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Morphology of the Small Intestine of Albino Wistar Rats Following Long Term Administration of Nevirapine

Umoren EB* and Osim EE

Department of Physiology, College of Medical Sciences, University of Calabar, Calabar, Nigeria

Abstract

Background: Nevirapine (NVP) is an antiretroviral medication that prevents human immunodeficiency virus (HIV) cells from multiplying in the blood. This study was undertaken to ascertain whether NVP administration affects intestinal morphology using albino Wistar rats.

Materials and methods: Sixty adult albino Wistar rats were used for the study. Rats in the control group (n=30) were fed normal rodent chow, while the NVP group (n=30) were fed by gavage NVP (0.4 mg/kg body weight) twice daily (7:00 am and 6:00 pm) in addition to normal rodent chow for 12 weeks. All animals were allowed free access to clean drinking water. Morphological examination of tissues (duodenum, jejunum and ileum) was done.

Results: Gross morphology of the duodenum in the NVP-treated group showed hypertrophy of the Bruner's glands within the sub-mucosa as compared to control where the tissues appeared intact. Gross morphology of the jejunum in the NVP-treated group showed hyperplasia of mucosal cells and mild desquamation of epithelia, when compared to control the tissues appeared intact. Gross morphology of ileum in the NVP-treated group showed reductions in the density of Payer's patches and diffused areas of necrosis of mucosal epithelium when compared to control where tissues appeared intact.

Conclusion: From the result of the study, long term administration of NVP may cause disorganization of the morphology of small intestine in albino Wistar rats.

Keywords: Nevirapine; Duodenum, Jejunum; Ileum

Abbreviations: ARV: Antiretroviral; H&E: Haematoxylin and eosin; HAART: Highly Active Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; HIV-1: Human Immunodeficiency Virus-Type 1; NNRTI: Non-nuceosidereverse Transcriptase Inhibitor; NVP: Nevirapine

Introduction

Nevirapine (NVP) is an antiretroviral (ARV) medication that prevents human immunodeficiency virus (HIV) cells from multiplying in the blood. NVP binds directly to reverse transcriptase and blocks the RNA-dependent and DNA-dependent polymerase activities by causing a disruption of the enzyme's catalytic site (Bertram 2004). Widespread use of highly active antiretroviral therapy (HAART) has led to dramatic reductions in morbidity and mortality among individuals infected with the HIV-1 [1,2]. It is now clear that long term remission of HIV-1 disease can be achieved using various combinations of ARV agents, which suppress plasma viral loads to less than the limit of quantification of the most sensitive commercially available assays [3,4]. The clinical and immunological stabilization of HIV disease that is possible thanks to the availability of a broad spectrum of ARV compounds has its caveats in adherence, resistance and toxicity problems [5,6]. When HIV disease is associated with a viral hepatitis, other pharmacological treatments are needed concurrently and if substance abuse is still present (including intake of alcohol, heroin and methadone), the risk of increased drug-drug interaction and end-organ toxicity is increased significantly especially because of the central role of liver tissue in drug metabolism [6,7]. NVP like many other ARV agents have side effect and toxicities which affect the gastrointestinal system [8,9].

Epithelial tissue consists of a flat sheet of closely adhering cells; one or more cells thick, with the upper surface usually exposed to the environment or to an internal space in the body [10]. Epithelium covers the body surface, lines body cavities, forms the external and internal linings of many organs, and constitutes most gland tissue. Since the extracellular material is so thin, there is therefore a possibility that NVP a protease inhibitor also, an anti-inflammatory drug [11] will affect the morphology of the small intestine. Since there is paucity of information regarding the effect of NVP on intestinal tissues, this study was therefore set out to examine possible effect of NVP administration on morphology of the small intestine using albino Wistar rats as a model.

Materials and Methods

NVP was obtained from Strides Arcolab Ltd., Bangalore, India

Experimental animals

Sixty albino Wistar rats of initial body weight between 50-125 g were used for this study. They were obtained from the animal house of Physiology Department, University of Calabar, Nigeria. They were kept in improvised plastic metabolic cages with wire net covers. The ethics for the use of experimental animals were strictly adhered to. They were maintained in the animal facility of the Physiology Department University of Calabar.

