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Effect of co-administration of artemether and nevirapine on haematological parameters in normal and immunosuppressed Wistar rats

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Artemether and nevirapine are effective drugs used in the treatment of malaria and as part of antiretroviral therapy, respectively in many parts of the world. However, concomitant use of these drugs may pose an adverse drug interaction that may alter their pharmacological effects. In this study, possible effect(s) of artemether-nevirapine co-administration on haematological indices in both normal and immune-compromised rats were investigated. Animals were divided into 6 groups of 6 rats per group. Groups 4, 5 and 6 received 30 mg/kg of nevirapine daily for 21 days. In addition, groups 5 and 6 received 5 and 10 mg/kg artemether, respectively while rats in groups 2 and 3 received artemether 5 and 10 mg/kg, respectively from day 15, for 7 days. This was repeated in immunosuppressed rats with an additional group receiving only dexamethasone and 3% v/v Tween 80. Rats in all groups received dexamethasone (20 mg/kg) and booster doses of 10 and 5 mg/kg on days 8 and 15, respectively, except the control group. All drugs administration were through intraperitoneal route. Data were analysed using analysis of variance (ANOVA), followed by Dunnett's post hoc test. P-values less than 0.05 were considered statistically significant. Artemether-nevirapine co-administration caused a significant decrease ($P < 0.05$) in packed cell volume (PCV), red blood cell (RBC), haemoglobin, lymphocyte as well as an increase in neutrophils in both normal and immunosuppressed rats. Findings from this study showed that concomitant administration of artemether and nevirapine altered PCV, RBC, haemoglobin, WBC, neutrophil and lymphocyte of both normal and immunosuppressed rats and this may induce some adverse effects on blood parameters.

Key words: Artemether, nevirapine, haematology, immunosuppression, rats.

INTRODUCTION

Concurrent use of two or more drugs is sometimes essential in order to achieve therapeutic goal(s) (Nwonu et al., 2008). However, when two or more drugs are administered together, there may be a possibility that one drug will alter the pharmacological effects of the other(s) (Perucca, 2006). With the increasing availability of new drugs, for example, artemether and their concomitant use with other drugs e.g.nevirapine), there has been a rise in

the potential for adverse drug interactions (Kuhlman and Muck, 2001). Artemether is a derivative of artemisinin, used as an effective drug in the treatment of malaria (Akomolafe et al., 2011). Nevirapine is one of the drugs routinely given as part of antiretroviral therapy in Nigeria (Idigbe et al., 2005) and other parts of the world (Boulle et al., 2008). However, concomitant use of these drugs may pose an adverse drug interaction that may alter their pharmacological effects. There exist pharmacokinetic interaction between artemether and nevirapine (Byakika-Kibwika *et al*., 2011) but there are no published studies on pharmacodynamic interaction involving these drugs, although the potential exists since nevirapine is a known inducer of human CYP3A4 and CYP2B6 isoenzymes, which metabolise a range of drugs and other zenobiotics. In addition, it was reported that the sub-class of antiretroviral drugs known as non-nucleotide reverse transcriptase inhibitors to which nevirapine belongs are metabolized to some extent by P_{450} enzymes, therefore exposing them to clinically significant drug interactions (Soyinka *et al*., 2009). Moreover, nevirapine has been reported to affect the pharmacokinetic profiles of artemether (Kredo *et al*., 2011), establishing the need for more clinical investigation. On the other hand, artemether is metabolized to dihydroartemisinin via cytochrome P450 CYP3A4, CYP2B6 and possibly CYP2A6 (Djimde and Lefevre, 2009). The presence of almost the same isoenzymes for artemether and nevirapine creates the potential for drug interaction on co-administration. The present study therefore aims at investigating the effect of artemether and nevirapine co-administration on haematological parameters in both normal and immunosuppressed Wistar rats.

