Nigerian Journal of Pharmaceutical and Applied Science Research, 5(3):1-6, December 2016

Effect of Disulphide Derivative of Dihydroartemisinin on the Haematological Indices of N-Methyl-N-Nitrosourea Injected Rats.

Imo Emmanuel Udoh[1*], Jonah Sydney Aprioku[2], Iyeopu Minakiri Siminialayi[3]

[1] Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

[2] Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, Nigeria

[3] Department of Pharmacology. Faculty of Basic Medical Sciences, University of Port Harcourt, Port Harcourt, Nigeria

ABSTRACT

Dihydroartemisinin (DHA) possesses anticancer activity but limited by its short half-life. Recently, a novel cancer chemotherapeutic agent, a disulphide derivative of DHA (sDHA) has been synthesized with the aim of extending its half-life. N-methyl-N-nitrosourea (MNU) has been largely used to induce cancer in the study of a variety of novel cancer chemotherapeutic agents. MNU induced carcinogenesis causes alterations in haematological parameters. This study evaluates the effect of sDHA on the haematological indices of MNU injected rats. MNU (50 mg/kg) was administered intravenously as single dose to different animal groups (n=10), followed ten days later by oral treatment with sDHA (37.42, 74.83 and 112.25 mg/kg), Tween 80 (0.3 ml) and distilled water (0.3 ml) once daily for 28 days. Animals were observed for 52 days after drug treatments after which the animals were sacrificed and blood collected for assay of haematological parameters [Hb, RBC, PCV, MCH, MCHC, PLT, WBC and WBC differentials (eosinophils, neutrophils, lymphocytes, monocytes and basophils)]. MNU significantly ($p<0.0001$) increased serum levels of WBC and differentials and decreased the levels of all red blood indices studied. Oral treatment with sDHA resulted in significant ($p<0.001$) dose-dependent reductions in the serum levels of MNU-induced elevations of WBC and differentials and increased MNU-induced reduction of PCV and PLT levels in a dose-dependent manner, and that of RBC, Hb, MCH and MCHC at the highest dose administered (112.25mg/kg). The study shows that sDHA has the potential to ameliorate the MNU-induced alterations in haematological indices in rats.

KEYWORDS: Disulphide-substituted dihydroartemisinin, N-Methyl-N-Nitrosourea, Haematological indices.

INTRODUCTION

Various drugs are used to kill neoplastic cells without damaging healthy surrounding tissues e.g. Doxarubicin, Bevacizumab, Sorafenib(Iqbal, 2015). Nevertheless, there exist some undesired adverse effects during and after therapy e.g. GIT disturbances, infertility and disturbed homeostasis (Iqbal, 2015). Furthermore many of these drugs unfortunately, are expensive and require frequent administration which has led to the search for anticancer drugs that are affordable while maintaining high safety profiles and efficacies. Dihydroartemisinin (DHA), the active metabolite of artemisinin is a well-known antimalarial drug which has been shown to possess potent anticancer activity against a variety of human cancer models(Jiao et al., 2009; Meyer and Cohen, 2011 and Tarun et al., 2014) but with the limitation of a short half-life (Ashton et al, 1998). The presence of endoperoxide group in its molecule, like other artemisinins has been shown to be responsible for its anti-cancer activity as well as its short half-life.

*Corresponding Author: Email: imoudoh2014@gmail.com Phone + 234(0)8161518553

In view of this, it is hypothesized that structural modification of the endoperoxide ring of dihydroartemisinin by substitution of the peroxide oxygen atoms with suitable atoms may resist superoxide dismutase (SOD) effect and extend its half-life. This has led to the synthesis of a disulphide derivative of DHA (sDHA)-sesquiterpene lactol endodisulphide (Bassey, 2015). The new drug was synthesized through structural modification of DHA by replacing its peroxide oxygen atoms with disulphide, or persulphide (Bassey, 2015).With the synthesis of this new compound, evaluation of its pharmacokinetic, pharmacodynamic, toxicity and anticancer profiles becomes imperative.

