[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

Original Article

Hematoxicity of Amodiaquine in Sprague-Dawley Rats

W. A. Saka, R. E. Akhigbe, A. O. Akinola, O. M. Azeez

Department of Physiology, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

ABSTRACT

Objective: The use of amodiaquine (AQ) and its associated toxic effect has been a major public health concern since cases of life-threatening agranulocytosis and hepatic toxicity were reported during its prophylactic use. The objective of this study was to evaluate the hematological safety profile of AQ therapy. **Materials and Methods:** Sprague-Dawley rats were randomly distributed into four groups (n=5). Group 1 was the control, while groups 2, 3, and 4 received AQ treatment for 14 days at varying doses of 5 mg/kgBW, 10 mg/kgBW, and 15 mg/kgBW daily, respectively. **Results:** Following treatment, hematological variables were comparable in all groups (P>0.05). **Conclusion:** This study provides evidence to support the use of AQ in the treatment of uncomplicated malaria. However, to prevent emergence of local drug resistance, it should be used as part of a combination therapy. Monitoring for adverse effects is suggested.

Key words: Amodiaquine, hematological parameters, malaria, toxicity

INTRODUCTION

Amodiaquine (AQ), a 4-aminoquinoline related to chloroquine (CQ), is commonly used as an antimalarial and anti-inflammatory agent.[1] It is used as prophylaxis as well as chemotherapy in acute malarial attacks in nonimmune subjects. Although resistance to AQ has been reported, it remains effective against some chloroquine-resistant strains (CRS). AQ has also been tried with variable success in the treatment of giardiasis, hepatic amoebiasis, lepra reactions, lupus erythematosus, rheumatoid arthritis, and urticarial.[2]

AQ, a congener of CQ, was withdrawn from use in some parts of the world because of fatal side effects, notably agranulocytosis and hepatitis, which occurred mainly in nonimmune adults taking the drug for prophylaxis.[3] AQ has been reported to cause direct bone marrow stem cell toxicity,[4] whereas other studies have detected little direct toxicity to peripheral cells at therapeutic concentrations.[5] Polymorphonuclear toxicity, however, has been observed in the presence of AQ-specific serum components, indicative of an indirect immunological mechanism for the agranulocytosis. The mechanism underlying the well-known side effects of AQ (agranulocytosis and hepatitis) is direct toxicity or immune-mediated hypersensitivity.[6]

Despite it being no longer recommended in the United States and some other parts of the world for chemoprophylaxis of *Plasmodium falciparum* malaria because of its associated hepatic toxicity and agranulocytosis, its inexpensiveness and substantial activities in CRS has encouraged its use in endemic areas with few alternatives to be debated and re-evaluated.[3] This study extensively evaluated the hematological safety profile of AQ treatment.

Access this article online

Quick Response Code:

Website: www.toxicologyinternational.com

DOI: 10.4103/0971-6580.97197

MATERIALS AND METHODS

Animals

Sprague-Dawley rats were used for the experiment. They were housed in standard rat cages under laboratory

Address for correspondence: Mr. R. E. Akhigbe, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. E-mail: akhigbemcroy@yahoo.com

[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

conditions with 12:12-hour light/dark cycle at 25°C±2. The animals were allowed to acclimatize for two weeks.[7]

Treatment

Rats were randomly distributed into four groups (n=5). Group 1 was the control, while groups 2, 3, and 4 received AQ treatment (Camoquine®, Parke-Davis Laboratories, United Kingdom) for 14 days at varying doses of 5 mg/kgBW (low dose), 10 mg/kgBW (normal dose), and 15 mg/kgBW (high dose) daily, respectively. All rats were allowed free access to standard rat chow and water.

Hematological evaluation

Blood samples were collected and dispensed into tubes containing lithium-heparin anticoagulant. Red blood cells and total white blood cell were counted by Neubauer's improved hemocytometer using Hyem's and Turks solution as a diluting fluid, respectively. Differential white blood cell (DWBC) count was estimated by standard laboratory method using Lieshman's stain. Hemoglobin was estimated by Shalis method. Packed cell volume was estimated using a microhematocrit reader after centrifuging the blood sample at 3 000 rpm for 30 minutes.[8] Mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated respectively using standard formula described by Dacie and Lewis[9] and Joshi *et al*.[10]

Statistical analysis

Data are expressed as mean±standard deviation (SD). Statistical analyses were done by ANOVA, followed by Duncan's multiple range test for pairwise comparison. Analyses of data were done using the SPSS software (SPSS Inc., Chicago, USA). $P<0.05$ was set as the level of significance.

Ethics

All animals received humane care in compliance with the institution's guideline and criteria for humane care as outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

RESULTS

Hematological changes in test groups and the control group are presented in Table 1. Hematological variables were comparable in all groups ($P>0.05$).