METHODOLOGY

Experimental animals

Seventy-eight adult male and female Wistar rats (180 to 230 g) housed in a temperature-controlled environment with approximately 12 h light and 12 h dark cycle, with free access to standard rat feed and water *ad libitum* were used for this study. They were bred in the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The use and handling of the animals were in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals (UND/World Bank/WHO, 2001) and was approved by the Institutional ethical committee on the use of laboratory animals.

Drugs

Nevirapine (Hetero Drugs Limited, India) was dissolved in 3% v/v Tween 80 according to the required concentration for administration in rats and administered immediately. Artemether (Haupt Pharma Livron, France) was diluted using the same solvent. Dexamethasone (JinLing Pharmaceutical, China) was the immunosuppressive agent used. All drugs were freshly prepared before use.

Experimental design

Animals used were divided into 6 groups of 6 animals per group (males and females). Groups 4, 5 and 6 received 30 mg/kg of nevirapine daily for 21 days. In addition, groups 5 and 6 received 5 and 10 mg/kg artemether, respectively while rats in groups 2 and 3 received artemether 5 and 10 mg/kg, respectively from day 15 for 7 days. This experiment was repeated in immunosuppressed rats with an additional group that received only dexamethasone and 3% v/v Tween 80. Rats in all groups received dexamethasone (20 mg/kg) and booster doses of 10 and 5 mg/kg on days 8 and 15, respectively, except those rats in group 1 that received 3% v/v Tween 80. All drugs administration was through intraperitoneal route. On the 22nd day of the experiment, all the experimental rats were humane-killed under light chloroform anaesthesia and blood samples were collected into EDTA bottles for parametric estimation of CD_4^+ Count (using Partec Cyflow Counter, Japan), red blood cell (RBC), white blood cell, lymphocyte, neutrophil (Neut), packed cell volume (PCV), haemoglobin (Hb) and platelets (PLT) using Sysmex KX 21N Haematological Auto Analyser Machine (Japan). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were accordingly determined.

Statistical analysis

The research results were calculated as means ± standard error of mean (SEM). Test of significance was carried out using analysis of variance (ANOVA) with Dunnet's post-hoc test. Values of $P < 0.05$ were considered statistically significant and results were presented as tables and figures.

RESULTS

The result showed that artemether-nevirapine co-administration caused a significant decrease ($p < 0.05$) in PCV, RBC, lymphocyte and haemoglobin in both normal and immunosuppressed Wistar rats while NEU increased significantly in normal Wistar rats in a dose-related manner (Tables 1 and 2). WBC also decreased significantly in normal Wistar rats but decreased in immunosuppressed Wistar rats, In addition, platelets were also decreased in normal rats, though not statistically significant. Other haematological indices measured (MCV, MCH and MCHC) were also not statistically different ($P > 0.05$) from the control. Weekly

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Table 1. Effect of Co-administration of artemether-nevirapine on haematological Indices and CD4 count in normal rats

NVP	T_{80}	T_{80}	T_{80}	NVP	NVP	NVP
ART	T_{80}	ART_5	ART_{10}	T_{80}	ART_5	ART_{10}
CD4 (cells/µl)	4.0±0.8	4.0±0.6	3.2±0.5	4.2±0.6	3.7±0.4	3.7±0.3
WBC (x10^3/µl)	18.4±3.4	11.7±2.2[a]	12.2±2.0[a]	12.5±2.3[a]	16.1±1.2	12.2±1.2[a]
LYMP (%)	73.9±1.6	77.2±3.6	72.9±2.4	61.0±3.5[a]	60.9±4.9[a]	57.9±2.9[a]
NEUT (%)	19.2±3.9	20.0±3.5	23.8±1.9	35.2±3.4[a]	35.4±4.5[a]	37.7±3.3
RBC (x10^6/µl)	7.8±0.3	7.4±0.3	7.2±0.1	7.9±0.2	7.5±0.2	6.8±0.3[a]
PCV (%)	46.3±1.2	44.4±2.1	42.7±0.9	45.2±0.9	42.4±1.1	38.5±0.9[a]
Hb (g/dl)	14.2±0.3	13.7±0.4	13.4±0.3	13.9±0.3	13.4±0.3	12.5±0.3[a]
PLT (×10^3/µl)	982.8±45.5	111.5±26.5	992.5±57.5	971.8±1.4	113.8±96.9	138.9±54.7
MCV (fl)	58.4±1.3	60.4±1.3	59.1±0.9	57.5±1.6	56.9±0.7	56.7±0.9
MCH (pg)	18.1±0.4	18.6±0.1	18.5±0.3	17.7±0.0	18.0±0.3	18.5±0.4
MCHC (g/dl)	31.5±0.4	30.9±0.7	31.3±0.3	30.9±0.5	31.7±0.2	32.6±0.2