N-methyl-N-Nitrosourea (MNU), one of the most utilized chemical carcinogens, has been largely used in the study of a variety of novel cancer chemotherapeutic agents (Macejová and Brtko, 2001). MNU is a direct-acting alkylating N-Nitroso compound (Golding *et al*., 1997) used to induce cancer in various organs in a variety of animal species including rodents, pigs, dogs, and rabbits, and in addition to that it is used as organ-specific animal models for human cancer (Tsubura *et al*., 2011). MNU-induced carcinogenesis causes alterations in haematological parameters (Mani, 2014) resulting in anemia from bleeding, nutritional deficiencies, bone marrow damage, tumor infiltration in bone marrow, and the malignant process itself. The inflammatory cytokines associated with tumor genesis, such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), can inhibit the proliferation of erythrocytic progenitors (Varlotto and Stevenson, 2005).

Blood contains a variety of cells in appropriate proportion. Any kind of severe abnormality (especially cancer) has a direct impact on blood parameters so it is necessary to study the changes in hematological parameters at regular intervals during chemotherapy as an indicator of treatment outcomes. This study is aimed at evaluating the effect of disulphide derivative of dihydroartemisinin on the haematological indices of N-Methyl-N-Nitrosourea injected rats.

MATERIALS AND METHODS

Equipment

Hematology Auto-Analyzer (Sysmex 1-5-1 Wakinohama-Kaigandori Chuo-ku, Kobe 651-0073, Japan).

Drugs, Reagents and Chemicals

Pure powder of disulphide derivative of DHA (sDHA) was obtained from the Department of Medicinal and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria.

N-methyl-*N*-Nitrosourea (MNU) (Sunglong Biotech Co. Ltd, Zhejiang, China), Cyclophosphamide tablets (Endoxan®) (ZydusCadila, Ahmedabad – 380015, Gujarat, India) purchased from Amela Pharmacy, Uyo, Akwa Ibom State. Ketamine Hydrochloride (Merck, Darmstadt, Germany), xylazine (Taj Pharmaceutical Limited, Mumbai,India), polysorbate 80(Tween® 80 (Sigma-Aldrich and Merck, KGaA, Darmstadt, Germany) and distilled water obtained from department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo.

Animals

Adult Sprague-Dawley rats aged 6-8 weeks old, weighing 180-200 g were used for the study. They were housed in plastic cages and maintained under standard conditions (12 h light-dark cycle, and room temperature). The rats were given free access to clean water and commercial rodent chow and allowed to acclimatize for two weeks before commencement of experiment. Experimental procedure was approved by the Ethics Committee on Animal Experiments of the College of Health Sciences, University of Port Harcourt, Nigeria, dated 24th April, 2011. All experiments were conducted according to the guidelines for care and use of laboratory animals (CCAC, 2009)

Method

Experimental Design

Seventy rats (180-200 g) were randomly distributed into seven groups (labeled as groups 1-7).containing 10 animals per group. Methyl-*N*-Nitrosourea (MNU) was administered as a single dose (50 mg/kg) through the jugular vein in groups 1 to 5 (Parikh, 2005). Ten days after MNU administration, group 2-5 animals were treated, respectively with three (3) different concentrations of disulphide derivative of DHA (sDHA) (37.42mg/kg, 74.83mg/kg, and 112.25mg/kg) and cyclophosphamide (0.71mg/kg) which served as the positive control group. The sixth group was given Tween 80 (0.2 ml) and the seventh group received distilled water only and served as a negative control group. All agents except MNU were administered orally once daily. MNU was dissolved in physiological saline (1 % w/v) buffered to pH 5.0 with 3 % acetic acid, whereas the drugs, were dissolved in Tween 80 (30 %). The doses of sDHA corresponded to 10, 20 and 30 % of its LD_{50} (Udoh, 2016). After the drug administrations, the rats were observed without treatment for additional 52 days.

