Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 5 (3): 181-187, 2010
ISSN 1815-9923 / DOI: 10.3923/ajb.2008.19.25

Adverse Hepatic Effects Associated with Administration of Antiretroviral Drugs (Nevirapine, Lamivudine and Stavudine) to Albino Rats: Implication for Management of Patients with HIV/AIDS*

[1]R.A. Umar, [1]S.W. Hassan, [1]M.J. Ladan, [1]I.K. Matazu, [2]B. Shehu,
[1]R.A. Shehu, [3]L.G. Muhammed and [1]F.I. Molabo
[1]Department of Biochemistry, Usmanu Danfodiyo University,
P.M.B. 2346, Sokoto, Nigeria
[2]Laboratory Services Unit, Health Services Management Board, Sokoto, Nigeria
[3]Veterinary Public Health Laboratory,
Department of Veterinary Microbiology and Public Health,
Faculty of Veterinary Medicine, Usmanu Danfodiyo University,
P.M.B. 2346, Sokoto, Nigeria

Abstract: We studied the effects of acute and sub-chronic oral administration of nevirapine, lamivudine and stavudine on liver function in albino rats. Acute administration of nevirapine resulted in significant ($p<0.05$) increases in activities of Aspartate amino transferase (AST) and Alanine amino transferase (ALT). Total proteins, albumin and globulin were significantly lowered. Upon sub-chronic administration of nevirapine, only AST and ALT activities were significantly raised. Acute administration of lamivudine was associated with significantly ($p<0.05$) lower albumin and globulin and higher total bilirubin and conjugated bilirubin levels. There were no significant differences ($p>0.05$) in liver function profiles associated with sub-chronic administration of the drug. However, acute and sub-chronic administrations of stavudine were not associated with significant ($p>0.05$) changes in liver function profiles. We conclude that while the use of stavudine is safe, acute and sub-chronic oral administration of nevirapine and lamivudine are associated with hepatotoxicity and hepatoprotective agents should be incorporated in the treatment regimens employing these drugs to avert life-threatening complications.

Key words: Adverse hepatic effects, administration, antiretroviral drugs, hepatotoxicity, HIV/AIDS management, albino rats

INTRODUCTION

Since the first report of cases of Acquired Immune Deficiency Syndrome (AIDS) by Gottlieb *et al*. (1981) in the USA and with 800,000 new infections occurring daily (Goulder and Watkins, 2004) the disease has become a global pandemic. By the year 2006, AIDS had infected more than 65 million people out 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 (WHO, 2005). In Nigeria, the acclaimed heart beat of Africa, the prevalence of HIV infection has been increasing steadily; from 1.8% in 1991 to 3.8% in 1993, 4.5% in 1996, 5.4% in 1999 and 5.8% in 2001 (Anonymous, 2001). Until recently (with the advent of antiretroviral therapy) HIV infection was a death sentence, in most parts of the world it still is (Goulder and Watkins, 2004) due to non-availability of the anti AIDS drugs,

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

*Originally Published in Asian Journal of Biochemistry, 2008

ignorance, financial incapacity or other factors. 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).

The deployment of antiretroviral drugs within the concept of 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 line with its mandate, the WHO has committed itself to the 3-by-5 target for anti-retroviral therapy which aims to ensure that 3 million HIV-infected people in developing countries have access to anti-retroviral therapy by the end of 2005 (Haines, 2003). This measure, by making anti-retroviral drugs available to people living with HIV/AIDS, will go along way in preventing transmission of the disease and improving the quality of life of the victims.

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, lipodystropy syndrome, gastrointestinal symptoms, hyperglycaemia (Schambelan *et al*., 2002; Anonymous, 2003). The most common and troublesome toxicities of nucleoside transcriptase analogues are notably hepatotoxicity (Schambelan *et al*., 2002), anaemia and neutropenia (Fistche *et al*., 1990), hepatic steatosis and osteonecrosis (Schambelan *et al*., 2002). These reports coupled with limited knowledge of HAART have generated confusion and loss of confidence amongst the population in Africa which militate against the acceptance and compliance to these drugs (Anderson, 2005; Gebrekristos *et al*., 2005). For example, in Durban, South Africa, of the women attending an AIDS clinic about 50% are HIV positive but none receives antiretroviral therapy (Goulder, 2006).

African studies on the safety of these drugs are few. In an earlier communication (Umar *et al*., 2007) we have reported the effect of administration of some antiretroviral drugs on haematological profiles. In the present preliminary report, we assessed the safety of the use of antiretroviral drugs (nevirapine, lamivudine and stavudine) based on the effects on liver function profiles in albino rats.