n= 6. Data above are means ± SEM, statistically significant at p<0.05, when subjected to Analysis of variance (ANOVA) followed by Dunnett's post-hoc test, WBC = White Blood Cell, LYMP = Lymphocyte, NEUT = Neutrophil, RBC = Red Blood Cell, PCV= Parked Cell Volume, Hb = Haemoglobin, PLT = Platelet, MCV =Mean Cell Volume, MCH = Mean Corpusular Haemoglobin, MCHC = Mean Corpusular Haemoglobin Concentration, ART_5 = Low Dose of Artemether (5 mg/kg), ART_{10} = High Dose of Artemether (10 mg/kg), NVP = Nevirapine (30 mg/kg), T_{80} = 3%v/v Tween 80.

Table 2. Effect of artemether on haematological Indices and CD4 Count in immunosuppressed rats receiving nevirapine.

DEX	T_{80}	DEX	DEX	DEX	DEX	DEX	DEX
NVP	T_{80}	T_{80}	T80	T_{80}	NVP $_{30}$	NVP	NVP
ART	T_{80}	T_{80}	ART_5	ART_{10}	T_{80}	ART_5	ART_{10}
CD4 (cells/µl)	4.2±0.6	3.4±0.6	4.2±0.4	4.5±1.6	4.6±0.7	4.3±0.5	10.3±4.7
WBC (×10^3/µl)	10.5±0.9	11.6±2.0	12.4±0.8	9.7±2.2	13.5±2.4	11.9±1.9	15.8±5.4
LYMP (%)	76.8±1.6	38.2±4.2[a]	56.9±4.2	54.9±3.8[a]	46.1±7.7[a]	41.8±5.2[a]	41.9±0.7[a]
NEUT (%)	14.3±2.5	16.7±9.6	28.3±8.9	22.5±11.4	7.6±1.1	17.6±3.5	16.5±1.6
RBC (×10^6/µl)	7.9±0.3	8.1±0.2	7.9±0.3	7.7±0.4	7.4±0.1	6.9±0.4	5.1±1.0[a]
PCV (%)	45.4±1.0	47.2±1.5	46.6±1.5	45.3±2.0	43.9±0.9	39.5±2.1	27.6±5.6[a]
Hb (g/dl)	13.7±0.3	14.4±0.4	14.0±0.4	13.9±0.6	13.6±0.4	12.0±0.7	9.3±1.7[a]
PLT (×10^3/µl)	92.9±1.0	84.2±68.5	88.6±89.5	94.4±50.9	90.3±63.9	96.3±28.7	80.6±1.7
MCV (fl)	57.1±0.9	58.6±1.2	59.5±0.9	58.8±1.1	59.4±1.2	57.6±1.8	58.9±1.2
MCH (pg)	17.5±0.5	17.8±0.4	17.9±0.4	18.0±0.5	18.2±0.3	17.6±0.6	17.9±0.3
MCHC (g/dl)	30.5±0.4	30.5±0.2	30.1±0.2	30.7±0.3	30.5±0.1	30.8±0.1	30.5±0.3