Table 2 shows the effect of AQ on red blood indices. There were no significant differences in MCV, MCH, and MCHC in all groups ($P>0.05$).

DWBC counts are shown in Table 3. There were no significant differences in all groups ($P>0.05$).

Table 1: Blood cells counts, packed cell volume, and hemoglobin concentrations in experimental groups

Variables	Group 1 Control	Group 2 Low dose	Group 3 Normal dose	Group 4 High dose	*P* values		
					1 *vs* 2	1 *vs* 3	1 *vs* 4
Red blood cell count (×10^{12}/l)	6.28 ± 3.18	6.92 ± 1.95	5.89 ± 0.47	7.51 ± 0.425	0.711	0.793	0.416
Packed cell volume (%)	44.83 ± 3.42	42.30 ± 3.85	43.90 ± 7.76	45.20 ± 1.83	0.304	0.812	0.837
Hemoglobin count (g/dl)	15.60 ± 0.94	13.63 ± 1.05	11.53 ± 1.95	14.60 ± 1.12	0.162	0.300	0.164
Total white blood cell count (×10^{9}/l)	7.10 ± 1.12	5.43 ± 2.97	7.60 ± 5.61	5.17 ± 2.33	0.274	0.850	0.133
Platelet count (×10^{9}/l)	449.67 ± 52.90	487.00 ± 21.30	371.67 ± 158.00	484.33 ± 113.00	0.182	0.327	0.551

Table 2: Blood indices in experimental groups

Variables	Group 1 Control	Group 2 Low dose	Group 3 Normal dose	Group 4 High dose	*P* values		
					1 *vs* 2	1 *vs* 3	1 *vs* 4
MCV (fl)	71.39 ± 8.77	61.13 ± 7.65	74.53 ± 5.41	60.18 ± 7.63	0.084	0.515	0.063
MCH (pg)	24.84 ± 4.70	19.70 ± 2.68	19.58 ± 3.80	19.47 ± 2.91	0.066	0.087	0.061
MCHC (%1 g/100ml)	34.80 ± 4.25	32.22 ± 2.01	26.26 ± 7.69	32.30 ± 2.91	0.255	0.062	0.309

MCV: Mean cell volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration

Table 3: Differential white blood cell counts in experimental groups

Variables	Group 1 Control	Group 2 Low dose	Group 3 Normal dose	Group 4 High dose	*P* values		
					1 *vs* 2	1 *vs* 3	1 *vs* 4
Neutrophils (%)	12.67 ± 2.68	10.67 ± 6.62	7.67 ± 4.92	8.33 ± 4.20	0.549	0.081	0.088
Eosinophils (%)	1.67 ± 0.74	3.13 ± 1.41	3.33 ± 0.74	2.33 ± 0.74	0.081	0.070	0.195
Basophils (%)	0.33 ± 0.33	0.33 ± 0.74	0.35 ± 0.72	0.40 ± 0.74	1.000	0.966	0.884
Monocytes (%)	7.00 ± 1.30	7.67 ± 2.68	7.88 ± 0.74	8.00 ± 1.30	0.629	0.224	0.258
Lymphocytes (%)	77.67 ± 4.16	78.67 ± 8.30	79.33 ± 1.99	81.00 ± 2.24	0.816	0.443	0.153

[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

DISCUSSION

Hematological changes in all the treated groups when compared with those of the control revealed that AQ has no significant effect on hematological parameters. Similarly, red blood indices were comparable in all groups. Although there were marginal changes in hematological variables and red cell indices, these were not statistically significant. This is in keeping with previous studies.[11,12] The results observed from this study suggest that AQ does not cause inhibition of hemopoiesis, reduction of growth factors, and other food utilization parameters associated with hemopoiesis or hemolysis.

This study also observed that there were no statistically significant changes in DWBC of all groups following AQ treatment. This is in consonance with previous studies[11,12] that observed marginal and within normal limits of hematological profile with no agranulocytosis in patients who received AQ treatment. However, this is in contrast with other studies[3] that reported agranulocytosis in adults taking AQ for prophylaxis. The variation observed might be due to the duration of treatment, as cases that reported AQ-induced agranulocytosis were associated with its long-term use as prophylaxis. Pharmacogenetic polymorphism might also be a contributing factor.

Results from this study corroborate previous studies[13-19] that reported the safety and efficacy of AQ treatment. This shows that AQ poses no hematological toxicity and thus supports its continued use in the treatment of uncomplicated malaria. However, we suggest that it is used as part of a combination therapy, preferably artemisinin-based combination therapy,[20] to prevent the development of local drug resistance. Monitoring for rare adverse effects is recommended.