***Corresponding author:** Elizabeth B Umoren, Department of Physiology College of Medical Sciences, University of Calabar, Calabar 540001, Nigeria, Tel: +2348067709327; E-mail: lizzyumoren@yahoo.com

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Experimental protocol

Thirty albino Wistar rats used for this study were randomly assigned into three groups of ten rats each; each group was further subdivided into two groups. Each group had control (n=5) and NVP-treated group (n=10). Group one was used to study the effect of NVP administration on the duodenum, group two was used to study the effect of NVP administration on the jejunum; while group three was used to study the effect of NVP administration on the ileum. Rats in all the three groups were fed ad libitum for twelve weeks and were kept free from drought at room temperature (28 ± 2oC and 12 hours light/dark cycles) throughout the feeding period, after which the samples were collected for analyses. The test group received oral administration of NVP (0.4 mg/kg body weight) once daily for 2 weeks after which the dosage was doubled by administering the drug twice daily (07:00 h and 18:00 h). The dosage of NVP administration was calculated based on the animal weight (50 g body weight) equivalence to adult human (60 kg). In this study, the dosing regimen was well tolerated.

Histopathological grading

This was done using an electron microscope. The three segments of the small intestine (duodenum, jejunum and ileum) from the three groups of NVP-treated rats were examined and the result was compared to their control respectively.

Morphological examination of tissues (duodenum, jejunum and ileum)

The preparation for microscopic examination was done according to the method of Wallington et al. [12] as used by Igiri et al. [13]. Rats were anaesthetized by inhalation of chloroform and were then decapitated. The small intestine was removed and placed in cold normal saline. The intestine was slit open and carefully rinsed in normal saline. The tissue blocks from the small intestine were fixed for 24 hours in Bouin' fluid after which they were dehydrated accordingly in ascending grades of ethanol one hour each i.e. 70%, 95% and absolute ethanol. The tissues were then cleared in two changes of xylene one hour each, thereafter, were infiltrated in molten paraffin wax at oven temperature of 58oC, and finally, embedded in pure paraffin wax and thin sections cut at 5 microns. Sections were floated on water bath and picked on albuminized slides and incubated for 6 hours at 37oC. Furthermore, sections were stained with haematoxylin and eosin (H&E) for 15 minutes. The sections were de-wax in xylene and taken through absolute ethanol, 95% and 70% rinsed in water, stained in haematoxylin for 15 minutes and rinsed in water. Sections were differentiated briefly in 1% acid alcohol, blued in running tap water for 30 minutes, counter stained in 1% aqueous eosin for 2 minutes, dehydrated in alcohol clear in xylene and mounted with DPX. The sections were then viewed under the microscope and photomicrographs taken.

Results

Effect of long term administration of NVP on the duodenumGross morphology of the duodenum among the rats in the control group showed normal intestinal mucosa, sub mucosa and muscularisexterna (Figure 1a and 1b). However, gross morphology of the duodenum among the rats in the NVP-treated group revealed hypertrophy of Brunner's glands within the sub-mucosa. The muscularisexterna appeared distorted (Figure 2a and 2b).

Effect of long term administration of NVP on the jejunum

Gross morphology of the jejunum among the rats in the control group showed normal intestinal mucosa, sub-mucosa and muscularisexterna (Figure 3a and 3b). However, gross morphology of the jejunum among the rats in the NVP-treated group revealed mucosal hyperplasia within the core of villi and mild desquamation of epithelia (Figure 4a and 4b).

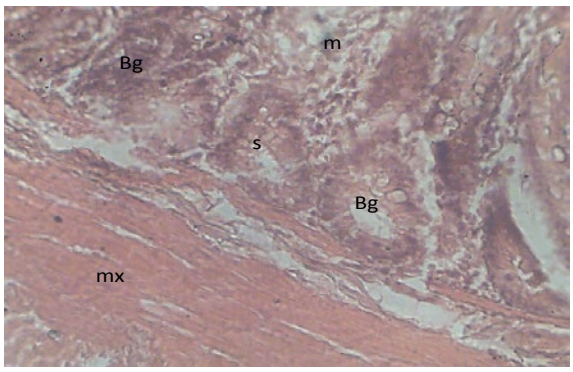

Figure 1a: Photomicrograph of duodenum of the control group. Showing intestinal mucosa (m), sub mucosa (s) and muscularis externa (mx).Within the sub mucosa are the Brunner's glands (Bg). Magnification x 25.