On the 81st day of the experiment, the animals were anaesthetized with ketamine/xylazine (150/10 mg/kg) and sacrificed. Blood was collected by cardiac puncture into EDTA bottles and used for assay of haematological parameters- haemoglobin (Hb), Red Blood Cells (RBC), packed cell volumes (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelets (PLT), White Blood Cells (WBC) and differentials (eosinophils, neutrophils, lymphocytes, monocytes and basophils) (Qchei and Kolhakaar, 2008).

Hematological parameters analysis

White blood cell (WBC), red blood cell (RBC), platelet, and WBC differential (neutrophil, lymphocyte, monocyte and eosinophil, and basophil) counts were determined using a Hematology Auto-Analyzer (Sysmex 1-5-1 Wakinohama-Kaigandori Chuo-ku, Kobe 651-0073, Japan). The levels of hemoglobin (Hb), packed cell volume (PCV),mean corpuscular hemoglobin(MCH), and mean corpuscular hemoglobin concentration(MCHC) were equally measured.

Statistical analysis

The results are presented as mean±SEM for each group. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnette's test for pair wise comparisons. Data were analyzed using GraphPad Prism 5 software and values were considered significant at $p < 0.05$.

RESULTS

Hematological parameters

There was significant ($p<0.0001$) decrease in serum levels of Red blood cell (RBC), haemoglobin (HB), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet (PLT) in MNU-treated group of animals compared to control (Table 1).In all the MNU+sDHA-treated groups there was significant ($p<0.001$) increase in PCV and PLT in a dose-dependent manner while significant ($p<0.001$) increase in serum levels of RBC, Hb, MCH and MCHC was observed only with the highest dose of sDHA administered compared to MNU-treated group (Table 1).

Serum levels of white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils and basophils were significantly ($p<0.0001$) increased in MNU-treated group of animals compared to control (Table 2), while in all the MNU+sDHA-treated groups, the serum levels of WBC, neutrophils lymphocytes monocytes, eosinophils and basophils decreased significantly ($p<0.001$) compared to the MNU-treated group in a dose dependent manner (Table 2).

Table 1: Serum levels of haematological parameters (Red blood indices) following administration of disulphide-substituted dihydroartemisinin DHA (sDHA) in *N*-Methyl-*N*-Nitrosourea-MNU (50mg/kg)-treated Sprague-Dawley rats.

Groups	RBC (10^6/µl)	Hb (g/dl)	PCV (%)	MCH (pg)	MCHC (g/dl)	PLT (10^3/µL)
Control(Distilled water)	7.82±0.12	14.78±0.51	41.80±2.65	19.20±0.58	35.04±0.50	785.±42.01
30% Tween	7.94±0.59	15.05±0.35	42.20±1.39	18.27±0.35	33.94±1.09	778±48.84
MNU (50mg/kg)	4.56±0.40[a]	10.60±0.10[a]	24.00±1.92[a]	12.81±1.19[a]	18.52±0.50[a]	398.±38.52[a]
MNU+37.42mg/kg sDHA	3.98±0.10[b]	10.29±0.30[b]	25.40±2.77[b]	11.20±0.14[b]	15.45±0.76[b]	470.±38.47[b]
MNU+74.83mg/kg sDHA	4.17±0.00[b]	10.39±0.36[b]	28.40±1.50[b]	11.39±0.17[b]	17.80±1.58[b]	469.±33.88[b]
MNU+112.25mg/kg sDHA.	4.67±0.60[b]	12.43±0.25[b]	31.00±1.23[b]	13.48±0.30[b]	29.05±2.39[b]	541.±11.14[b]
MNU+0.71mg/kg Cyclophosphamide	7.46±0.20[b]	15.14±0.47	41.60±1.72	16.46±0.38[b]	34.47±0.42[b]	778.±30.07

Values are Mean ± SEM of 10 rats in a group. [a]Significantly different compared to control group ($p<0.0001$), [b] Significantly different compared to MNU-treated group ($p<0.001$). RBC=Red blood cells, HB=Haemoglobin, PCV=Packed cell volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration, PLT=Platelet.