n = 6. Data above are means ±SEM, statistically significant at p<0.05, when subjected to Analysis of variance (ANOVA) followed by Dunnett's post-hoc test. WBC = White Blood Cell, LYMP = Lymphocyte, NEUT = Neutrophil, RBC = Red Blood Cell, PCV= Parked cell volume, HB = Haemoglobin, PLT = Platelet, MCV =Mean cell volume, MCH = Mean Corpusular Haemoglobin, MCHC = Mean Corpusular Haemoglobin Concentration, DEX = Dexamethasone, ART_5 = Low dose of artemether (5 mk/kg), ART_{10} = High dose of artemether (10 mg/kg), NVP = Nevirapine (30 mg/kg).

changes in PCV of both normal and immunosuppressed rats were also recorded; this was statistically significant (P < 0.05) in both normal (day 21 only) and immunosuppressed Wistar rats (days 14 and 21) in artemether-nevirapine co-administered groups (Figures 1 and 2).

DISCUSSION

This study showed a dose-dependent decrease (P < 0.05) in some of the haematological parameters measured (RBC, PCV, Hb, WBC Lymp and platelets) in artemether and nevirapine co-administration. Decreased

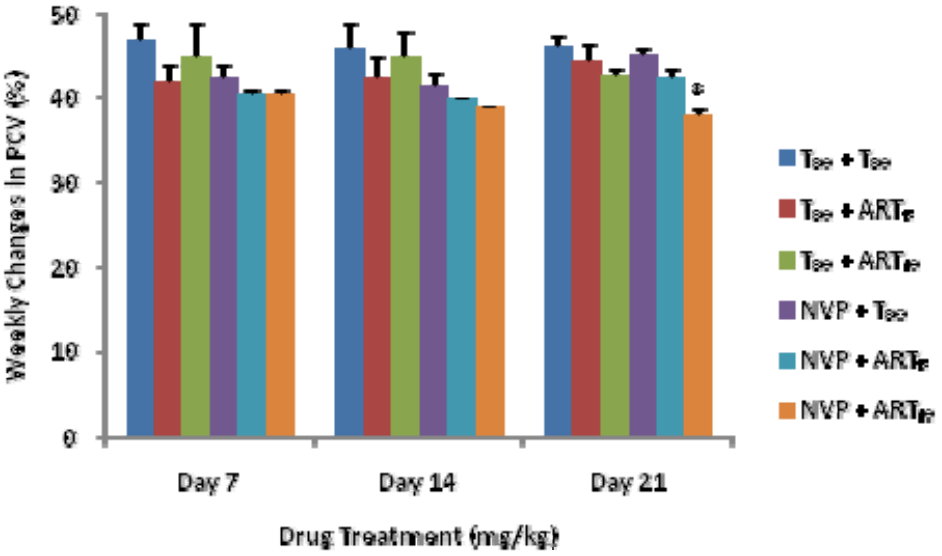

Figure 1. Weekly Changes in PCV on Co-administration of artemether and nevirapine in normal rats. n= 6, data are means ± SEM, *statistically significant at p<0.05 compared with the control (3% Tween 80) when subjected to Analysis of variance (ANOVA) followed by Dunnett's post-hoc test. ART_5 = lower dose of artemether (5 mg/kg), ART_{10} = Higher dose of artemether (10 mg/kg), ART = Artemether, NVP = nevirapine.