MATERIALS AND METHODS

Drugs and Source

The three 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. 04-6335, 04-6232 and 04-6334 for Stavudine®, Lamivudine® and Nevirapine®, respectively.

Reagents

All reagent kits used in the study were obtained from Randox Laboratories, U.K.

Animals and Treatment

Thirty male albino rats (Wistar Strains) weighing 200-269 g were obtained from Veterinary Research Institute Vom, Jos, Plateau state, Nigeria. The animals were housed singly in clean cages and fed on chicken mash (Bendel Foods Ltd., Nigeria) for 2 weeks to acclimatize prior to the experiment. They were subsequently weighed and randomly assigned into four treatment and control groups (5 groups of 6 rats each). For treatment groups, each subgroup was labelled to represent the drugs to

be administered. In humans (adults) the therapeutic doses of the drugs were 200, 600 and 350-400 mg daily for stavudine, lamivudine nevirapine, respectively. To create a rat model for the study we assumed an average human being weighs 70 kg and calculated the corresponding therapeutic doses for the rat models. The drugs and dosages were as follows: stavudine® 0.57, lamivudine® 2.06 and nevirapine® 1.54 mg kg^{-1} body weights orally, daily for 1 and 4 weeks, respectively to represent acute and sub-chronic dosing. This enabled us to study the effects of the drugs in short (1 week) and medium (4 weeks) terms. The control group was given 0.9% normal saline as placebo. At the end of one and 4 weeks, the animals were sacrificed painlessly under chloroform anesthesia. Blood samples (1 mL) were collected in vacutainer tubes and allowed to clot for 30 min before it was centrifuged for 10 min at 3000 rpm to obtain sera for the analyses.

Liver Function Tests

Aspartate and alanine amino transferase activities were assayed according to the method of Reitman and Frankel (1957), total proteins were determined by Biuret method of Whicher *et al*. (1994) using Biuret reagent of King (1951), albumin was determined using bromocresol green binding method, globulin was obtained by subtracting the albumin value from the total protein, total and direct bilirubin were determined as described by Perry *et al*. (1983).

Statistical Analysis

Statistical analysis was performed using Graph pad Instat version 3.02 (San Diego, USA).The data were 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 considered statistically significant.

RESULTS

The effect of acute and sub-chronic administrations of stavudine®, lamivudine and nevirapine® on liver functions in rats are presented in Table 1 and 2.

Stavudine

Acute and sub-chronic administrations of stavudine did not produce significant ($p>0.05$) changes in liver function profiles.

Lamivudine

Acute administration was associated with significantly ($p<0.05$) lower globulin and albumin levels and raised total bilirubin and conjugated bilirubin levels. Total proteins were non significantly raised. There were no significant differences ($p>0.05$) in liver functions parameters with sub-chronic administration of the drug.

Table 1: Effect of acute administration of stavudine, lamivudine and nevirapine on liver functions in rats

Parameters	Control	Stavudine	Lamivudine	Nevirapine
ASAT (UL^{-1})	30.70±0.50	32.75±4.03	31.50±3.42	36.50±4.04*
ALAT (UL^{-1})	17.25±0.50	19.75±2.36	18.50±1.29	21.25±2.50*
T. proteins (g dL^{-1})	4.43±0.62	3.33±0.80	3.25±0.90	3.03±0.35*
Albumin (g dL^{-1})	3.65±0.70	2.48±0.70	2.68±0.94	2.45±0.31*
Globulins (g dL^{-1})	0.78±0.10	0.85±0.47	0.58±0.05*	0.58±0.10*
T. bilirubin (mg dL^{-1})	0.28±0.06	0.32±0.09	0.62±0.18*	0.44±0.10*
D. bilirubin (mg dL^{-1})	0.16±0.02	0.15±0.02	0.37±0.07*	0.17±0.08

Values are Means±SD, *: Indicates significant difference ($p<0.05$), ASAT = Aspartate amino transferase, ALAT = Alanine amino transferase, T = Total, D = Direct, UL^{-1} = Units per liter

Table 2: Effect of sub-chronic administration of stavudine, lamivudine and nevirapine on liver functions in rats