Table 2: Serum levels of haematological parameters (White blood cell and differentials) following administration of disulphide-substituteddihydroartemisinin DHA (sDHA) in *N*-Methyl-*N*-Nitrosourea-MNU (50mg/kg)-treated Sprague-Dawley rats.

Groups (10^3/µl)	WBC	Neut (10^3 /µl)	Lymp(10^3/µl)	Mon(10^3/µl)	Eosin(10^3/µl)	Baso(10^3/µl)
Control (Distilled water)	5.77±0.39	1.38±0.11	5.26±0.51	0.08±0.00	0.03±0.01	0.10±0.0
30% Tween 80	6.71±0.17a	1.32±0.16	4.52±0.12	0.06±0.01	0.11±0.00.3	0.10±0.0
MNU (50mg/kg).	8.80±0.52[a]	2.90±0.10[a]	8.65±0.39[a]	0.19±0.01[a]	0±0.03[a]	0.38±0.06[a]
MNU+37.42mg/kg sDHA	8.73±0.17[b]	2.56±0.16[b]	8.53±0.25[b]	0.14±0.01	0.23±0.01[b]	0.24±0.02[b]
MNU+74.83mg/kg sDHA	8.51±0.14[b]	2.38±0.18[b]	7.86±0.24[b]	0.14±0.01[b]	0.22±0.01[b]	0.12±0.02
MNU+112.25mg/kg sDHA	7.82±0.16[b]	2.28±0.15[b]	7.31±0.12[b]	0.12±0.04[b]	0.21±0.01[b]	0.10±0.00
MNU+0.71mg/kg Cyclophosphamide	7.35±0.08[b]	1.44±0.10	6.32±0.49[b]	0.12±0.01[b]	0.17±0.01[b]	0.10±0.00

Values are Mean ± SEM of 10 rats in a group. [a]Significantly different compared to control group ($p<0.0001$), [b] Significantly different compared to MNU-treated group ($p<0.001$). WBC=White blood cell, Neut=Neutrophils, Lymp=Lymphocytes, Mon=Monocytes, Eosin=Eosinophils, Baso=Basophils.

DISCUSSION

The pharmacological activity of artemisinins is directly linked to their structures (Li, 2012andRajkhowa et al., 2013). Even minor structural changes of the drugs could profoundly modify their pharmacodynamics and pharmacokinetics, as well as their toxicological indices. Recently, Bassey (Bassey, 2015) has synthesized disulphide substituted derivative of DHA which is believed to have a longer half-life than the parent drug. The present study reports the effect of the disulphide derivative of dihydroartemisinin (sDHA) on the haematological indices of MNU injected rats .N-methyl-N-nitrosourea (MNU), one of the most utilized chemical carcinogens, has been largely used in the study of effect of a variety of novel cancer chemotherapeutic agents on animals (Macejová and Brtko, 2001).

Blood is composed of erythrocytes, leucocytes, thrombocytes and plasma. The three main functions of erythrocytes or red blood cells (RBC) are Oxygen distribution to the periphery from the lungs through the pulmonary capillaries, removal of carbon dioxide from the tissues back to the lungs through the systemic capillaries and maintenance of acidic and basic values of the body. Platelets on their part are involved in hemostasis, thrombosis, clot retraction, vessel constriction and repair, inflammation including promotion of atherosclerosis, host defense and even tumor growth/metastasis. White blood cells or Leucocytes are the main cells of the immune system that provide innate and specific adaptive immunity; divided into basophils, neutrophils, eosinophils, lymphocytes and monocytes (Muriithi et al., 2015).

