



Asian Journal of **Biochemistry**

ISSN 1815-9923



Academic
Journals Inc.

www.academicjournals.com

**Administration of Antiretroviral Drugs
(Lamivudine, Nevirapine and Stavudine) has no Untoward
Effect on Haematological Profile in Albino Rats**

R.A. Umar, M.J. Ladan, S.W. Hassan, Y. Sa'id, A.Y. Abbas and I.B. Oduolisaeme
Department of Biochemistry, Usmanu Danfodiyo University,
P.M.B. 2346, Sokoto, Nigeria

Abstract: The sub-Saharan African region is home to the greatest burden of Human Immunodeficiency Virus infection and Acquired Immunodeficiency Syndrome (HIV/AIDS). Appropriate management of infectious diseases is the most cost effective intervention to reduce the burden of disease. Highly Active Anti Retroviral Therapy (HAART) is the mainstay for inhibiting the multiplication of the causative agent and improving quality of life of the victims. There is some doubt in the region regarding the safety of the drugs in the anti AIDS pharmacy. Local studies on the safety of these agents are few. We studied the effects of acute and sub-acute oral administration of Lamivudine, Nevirapine and Stavudine on haematological profiles in albino rats. The rats were administered acute and sub-acute doses of Lamivudine, Nevirapine and Stavudine. Packed Cell Volume (PCV), total white blood cell count and differentials were determined by standard haematological methods. There were no significant differences ($p < 0.05$) in the parameters except in PCV, eosinophils, neutrophils and leucocyte count associated with nevirapine and leucocytes and eosinophils associated with lamivudine. We conclude that acute and sub-acute oral administration of these drugs is associated with few haematological abnormalities and is therefore safe.

Key words: HIV/AIDS, anti retroviral therapy, haematological profile, rats

Introduction

The first report of cases of Acquired Immune Deficiency Syndrome (AIDS) appeared in the Morbidity and Mortality Weekly Report (1981) in the USA. Twenty-five years later, AIDS had become a worldwide epidemic infecting more than 65 million people of which 25 million have died (Fauci, 2006). Of the people infected with the disease, 44.2 million (68%) are in Sub-Saharan Africa, making it the region with the highest overall AIDS prevalence rate in the general adult (15-49 years) population (World Health Organization, 2005). The prevalence of HIV has been increasing steadily in Nigeria from 18% in 1991 to 3.8% in 1993, 4.5% in 1996, 5.4% in 1999 and 5.8% in 2001 (National Prevalence Survey, 2001). Appropriate management of infectious diseases is one of the most cost effective interventions to reduce the global burden of disease.

The introduction of Highly Active Antiretroviral Therapy (HAART), a cocktail of nucleoside and non-nucleoside analogues capable of inhibiting reverse transcriptase and proteases, in industrialized countries during the mid-1990s led to well documented reductions in the risk of AIDS-defining illness and AIDS related mortality (Anonymous, 2003).

Corresponding Author: Dr. R.A. Umar, Department of Biochemistry, Usmanu Danfodiyo University,
P.M.B. 2346, Sokoto, Nigeria
Tel: +234 803615 4828 Fax: +234 602 355 519

The deployment of antiretroviral drugs within the concept of Highly Active Antiretroviral Therapy (HAART) for the treatment of HIV/AIDS revolutionized the management of the disease by suppressing viral loads to non-detectable levels, improving immune status and reducing the incidence of opportunistic infections, resulting in a dramatically improved clinical course and survival in infected patients (Anonymous, 2003). With AIDS becoming a global emergency, antiretroviral drugs became the most effective health care intervention (Anonymous, 2003). In most Sub-Saharan African countries there was access neither to prevention nor to treatment. But with the interventions like the Global Funds to Fight AIDS, Tuberculosis and Malaria (GFATM) in 2002, under the umbrella of the United Nations things have started to get better.

However, increasing reports of adverse clinical events and toxicities have diminished the enthusiasm generated by HAART. Some of the clinical events include AIDS-related insulin resistance, lipodystrophy syndrome, gastrointestinal symptoms, hyperglycaemia, which is observed in 30-80% of patients who are well controlled with HAART (Schambelan *et al.*, 2002; Anonymous, 2003). Haematologic abnormalities are among the most common manifestations of HIV infections and AIDS dominated by peripheral blood cytopenias (Lundgreen, 1994). The most common and troublesome toxicities of nucleoside transcriptase analogues are notably anaemia and neutropenia (Fische *et al.*, 1990). These reports coupled with limited knowledge of HAART have generated confusion and loss of confidence amongst the population in Africa which may militate against the acceptance and compliance to these drugs (Anderson, 2005; Gebrekristos *et al.*, 2005). Local studies on the safety of these drugs are few. This study aimed to assess the safety of the use of antiretroviral drugs (Lamivudine, Stavudine and Nevirapine) based on the effects on haematological profiles of albino rats.