REFERENCES

1. AFHS. American Hospital Formulary Service. Drug information 88. In: McEvoy GK, editor. Bethesda, MD. 1988.
2. Winstanley P, Edwards G, Orme M, Breckenridge A. The disposition of amodiaquine in man after oral administration. Br J Clin Pharmacol 1987;23:1-7.
3. Olliaro P, Mussano P. Amodiaquine for treating malaria. Cochrane Database Syst Rev 2003;(2):CD000016.
4. Rhodes EG, Ball J, Franklin IM. Amodiaquine induced agranulocytosis: Inhibition of colony growth in bone marrow by antimalarial agents. Br Med J (Clin Res Ed) 1986;292:717-8.
5. Ellis ME, Steed AJ, Addison GM. Neutropenia associated with dual antimalarial chemoprophylaxis; use of bone marrow culture as an aid in further drug management. J Infect 1987;15;147-52.
6. Clarke JB, Neftel K, Kitteringham NR, Park BK. Detection of antidrug IgG antibodies in patients with adverse drug reactions to amodiaquine. Int Arch Allergy Appl Immunol 1991;95:369-75.
7. Akhigbe RE, Olatunji LA, Soladoye AO, Oyeyipo IP. Effect of angiotensin 1-converting enzyme inhibitor, captopril, on body weight and food and water consumption in oral contraceptive-treated rats. Am J Biochem Mol Biol 2011;1:95-100.
8. Akhigbe RE, Azeez OM, Ige SF, Oyeyipo IP, Ajao FO, Soladoye AO. Hemorheological effect of long-term administration of oral contraceptive in rats. Int J Pharmacol 2008;4:403-6.
9. Dacie SIV, Lewis SM. Practical haematology 7th ed. Livingston, London, Melborne and New York: J and A Churchill Ltd; 1991.
10. Joshi PK, Bose M, Harish D. Changes in certain haematological parameters in a siluroid catfish Clariasbatrachus (Linn) exposed to cadmium chloride. Pollution Resources 2002;22:129-131.
11. Molta NB, Oguche S, Pam SD, Omalu IC, Afolabi BM, Odujoko JB, *et al*. Amodiaquine treatment of uncomplicated malaria in children, in an area of chloroquine-resistant Plasmodium falciparum in north-central Nigeria. Ann Trop Med Parasitol 2003;97:663-9.
12. Massaga JJ, Lusingu JP, Makunde R, Malebo HM, Chile MM, Akida JA, *et al*. Biological and haematological safety profile of oral amodiaquine and chloroquine in healthy volunteers with or without Plasmodium falciparum infection in northeast Tanzania. Tanzan J Health Res 2008;10:144-50.
13. Luzzi GA, Peto TE. Adverse effects of antimalarials. An update. Drug Saf 1993;8:295-311.
14. Olliaro P, Nevill C, LeBras J, Ringwald P, Mussano P, Garner P, *et al*. Systematic review of amodiaquine treatment in uncomplicated malaria. Lancet 1996;348:1196-201.
15. Kremsner PG, Krishna S. Antimalarial combinations. Lancet 2004;364:285-94.
16. Mutabingwa TK, Anthony D, Heller A, Hallett R, Ahmed J, Drakeley C, *et al*. Amodiaquine alone, amodiaquine+sulfadoxine-pyrimethamine, amodiaquine+artesunate, and artemether-lumefantrine for out patient treatment of malaria in Tanzanian children: A four-arm randomized effectiveness trial. Lancet 2005;365:1474-80.
17. Obua C, Gustafsson LL, Aguttu C, Anokbonggo WW, Ogwal-Okeng JW, Chiria J, *et al*. Improved efficacy with amodiaquine instead of chloroquine in sulfadoxine/pyrimethamine combination treatment of falciparum malaria in Uganda: Experience with fixed-dose formulation. Acta Trop 2006;100:142-50.
18. Oyakhirome S, Pötschke M, Schwarz NG, Dörnemann J, Laengin M, Salazar CO, *et al*. Artesunate – amodiaquine combination therapy for falciparum malaria in young Gabonese children. Malar J 2007;6:29.
19. Ogungbamigbe TO, Ojurongbe O, Ogunro PS, Okanlawon BM, Kolawole SO. Chloroquine resistant Plasmodium falciparum malaria in Osogbo Nigeria: Efficacy of amodiaquine+sulfadoxine-pyrimethamine and chloroquine+chlorpheniramine for treatment. Mem Inst Oswaldo Cruz 2008;103:79-84.
20. WHO. Position of WHO roll back malaria department on malaria treatment policy. Geneva: WHO; 2006. Available from: http://rbm.who.int/rbm/Attachment/20050418/malariaTreatmentPolicyMarch2005.pdf] website 2003. [Last accessed on 2006 Jan 29].

How to cite this article: Saka WA, Akhigbe RE, Akinola AO, Azeez OM. Hematoxicity of amodiaquine in sprague-dawley rats. Toxicol Int 2012;19:112-4.

Source of Support: Nil. **Conflict of Interest:** None declared.