The actual physiological status of organisms can be diagnosed through use of blood parameters. For normal functioning of the body of an organism, it must keep its blood composition and constituent under natural conditions (Rodrigues and McNeill, 1992). Increase in number of leukocytes and decrease in erythrocytes in the blood is often an indicator of disease condition e.g of neoplastic or autoimmune in origin (Ali, 2014).Therefore testing of hematological indices can be used to determine both the extent of deleterious effect of diseases or foreign compounds on the blood composition and the ameliorating effect of a chemotherapeutic agent.

In this study MNU significantly ($p<0.0001$) reduced serum level of all the red blood indices studied and significantly ($p<0.0001$) elevated the serum levels of WBC and differentials in the animals compared to control thus confirming its deleterious effect on heamatological parameters as reported by Mani (2014). sDHA significantly ($P<0.001$) increased the MNU-induced reduction in serum levels of Hb, RBC, PCV. MCH, MCHC, and PLT while significantly ($p<0.001$) reducing the elevated WBC and differential levels in a dose dependent manner. Since MCHC, MCH relate to individual red blood cells while Hb, RBC and PCV relate to the total population of red blood cells in the blood, the results imply that sDHA not only encouraged the incorporation of haemoglobin into red blood cells but

also helped to maintain the morphology and osmotic fragility of red blood cells.

The significant ($p<0.001$) increase in PCV is also an indication that the red blood cell volume was increased by sDHA treatment. An increase in the PCV suggests an increase in the oxygen carrying capacity of the blood. The significant ($p<0.001$) decrease in WBC, percentage neutrophil and percentage lymphocyte counts is an indication of the reduction in antibody dependent cellular toxicity by sDHA. Neutrophils and lymphocytes play a role in the antibody dependent cellular cytotoxicity to invading pathogens in the body (Muriithi et al., 2015). sDHA was also found to have reduced the serum level of basophils. Basophils are usually present only when there is severe infection.

CONCLUSION

In conclusion the present study showed that oral administration of the disulphide derivative of dihydroartemisinin (sDHA) in MNU-injected rats resulted in a significant improvement of erythrocytic parameter profiles. This may suggest that sDHA possesses erythropoietin protective activity and compounds that slow down the natural process of oxidative breakdown of erythrocyte and hence have a promising role in prevention of anemia. The significant decrease in total white blood cell and differential white blood cell counts in MNU-injected rats after oral administration of sDHA shows that it promotes and improves the immune-regulatory activities hence can be pursued for their clinical relevance in management of immunity- dependent disorders. The significant increase in platelet and their related parameters in MNU-injected rats after oral administration of sDHA has the potential to protect thrombopoietin production and can thus be used to manage hemostatic capacity of blood since platelets in blood mediate clotting mechanisms. This study shows that sDHA has the potential to ameliorate MNU-induced alterations in haematological parameters.

REFERENCES

Ali.S. (2014). Questions and guide to answers (Q&A) in internal medicine: give an account on compartment syndrome, hyperhomocysteinemia, brain natriuretic peptide, and viral autoimmune manifestations. *Egypt Journal of Internal Medicine*; 26:186-92

Ashton, M., Sy N.D., Huong, N.V., Gord,i T., Hai, T.N., Huong, D.X., Niêu, N.T. and Cng, LD. (1998). Artemisinin kinetics and dynamics during oral and rectal treatment of uncomplicated malaria. *Clinical Pharmacology and therapeutics* 63(4):482-493

Bassey, U.B. (2015). Synthesis, characteristics and antimalarial study of the deoxy and disulphide derivatives of dihydroartemisinin. *M.Sc Thesis*, Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria. Supervisor: Prof. E.E. Essien.

Golding, B, T., Bleasdale, C., Mcginnis, J., Watson, W. P.(1997). The mechanism of decomposition of N-methyl- N-nitrosourea (MNU) in water and a study of its reactions with 2′-deoxyguanosine, 2′-deoxyguanosine 5′-monophosphate and d(GTGCAC). *Tetrahedron* 53(11):4063-4082.

Iqbal, M., Younis, M., Shoukat, N., Shaikh, S, Akram, N., Abbas, R., Shahzad, K. A., Mazhar, K, Rehman, F. (2015). Hematological study of cancer patients with radio-chemotherapy. *Science letters*3(2):75-79.

