatory effect of dexamethasone, which is quantified by Ganong [21], and Haynes [22], to be 25 to 30 times more potent than with hydrocortisone may mitigate cellular damages. We however, did not observe this anticipated cellular stability with this level of irradiation-induced trauma, or dose of the drug. The group that had a combination therapy with dexamethasone and metronidazole was observed to show less neuronal degeneration, by the observation of a greater mean value of the widest diameter of the neurons of the anterior horn of the spinal cord, when compared with other experimental groups (Table 2.) The explanation for this observation is not obvious and may need further work to proffer the answer.

The effect of the anti-oxidant, and water soluble, free radical scavenging ability of ascorbic acid was also not enough to prevent vascular and neuronal damages in the setting of our experiment. Endogenous ascorbate in brain injury was reported by Tyurin *et al.* [23] to rapidly reduce nitroxide radical adducts to hydroxylamines, but in the course of the same reaction, ascorbate undergoes one-electron oxidation to form ascorbate radical. This would reduce the quantity of the antioxidant reserves in the central nervous system, thereby depleting the available physiological doses we gave. It is, however, possible that the damaging effect of direct radiation might have overwhelmed the protective potentials of these drugs which we gave at physiological doses, thereby masking any ameliorative potential at this level and setting of our investigation.

In conclusion, dexamethasone, ascorbic acid, and metronidazole, administered individually did not seem to protect the spinal cord from radiation injury at this level of our investigation. A combined use of dexamethasone and metronidazole, however, reduced the extent of neuronal damage as assessed by the widest diameter of the neurons of the anterior horn of the cord, thus indicating a need for further investigation.

Acknowledgements

We gratefully acknowledge the helpful roles of Mrs B.O. Akinlade and Mrs A.O. Ekisola of the Department of Radiotherapy, U.C.H., Ibadan, in getting our animals irradi-

ated. We thank Prof. S.O. Akpavie, formerly Head, Department of Veterinary Pathology, University of Ibadan, for his very helpful comments and assistance with photography.

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Received: 02/08/04
Accepted: 06/04/05