Materials and Methods

Drugs and Source

The 3 antiretroviral drugs used for the study were obtained from the Pharmacy, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. The drugs were produced for Evans Medical Plc. Nigeria by CIPLA Limited, Verma Goa, India, with National Agency for Food Drug Administration and Control (NAFDAC) Reg. no. 04-6232, 04-6334 and 04-6335 for Lamivudine®, Nevirapine® and Stavudine®, respectively.

Chemicals

All chemicals used in the study were of analytical grade.

Animals and Treatment

Forty albino rats (Wistar Strains), three weeks old, were obtained from Veterinary Research Institute Vom, Jos, Plateau state. The animals were housed singly in clean cages and fed on chicken mash (Bendel Foods Ltd, Nigeria) for 3 weeks to acclimatize prior to the experiment. They were subsequently weighed and randomly assigned into two treatment groups (16 rats per treatment group). Members of the treatment group were further divided into four subgroups (4 per group). Each subgroup was labelled to represent the drugs to be administered. The drugs and dosage regimen were as follows: lamivudine® 2.0, nevirapine® 3.3 and stavudine® 0.5 mg kg⁻¹ body weights orally, twice daily for 2 and 4 weeks, respectively to represent acute and sub-acute dosing. The control group was given 0.9% normal saline as placebo. The rats were weighed daily using a weighing balance (Mettler PC, Switzerland). At the end of two and four weeks, the animals were sacrificed painlessly under chloroform anesthesia. Blood samples (1 mL) were collected in vacutainer tubes containing about

0.1 mL of Ethylene Diamine Tetraacetic Acid (EDTA). Haematological analyses ranging from Packed Cell Volume (PCV), Total White Blood Cell Count (WBC) and differentials were conducted according to standard haematological methods as reported by Dacie and Lewis (1991).

Statistical Analysis

Statistical analysis was performed using Graph pad Instat version 3.02 (Graph pad Corp., San Diego, USA). The data were first described using descriptive statistics and analysis of variance (Benferroni compare all columns) was used to test for the level of significance between means. A p value of <0.05 was taken as statistically significant.

Results

Nevirapine

At acute administration, there was significant difference ($p < 0.05$) in the leucocyte and eosinophil counts when compared with controls. All other parameters (neutrophils, monocytes and PCV) were lower compared with the controls but the differences were not statistically significant. Upon sub acute administration PCV and neutrophils were significantly lower ($p > 0.05$) compared with the controls (Table 1).

Lamivudine

Acute administration was associated with significantly ($p < 0.05$) lower eosinophils and leucocytes levels when compared with the controls. There was no significant difference in the other parameters (neutrophils, monocytes, PCV) despite being lower than the controls. Sub-acute administration was not associated with statistically significant difference in any of the parameters (Table 2).

Stavudine

Upon acute administration monocytes counts were slightly higher compared with the controls. Other parameters were lower but the difference was not significant except for leucocyte count. Sub-acute administration did not result in significant decrease in any of the parameters.

Table 1: Effect of acute administration of Lamivudine, Stavudine and Nevirapine on haematological indices in rats

Parameters	Control	Stavudine	Lamivudine	Nevirapine
PCV (%)	32.25±3.30	32.00±2.83	29.50±7.90	29.75±5.56
TLC ($10^9 L^{-1}$)	4.00±2.16	3.00±0.83	2.25±0.50	2.25±0.57
Neutrophils ($10^9 L^{-1}$)	57.76±6.20	48.75±3.80	51.75±6.24	45.00±7.70
Leucocytes ($10^9 L^{-1}$)	36.25±2.87	43.00±13.10*	35.00±5.77	48.75±8.54*
Eosinophils ($10^9 L^{-1}$)	4.75±1.50	3.50±1.70	2.25±1.26*	2.50±1.29*
Monocytes ($10^9 L^{-1}$)	4.25±2.63	4.75±1.26	3.00±1.83	3.75±0.50

Values are Means±SD, * Indicates significant difference ($p < 0.05$), PCV = Packed Cell Volume, TLC = Total Leucocyte Count

Table 2: Effect of sub-acute administration of Lamivudine, Stavudine and Nevirapine on haematological indices in rats

Parameters	Control	Stavudine	Lamivudine	Nevirapine
PCV (%)	35.75±3.77	39.75±0.35	28.75±5.06	24.75±4.03*
TLC ($10^9 L^{-1}$)	3.00±2.15	2.75±0.96	2.20±0.58	2.50±0.56
Neutrophils ($10^9 L^{-1}$)	54.00±0.06	52.75±7.37	46.00±7.62*	51.75±1.71
Leucocytes ($10^9 L^{-1}$)	37.25±0.86	41.50±5.70	45.50±6.14	41.25±0.96
Eosinophils ($10^9 L^{-1}$)	3.25±1.70	2.50±1.00	3.75±0.95	3.50±1.30*
Monocytes ($10^9 L^{-1}$)	5.50±2.60	3.25±0.96*	3.51±1.29*	3.50±1.30*

Values are mean±SD, * Indicates significant difference ($p < 0.05$), PCV = Packed Cell Volume, TLC = Total Leucocyte Count