Jiao, Y., Ge, C.M., Meng, H.G., Cao, J.P., Tong, J., Fan, S.J. (2007).Dihydroartemisinin is an inhibitor of ovarian cancer cell growth. *Acta Pharmacol Sin*; 28(7):1045–56.

Li, Y. (2012).Qinghaosu (artemisinin): Chemistry and pharmacology. *Acta Pharmacol Sin*; 33(9):1141–6.

Macejova', D. and Brtko, J. (2001). Chemically induced carcinogenesis: A comparison of 1-Methyl-1-Nitrosourea, 7, 12-dimethylbenzanthracene, diethylnitroso-amine and azoxymethane models.*(Minireview)*. *Endocrine regulations*. vol. 35, 53-59

Mani, S., Ram, B.R., Kuldeep, D., Babu, L.J., Subramaniyam, S., Gopikunte,J.R., Inbaraj, S., Kuppusamy, K., Singaram,B. and Arumugam, G. (2014). Effect of *Morinda citrifolia*(Noni) Fruit Juice on Antioxidant, Haematoloical and Biochemical Parameters in N-Methyl-N-Nitrosourea (NMU) induced Mammary carcinogenesis in Sprague-Dawley Rats. *International Journal of Pharmacology*: 10(2):109-119.

Meyer, J.E., Cohen S.J. (2011). Beyond first-line therapy: Combining chemotherapy and

radioembolization for hepatic colorectal metastases. *Journal of Nuclear Medical Radiation Therapy*; 2:103.

Muriithi, N.J., Maina, G. S., Mugendi, N.M., Maina, M.B., Kiambi, M.J., Kelvin, J.K., Aliyu, U. A., John, M.K., Ann, N.W., Abdirahman, Y.A., Ngugi, M. P. and Eliud, N.M. (2015). Determination of Hematological Effects of Methanolic Leaf Extract of *S. incanum* in Normal Mice. *Pharm Anal Acta*; 6(10):429-435

Ochei, J., and Kolhatkar, A. (2008). Complete blood count: In Medical Laboratory Science Theory and Practice. 7th Edition Tata McGrawHill, pp 273 – 277.

Parikh, R.R., Gildener-Leapman, N., Narendran, A., Lin, H., Lemanski, N., Bennett, J.A, (2005). Prevention of N-Methyl-N-Nitrosourea-Induced Breast Cancer by a-Fetoprotein (AFP)-Derived Peptide, a Peptide Derived from the Active Site of AFP. *Clinical Cancer Research*; 11:8512-20.

Rajkhowa, S., Hussain, I., Hazarika, K.K.,Sarmah, P., Deka, R.C. (2013). Quantitative structure-activity relationships of the antimalarial agent artemisinin and some of its derivatives - a DFT approach. *Comb Chem High Throughput Screen*; 16(8):590-602.

Rodrigues, B., McNeill, J.H. (1992). The diabetic heart: metabolic causes for the development of cardiomyopathy. *Cardiovascular Research* 26: 913-922

Tarun, V., Yash, P., Diksha, J.(2014). An updated review on cancer.*International Journal of Pharmacological Research*; 4(2):91-102.

Tsubura, A., lai, Y., Miki, H., Sasaki, T., Uehara, N., Yuri,T. and Yoshizawa, K. (2011). Review: Animal Models of N-Methyl-N-nitrosourea-induced Mammary Cancer and Retinal Degeneration with Special Emphasis on Therapeutic Trials. *in vivo*25: 11-22

Varlotto, J., Stevenson, M.A.(2005). Anemia, tumor hypoxemia and the cancer patient. *Int J RadiatOncolBiolPhys*;. 63: 25-36. 3.

CCAC- Canadian Council On Animal Care. The Care and Use of Farm Animals in Research, Teaching and Testing. 12-15. Ottawa; 2009.