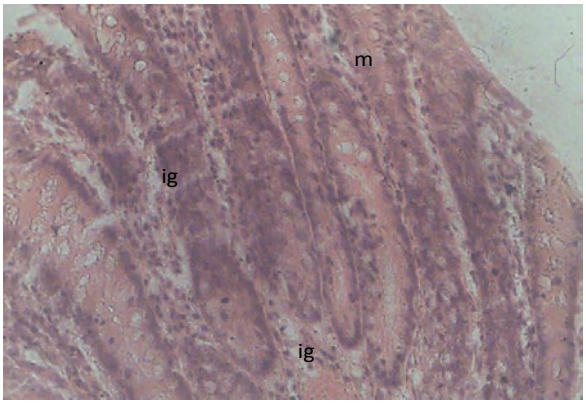

Figure 1b: Photomicrograph of the Jejunum of the control group. Showing the mucosa (m), submucosa (s) and muscularis externa (mx) appear normal. Magnification x 25.

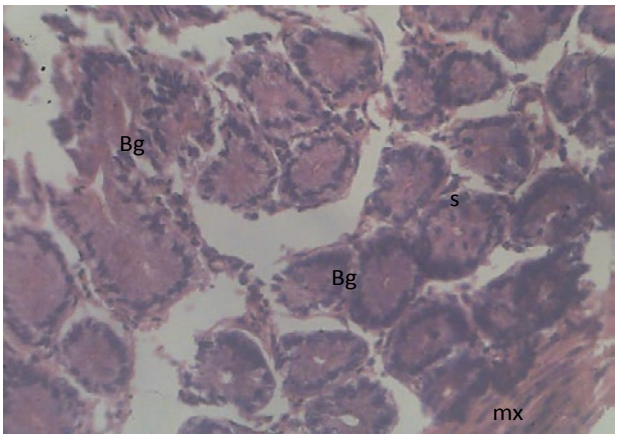

Figure 2a: Photomicrograph of duodenum of the NVP-treated group. Showing hypertrophy of the Brunner's glands (Bg) within the submucosa (S). Mx = muscularis externa. Magnification x 25.

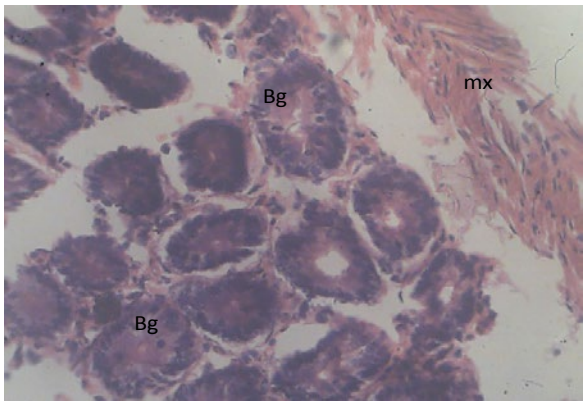

Figure 2b: Photomicrograph of duodenum of the NVP-treated group. Showing hypertrophy of Brunner's glands (Bg). Mx = muscularis externa. Magnification x 25.

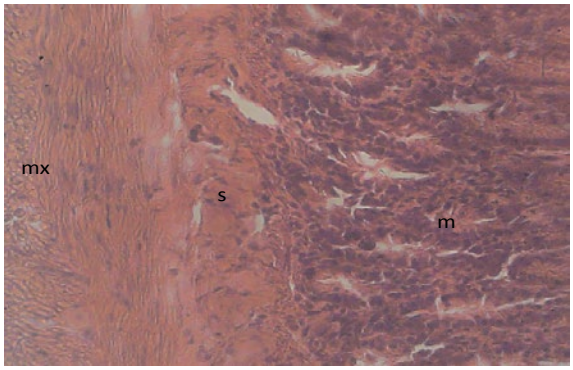

Figure 3a: Photomicrograph of the Jejunum of the control group. Showing the mucosa (m), submucosa (s) and muscularis externa (mx) appear normal. Magnification x 25.

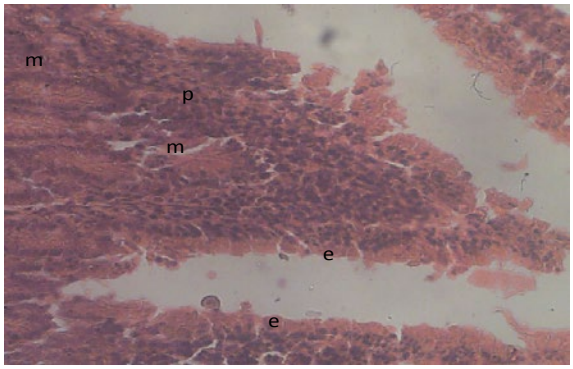

Figure 3b: Photomicrograph of jejunum of control group. Showing mucosa of the jejunum seen to be thrown into folds, with its core made of lamina propia (p) and the submucosal (s) tissue carrying blood vessels within. Magnification x 25.