Parameters	Control	Stavudine	Lamivudine	Nevirapine
ASAT (UL^{-1})	84.50±7.94	85.75±8.02	95.75±10.24*	118.30±15.90*
ALAT (UL^{-1})	33.00±5.35	35.75±9.22	31.00±1.41	42.75±3.86*
T. Proteins (g dL^{-1})	4.15±0.64	4.22±0.15	3.68±0.35	3.83±0.28
Albumin (g dL^{-1})	3.48±0.49	3.60±0.12	3.10±0.29	3.25±0.24
Globulins (g dL^{-1})	0.68±0.15	0.63±0.05	0.50±0.14	0.58±0.05
T. bil. (mg dL^{-1})	0.75±0.02	0.78±0.10	0.79±0.05	0.74±0.01
D. bil. (mg dL^{-1})	0.13±0.01	0.16±0.10	0.14±0.01	0.15±0.02

Values are mean±SD, *: Indicates significant difference ($p<0.05$), ASAT = Aspartate amino transferase, ALAT = Alanine amino transferase, T = Total, T. bil. = Total bilirubin, D. bil. = Direct bilirubin, UL^{-1} = Units per liter

Nevirapine

At acute administration Nevirapine resulted in significant ($p<0.05$) increases in activities of aspartate amino transferase (AST) and alanine amino transferase (ALT). Total proteins, albumin and globulin were significantly lowered. Upon sub-chronic administration of Nevirapine, only AST and ALT activities were still significantly raised. Total proteins, albumin and globulin levels improved, while levels of total and direct bilirubin were almost similar to the controls.

DISCUSSION

In the face of the global AIDS pandemic advancement in the treatment of the disease has been strikingly impressive, for example patients on antiretroviral therapy now live at least 13-14 years longer than those without the therapy (Walensky, 2006). But problems still remain. Hepatotoxicity has arisen as a major side effect of antiretroviral drugs limiting their use in treatment regimens. Severe hepatic reactions attributed to nevirapine as part of HAART or in post-exposure prophylaxis regimens have been reported (Anonymous, 2000; Johnson and Baraboutis, 2000; Martinez *et al*., 2001). Hepatotoxicity has been reported for all antiretroviral classes and nevirapine was attributed the highest risk (Sulkwoski *et al*., 2000; Reisler *et al*., 2001). But our study has not encountered adverse hepatic effect of stavudine and it may be one of the safest drugs in the anti AIDS armamentarium.

Most reports on the hepatic toxicity of nevirapine show that the abnormality of liver function tests are reversible after discontinuation of the drug (Johnson and Baraboutis, 2000; Martinez *et al*., 2001; Piliero and Purdy, 2001). Present study is the first to show that even on continuation of treatment, by four weeks, hepatic function remarkably improves. Lamivudine normalizes alanine amino transferase levels and reduces hepatic inflammation (Hache and Villeneure, 2006). A potential criticism of this work may be our failure to assay for alkaline phosphatase (ALP) activity in our model. ALP is a marker enzyme for plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974), but ALAT and ASAT are more specific markers of necrotic injury and cholestasis (Speech and Liehr, 1983; Lott and Wolf, 1986). Hence, ALP being ubiquitous, failure to assay it may not adversely affect or invalidate the conclusions that can be drawn from the study.

The liver being the organ chiefly involved in drug metabolism is endowed with drug metabolizing enzymes for the purpose. Drugs are metabolized through two phases of reactions. In some instances such reactions convert drugs into reactive forms and hence toxicity results.

Drug-induced toxicity is the most frequent reason for the withdrawal of a drug from the market and accounts for more than 50% of cases of acute liver failure in the United States (Reed, 2001). The exact mechanisms by which nevirapine and lamivudine cause adverse hepatic events have not been elucidated, but Lee (2003) reports that drug-induced liver injury occurs via at least 6 mechanisms involving various intracellular organelles, with consequent disruption of intracellular calcium homeostasis, decline in ATP levels and finally hepatocyte swelling and rupture (Beaunc, 1987; Yun *et al*., 1993). Other cells within the liver may be the target of drug-induced liver damage or modulate incipient reaction. For example, kupffer cells may activate cytokines which may amplify