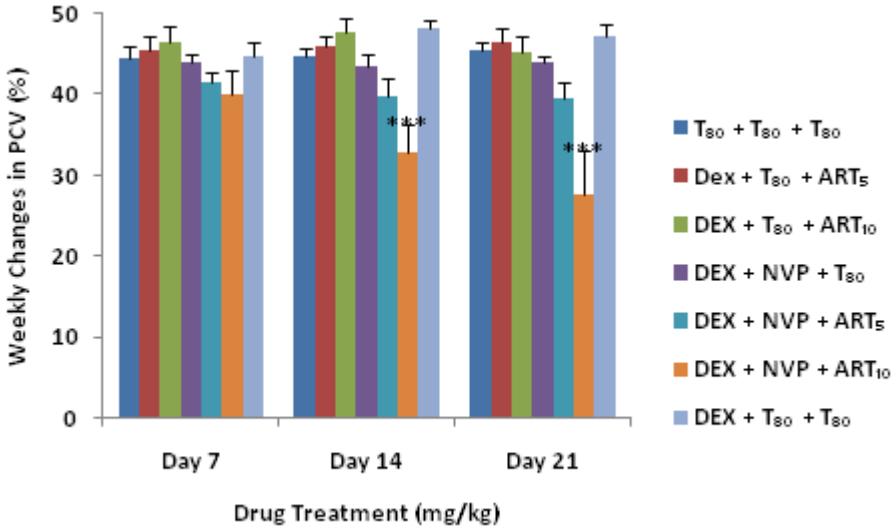

Figure 2. Weekly Changes in PCV on Co-administration of artemether and nevirapine in immunosuppressed rats. n= 6, data are means ±SEM, *statistically significant at p<0.001, cp<0.0001compared with the control (3% Tween 80) when subjected to Analysis of variance (ANOVA) followed by Dunnett's post-hoc test. DEX= Dexamethasone (Immunosuppressive agent), ART_5 = lower dose of artemether (5 mg/kg), ART_{10} = Higher dose of artemether (10 mg/kg), ART = Artemether, NVP = nevirapine.

in lymphocytes observed in both normal and immunosuppressed rats may be due to the fact that immune system is been compromised as a result of concurrent artemether and nevirapine administration in normal rats or may be due to the administration of dexamethasone in immunosuppressed Wistar rats.

Increased neutrophils observed is suggestive of increased infection as a result of drug administration. The significant decreased in RBC was an indication that there was a destruction of matured RBCs. Co-administration of artemether and nevirapine induced anemia as this effect was observed in decreased PCV. Haematological parameters are of diagnostic significance in routine clinical evaluation of the state of health (Sule et al., 2012); and therefore can be used to monitor several disease progression such as HIV/AIDS especially when viral load testing and CD4 cell count monitoring are not readily available (Chen et al., 2007). Blood has been re-ported to be the most easily accessible diagnostic tissue (Maina et al., 2010); therefore employed to evaluate the effect of concomitant use of artemether and nevirapine in this study. Several studies have reported the effects of nevirapine when used alone (Adaramoye et al., 2012) or in combination with other drugs (Odunukwe et al., 2005; Umar et al., 2008) such as pharmacokinetic effect of artemether and nevirapine (Byakika-Kibwika et al., 2011). A previous study by Cinque et al. (1993) showed that Non-nucleoside reverse-transcriptase inhibitor (NNRTI) such as nevirapine was associated with several complications including anaemia in a dose dependent manner. Thus, the present study where artemether was co-administered with nevirapine is in line with that report.

It has been reported that the use of artemether may potentiate haematological abnormalities such as anaemia (Osonuga et al., 2012), as observed when it was co-administered with nevirapine in this study. The increase in both WBC (leucocytosis) and lymphocytes (lymphocytosis) is a sign of immunological response to a trauma caused or induced by drugs (Guyton and Hall, 2006). Dexamethasone used in this study may also be attributed to the changes in immunological status observed. High dose artemether and nevirapine co-administration has been demonstrated in this study to induce anaemia and caused reduction in immunity in both normal and immunosuppressed Wistar rats. Therefore caution with close monitoring should be observed when artemether and nevirapine are co-administered especially in individuals that are susceptible to anaemia as well as immunocompromised patients.

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Conflict of interest

There is no conflict of interest as regard this study.

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