Effect of long term administration of NVP on the ileum

Gross morphology of the ileum among the rats in the control group showed normal mucosa, within which was seen the Payer's patches limited externally by the sub mucosa and muscularisexterna (Figure 5). However, gross morphology of the ileum among the rats in the NVP-treated group revealed reductions in density of the Payer's patches and diffused areas of necrosis of mucosal epithelium (Figure 6).

Discussion

Effects of long term administration of NVP given through oral gavage on morphology of the small intestine of albino Wistar rats were studied. The results obtained from morphological examination revealed

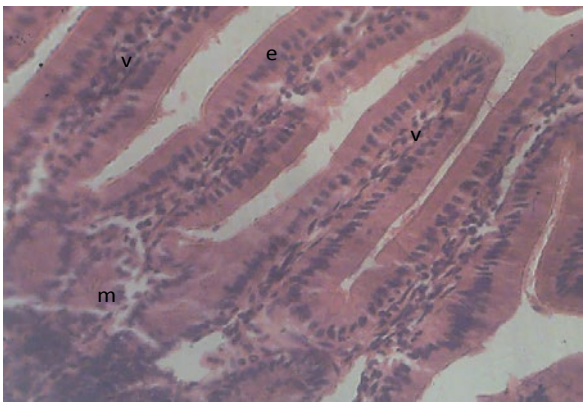

Figure 4a: Photomirogaph of jejunum of NVP-treated group. Showing mucosa (m) of jejunum with a continuum in the epithelium (e) which is composed of simple columnar cells. V= villi. Magnification x 25.

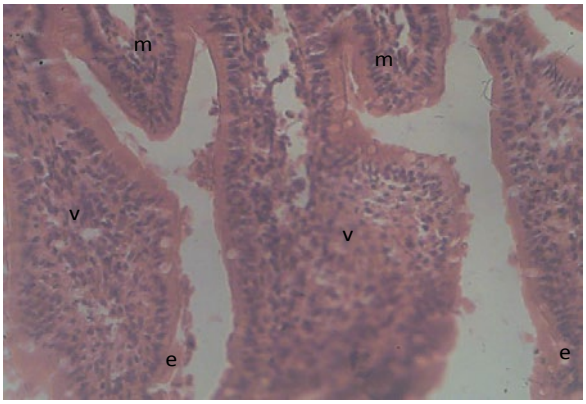

Figure 4b: Photomicrograph of jejunum of NVP-treated group. The mucosa (m) of jejunum in this treated group showed hyperplasia of cells within the core of villi and mild desquamation of epithelia (e). Magnification x 25.

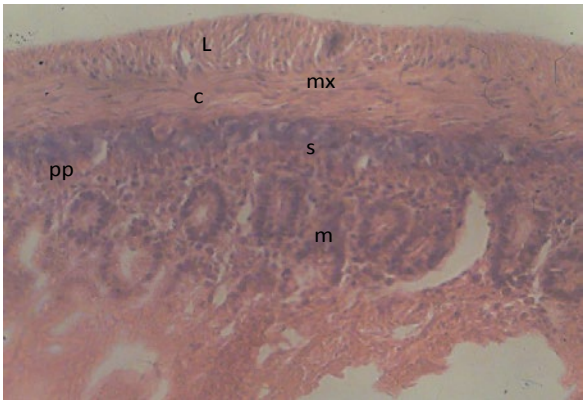

Figure 5: Photomicrograph of the ileum of control group. Showing the mucosa within which are seen the Payer's patches (PP), limited externally by the submucosa (s) and the muscularis externa (mx). L=longitudinal, C=circular layers of the muscularis externa. Magnification x 25.