Discussion

In this study the effect of some antiretroviral drugs on the haematological parameters of experimental rats were examined. Despite statistically significant reduction in PCV, eosinophils and leucocyte levels induced by acute and or sub-acute administration of nevirapine and lamivudine no serious effects on haematological parameters were encountered. Previous study by Janet *et al.* (1995) showed that toxic effect of antiretroviral drugs leads to anaemia and neutropenia. These could be as a result of these drugs interfering with the progenitor cells of the bone marrow leading to suppression of their activity. Neutropenia has been described in 16% of lamivudine and nevirapine recipients compared with 2% of those on placebo (Van Leeuwen, 1995). Nevirapine, a non-nucleoside reverse transcriptase inhibitor was more toxic than nucleoside reverse transcriptase inhibitors and was associated with several complications including anaemia in a dose dependent manner (Cinque *et al.*, 1993). Neutropenia is also common in the advanced stages of HIV and is often caused by concomitant myelosuppression from drug therapy (Van Leeuwen, 1995).

A previous study by Ejele *et al.* (2004) showed that incidences of relative prevalence of anaemia, leucopenia and neutropenia declined respectively from baseline values of 84.3, 11.4 and 24.3% to post treatment values of 75.7, 11.4 and 7.1%. Consequently the prevalence of anaemia, leucopenia and neutropenia increased from baseline values of 96.0 and 6.7% after 12 weeks observational period in untreated controls. Our findings of low levels for Packed Cell Volume (PCV) and leucocytes (WBC) is at variance with high PCV and leucocytes reported by Odunukwe *et al.* (2001), although the drugs were administered together. The reported increase could be as a result of different mechanism of actions of these drugs acting together to improve erythropoiesis. This would be a boost to AIDS treatment as usually the drugs are administered in combination to achieve the goal of inhibiting viral replication, fusion and integration. Stavudine, Nevirapine and Lamivudine administration are not associated with so serious adverse effects as to militate against their deployment in treatment of AIDS patients. More so as the effects on haematology could be mitigated by inclusion in the treatment protocol of agents that could improve the erythropoietic process. There is therefore no scientific basis for confusion or rejection of the drugs currently in the AIDS armamentarium.

References

- Anderson, N., 2005. Western and traditional African medicine: Working together on AIDS. *Br. Med. J.*, 331: 785-786.
- Anonymous, 2003. Study confirms effectiveness of antiretroviral drugs for HIV patients. *Lancet*, 362: 1267-1274.
- Cinque, P., G. Lando and A. Lazzam, 1993. Long term treatment with zidovudine in patients with immune deficiency virus (HIV) associated with thrombocytopenia mode of response and correlation with markers of HIV replication. *Eur. J. Haematol.*, 50: 17-26.
- Dacie, J.V. and S.M. Lewis, 1991. *Practical Haematology*. 7th Edn., ELBS, Churchill Livingstone, England, pp: 37-85.
- Ejele, O.A., C.A. Erhabor and A. Nwauche, 2004. Haematologic effects of some antiretroviral drugs in patients with AIDS. *Sahel Med. J.*, 8: 25-29.
- Fauci, A.S., 2006. Editorial: Twenty five years of HIV/AIDS. *Science*, pp: 313-409.
- Fische, M.A, C.B. Parker and C. Pettineli, 1990. A randomized controlled trial of reduced daily dose of zidovudine in patients with AIDS. *N. Eng. J. Med.*, 323: 200-202.
- Gebrekrstos, H.T., K.P. Mlisane and Q. AbdelKarim, 2005. Assessing patients' readiness to start antiretroviral therapy. *Br. Med. J.*, 331: 772-773.

- Janet, E., S.C. Benoit and J. Jemsek, 1995. Treatment with Lamivudine, Zidovudine or both in HIV Positive patients. *N Eng. J. Med.*, 323: 181-183.
- Lundgreen, J.D., 1994. Comparison of long term prognosis of patients with AIDS treated with Zidovudine, *JAMA*, pp: 271.
- Morbidity and Mortality Weekly Report, 1981. Centre for Disease Control and Prevention, Atlanta, Georgia.
- National HIV/AIDS Prevalence Survey, 2001. HIV/Syphilis sentinel sero prevalence survey in Nigeria. Information for Policy Makers, pp: 30-50.
- Odunukwe, N., N.E.O. Idigbe, T.A. Adewole, D.I. Onwuyekwe and A. Audu, 2001. Abstract of Clinical Research. Federal Ministry of Health, Lagos, Nigeria.
- Schambelan, M., C. Benson and A. Carr, 2002. International AIDS Society. USA Panel. Acquired Immune Deficiency Syndrome, 9: 30-32.
- Van Leeuwen, R., 1995. Evaluation of safety and efficacy of Lamivudine in patients with asymptomatic or mildly symptomatic human immunodeficiency virus infection *J. Infect. Dis.*, 26: 1166-1171.
- World Health Organization, 2005. World's AIDS and Tuberculosis Situation. Tuberculosis Department, WHO, Geneva.