liver injury or macrophages may augment injury, produce fibrosis or granulomas (Lee, 2003). Decreases in serum total proteins are an indication of hepatotoxicity (Abatan *et al*., 1996). Increases in activities of ALAT and ASAT directly reflect a major permeability problem or cell rupture (Benjamin, 1978). ASAT exhibits high activity in cytoplasm, mitochondrion and microsomes of liver, heart, kidney and brain (Benjamin, 1978; Ringer *et al*., 1979). ALAT is hepato-specific principally found in the cytosol of hepatocytes. Elevated ALAT levels are associated with acute liver and cholestatic disease (Wolf, 2003). Elevated levels of conjugated bilirubin occur in intrahepatic cholestasis (Wolf, 2003). Defect in metabolism of bilirubin generally reflects defective metabolic capacity of the liver (Schreiber, 2004). As indicated by the results out of the three antiretroviral drugs only nevirapine is associated with significant activities of ALAT and ASAT upon both acute and sub-chronic administration. Elevated activities of these enzymes indicate cell damage which might have resulted from several mechanisms; generation of toxic species, peroxidation of membranes etc. Nevirapine and lamivudine perhaps depress protein synthesis as indicated by the decreased levels of total proteins, albumin and globulins.

Nevirapine also imperils the excretory capacity of the liver as shown by the significant increases in levels of total bilirubin. Present results are in agreement with report by Bartlett and Gallant (2003) on the toxicity of nevirapine.

Administration of drugs that are hepatotoxic to HIV/AIDS patients may have untoward consequences. The lives of patients are put at risk. One study reports fatal case resulting from liver failure consequent to nevirapine toxicity (Anonymous, 2000). A damaged liver may fail to metabolize drugs and thus prolong their stay in circulation and cause further toxicity or drugs that need to be metabolized to active forms may not be so converted. There is high chance that a drug may be withdrawn due to its toxicity or the patient may fail to comply with the treatment regimen both of which may adversely affect the outcome of treatment.

We conclude that nevirapine and lamivudine are associated with hepatic adverse events and must be used with caution and close monitoring of liver enzymes may be critical in preventing life threatening events. Inclusion in the treatment regimen of agents which are hepatoprotective should be seriously considered. Further work is on going in our laboratory to assess the specific nature of the hepatic toxicity through histopathological investigations.

REFERENCES

Abatan, M.O., R.O.A. Arowolo and O. Olorunsogo, 1996. Pathological effects of *Lantana camara* and *Dichapetalum madagascasiense* in goats. Trop. Vet. Med., 14: 127-132.

Anderson, N., 2005. Western and traditional African medicine: Working together on AIDS. Br. Med. J., 331: 785-786.

Anonymous, 2000. Serious adverse events attributed to nevirapine regimens for post-exposure prophylaxis after HIV exposures world wide. Morbidity and Mortality Weekly Report, Center for Disease Control, 49: 1997-2001.

Anonymous, 2001. HIV/Syphilis Sentinal Sero Prevalence Survey in Nigeria. Information for Policy Makers, pp: 30-50.

Anonymous, 2003. Study confirms effectiveness of antiretroviral drugs for HIV patients. Lancet, 362: 1267-1274.

Bartlett, J.G. and J.E. Gallant, 2003. Natural history and classification in management of HIV infection. John Hopkins University, Baltimore.

Beaunc, P., P.M. Dansette and D. Mansuy, 1987. Human anti-endoplasmic reticulum auto antibodies appearing in a drug-induced hepatitis are directed against human liver cytochrome P-450 that hydroxylates the drug. Proc. Nat. Acad. Sci. USA., 84: 551-555.

Benjamin, M.N., 1978. Outline of Veterinary Clinical Pathology. University Press, Iowa, USA., pp: 229-232.

Fauci, A.S., 2006. Editorial: Twenty five years of HIV/AIDS. Science, 313: 409-409.

Fistche, 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.

Gebrekristos, H.T., K.P. Mlisane and Q. AbdelKarim, 2005. Assessing patients'readiness to start antiretroviral therapy. Br. Med. J., 331: 772-773.

Gottlieb, M.S., H.M. Schanker and A. Fan, 1981. Pneumocystis pneumonia in Los Angeles. Morbidity and Mortality Weekly Report. Center for Disease Control and Prevention, Atlanta, Georgia, 30: 250-252.

Goulder, P.J. and D.I. Watkins, 2004. HIV and SIV CTL escape: Implication for vaccine design. Nat. Rev. Immunol., 4: 630-640.

Goulder, P.J., 2006. A deadly struggle: HIV versus the human immune system. Wellcome Science, pp: 21-23.

Hache, C. and J. Villeneure, 2006. Lamivudine treatment in patients with chronic hepatitis B and cirrhosis. Exp. Opin. Pharmacother., 17: 835-843.

Haines, A., 2003. Editorial. Shaping the future of global health. Bull. World Health Organ., 81: 855-855.