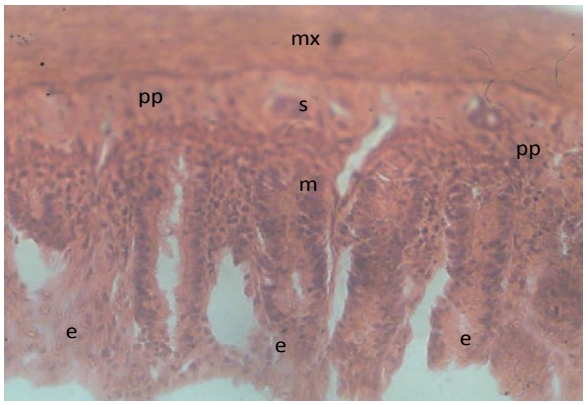

Figure 6: Photomicrograph of Ileum of NVP-treated group. Showing reduction in density of the Payer's patches (pp) and diffused areas of necrosis of mucosal epithelium (e). The submucosa (s) and muscularis externa (mx) appeared normal. Magnification x 25.

that the duodenum, jejunum and ileum of NVP-treated rats had hypertrophy of the Bruner's glands within the sub mucosa; hyperplasia of the mucosal cells and mild desquamation of the epithelia; reductions in density of the Payer's patches with diffused areas of necrosis of the mucosal epithelium, respectively, when compared to control groups of rat, the different segments of the intestinal tissues appeared normal.

Although, there is paucity of information with regards to the effect of NVP administration on the morphology of small intestine, there are series of reports as regards the toxicity of NVP a non-nucleoside reverse transcriptase inhibitor (NNRTI) on the liver, mitochondria, muscle and bone [14-17] had earlier reported that a direct or immune-mediated hepatic involvement seemed to be caused by NNRTIs, while [18-20] reported that the administration of nucleoside analogues acts via mitochondrial abnormalities prompting hepatosteatosis, lactic acidosis, and muscle and bone toxicity. Den Brinker et al. [21] reported that protease inhibitors seemed to be the main cause of glucose and lipid abnormalities.

The duodenum has prominent duodenal (Brunner) glands in the sub mucosa. They secrete an abundance of bicarbonate-rich mucus, which neutralizes stomach acid and shields the mucosa from its corrosive effects [10]. Unarguably, the mucosa can be damaged via a number of substances including prostaglandin inhibitors and other anti-inflammatory drugs [22]. It could be that NVP a protease inhibitor and also an anti-inflammatory drug, may be acting as an inhibitor of prostaglandins-which could confer protection on the intestinal mucosa. Further work on the effect of NVP on prostaglandins would substantiate this study.

Umoren et al. [8] had earlier reported intestinal motility/transit stimulating actions of NVP in albino Wistar rats. Disruption and erosion of the intestine of rats fed NVP exposed the muscle coat of intestine to various yaso-active agents causing contraction. Tissue and erosion degeneration can cause leakage of electrolytes such as sodium, potassium and hydrogen ions into the muscle coat layer of the intestine leading to amplification of the electrical activity of intestine [23]. This can result in increased intestinal motility and transit [24] had reported increased intestinal transit in NVP-treated HIV patients. Also, Deborah et al. [9] had reported gastrointestinal manifestations with protease inhibitor and NVP treatment to include diarrhea. These findings may be due to tissue erosion and degeneration caused by NVP administration, since damage to the cyto-architecture of the small intestine results in the leakage of various ions, thus, affecting certain ion channels. Opening of calcium-sodium ion channels enhances calcium ion entry which causes contraction of smooth muscle cells [25]. Therefore, long term NVP administration may cause derangement of intestinal tissue leading to increased intestinal motility, contraction and transit [8]; these mechanisms can damage the intestinal mucosa. The circular folds of the intestine promote more thorough mixing and nutrient absorption. The core of the villus has a few smooth muscle cells that contract periodically. This enhances the mixing of chime in the intestinal lumen. Each absorptive cell of a villus has a fuzzy brush border that increases the absorptive surface area of the small intestine and contains brush border enzymes [10]. From the result of our laboratory studies [26] the NVP-treated rats had decreased nutrients (glucose + protein) absorption as compared to the control group. This could be a pointer to the fact that the intestinal tissues had been compromised. On the floor of the small intestine, between the bases of the villi, there are numerous pores that open into intestinal crypts which consist of absorptive and goblet cells like those of the villi Saladin [10]. Also, from the result of our laboratory studies (Umoren et al. [12]; unpublished results), the crypt height and crypt depths in the NVP-treated rats were significantly reduced respectively, when compared to their controls with normal crypt depth and crypt height. This also points to a possible intestinal mucosal damage caused by NVP administration, which could probably result in the abnormal (decreased) nutrients absorption.

In conclusion, the above results suggest that NVP administration may cause intestinal tissue erosion and degeneration in albino Wistar rat.

Limitations of the Study

The sample size (sixty rats) used in the study was small. Also, the choice of dose for the animal treatment though small was based on the weight equivalence of rat (50 g B/W) to adult physiological man (60 kg).

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Conflict of Interest Statement

The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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