Johnson, S. and J.G. Baraboutis, 2000. Adverse effects associated with the use of nevirapine in HIV post exposure prophylaxis for 2 health care workers. JAMA., 284: 2722-2723.

King, E.J., 1951. Determination of Proteins. In: Recent Advances in Clinical Pathology. Dyke, S.C. (Ed.), 2nd Edn., J and A Churchill, London.

Lee, W.M., 2003. Drug-induced hepatotoxicity. New Eng. J. Med., 349: 474-485.

Lott, J.A. and P.L. Wolf, 1986. Alanine and aspartate aminotransferase. Clinical Enzymology. Field Rich and Associates, New York, pp: 111-138.

Martinez, E., J.L. Blanco, A. Amaiz, J.B. Peres-Cuevas, A. Mocroft, A. Cruceta, M.A. Marcos, A. Milinkovic and M.A. Garcia-Viejo, 2001. Hepatotoxicity in HIV-1 infected patients receiving nevirapine-containing antiretroviral therapy. AIDS, 15: 1261-1268.

Perry, B.W., B.T. Doumas and D.D. Bayse, 1983. A candidate reference method for determination of bilirubin in serum: Test for transferability. Clin. Chem., 29: 297-301.

Piliero, P.J. and B. Purdy, 2001. Nevirapine-induced hepatitis: A case series and review of the literature. AIDS Read, 11: 379-382.

Reed, J.L., 2001. Apoptosis regulating proteins as targets for drug discovery. Trends Mol. Med., 7: 314-319.

Reisler, R., S. Liou, J. Servoss, G. Robbins, D. Theodore, R. Murphy and R. Chung, 2001. Incidence of hepatotoxicity and mortality in 21 adults antiretroviral treatment trials. Program and Abstracts of the 1st International AIDS Conference on HIV Pathogenesis and Treatment; Buenos Aires, Argentina. Abstract, 43: 8-11.

Reitman, D. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56-59.

Ringer, D.H. and L. Dabich, 1979. Haematological and Biochemical Parameters. In: The Laboratory Rats. Baker, H.J., J.R. Lindsay and S.H. Weisbroth (Eds.), Vol. 1 Acad. Press, London, pp: 105-116.

Schambelan, M., C. Benson and A. Carr, 2002. Acquired Immune Deficiency Syndrome. International AIDS Society. USA Panel, 9: 30-32.

Schreiber, M., 2004. Laboratory assessment of liver functions. www.pathology ubc.ca/path 425/systemic pathology/liver and biliary tract. Accessed 05/09/2005.

Speech, H.J. and H. Liehr, 1983. Of what values are SGOT/SGPT, GGT/AP and IgG/Ig A ratios in the differential diagnosis of advanced liver diseases? Z. Astroenterol., 21: 89-96.

Sulkwoski, M.S., D.L. Thomas, R.E. Chaisson and R.D. Moore, 2000. Hepatotoxicity associated with antiretroviral therapy in adults infected with HIV and the role of hepatitis C or B virus infection. JAMA., 283: 74-80.

Umar, R.A., M.J. Ladan, S.W. Hassan, Y. Sa'idu, A.Y. Abbas and I.B. Oduolisaeme, 2007. Administration of antiretroviral drugs (Lamivudine, Nevirapine and Stavudine) has no untoward effect on haematological profile in albino rats. Asian J. Biochem., 2: 147-151.

Walensky, R.P., A.D. Paltiei and E. Casina, 2006. The survival benefits of AIDS treatment in the United States. J. Infect. Dis., 194: 1-9.

Whicher, J., R.F. Ritchie and A.M. Johnson, 1994. New International Reference Preparation for Proteins in Human Serum (RPPHS). Clin. Chem., 40: 934-938.

Wolf, L.D., 2003. Update on the Clinical Liver Function Testing. San Diego Health System, University of California, USA.

WHO, 2005. World's AIDS and Tuberculosis Situation. Tuberculosis Department. WHO, Geneva.

Wright, P.J. and D.T. Plummer, 1974. The use of urinary enzyme measurements to detect renal changes caused by nephrotoxic compounds. Biochem. Pharmacol., 12: 65-68.

Yun, O.H., R.A. Okerholm and F.P. Guengerich, 1993. Oxidation of the antihistamine drug terfenadrine in human liver microsomes: Role of cyt. P450 3A4 in N-dealkylation and C-hydroxylation of drug. Metab. Dispos., 21: 403-409.