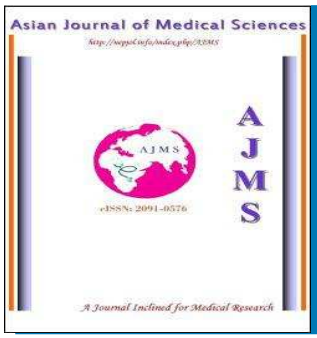

IN VITRO EFFECT OF ALLOPURINOL ON SICKLING RATE AND URIC ACID LEVEL IN SICKLE CELL ERYTHROCYTE

SHORT COMMUNICATION, Vol-4 No.3

Asian Journal of Medical Science, Volume-4(2013) http://nepjol.info/index.php/AJMS

Nnodim Johnkennedy. Department of Medical Laboratory Science, Faculty of Health Science, Imo State University Owerri, Imo State, Nigeria

CORRESPONDENCE:

Nnodim Johnkennedy
Department of Medical Laboratory Science,
Faculty of Health Science, Imo State University Owerri, Imo State, Nigeria
Email: johnkennedy23@yahoo.com
Tel: +2348034237000

"Allopurinol induces dehydration"

ABSTRACT

Objective: Allopurinol is a drug used for the prevention of gout. The in vitro effect of allopurinol on human plasma and erythrocyte of sickle cell haemoglobin(HbSS) were investigated on uric acid level and sickling rate respectively.

Methods: The determination was carried out on samples from 9-25years attending General Hospital Owerri. The plasma uric acid level and sickling rate were determined prior to and after incubation with 100mg standard dose of allopurinol.

Results: The plasma uric acid level was significantly decreased ($p<0.05$) when compared with the control while sickling rate was significantly increased when compared with the control ($p <0.05$).The results obtained demonstrated that allopurinol could hasten sickling rate and decrease uric acid level.

Conclusion: These observations suggest that allopurinol concentration in the circulation might induce marked increased in erythrocyte sickling in vivo and reduced the antioxidant ability of uric acid level in sickle cell disease. Consequently, slow recovery from a sickling crisis. This may be detrimental in the management of sickle cell disease

Key words: Allopurinol, uric acid, sickling rate, erythrocyte, *in vitro*

INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder caused by the substitution of valine for glutamic acid at the sixth position of the amino acid beta chain of the haem molecule[1] and characterized by the possession of sickle cell hemoglobin[2]. Hence, sickle cell hemoglobin is a type of hemoglobin occurring in the erythrocytes of sickle cell disease patients [3]. Sickle cell disease is a significant cause of morbidity and mortality among black individuals and descendants of the Negroid race[4]. Wilson *et al*[5] observed that the abnormal hemoglobin types precipitate in the erythrocyte upon deoxygenation and hence form crystals that distort the cell into characteristic sickle cell erythrocyte produce the major cause of all the crises experienced by sickle cell disease patients[6].

Sickle cell disease has been shown to produce higher uric acid levels in the sufferers. However, this higher than normal uric acid level is often within the normal reference range. It is said to confer a measure of adaptation to the sufferers in that uric acid has been demonstrated to have antisickling properties [7]. Allopurinol which is a xanthine oxidase inhibitor and blocks the synthesis of uric acid has been used as a drug for prevention of and long term treatment of gout[8]. Hence, it allows the excretion of purine as the more soluble xanthenes[9]. Allopurinol is a structural isomer of hypoxantine and enzyme inhibitor[10].In fact, it has been recognized that the abnormal viscoelastic properties of oxygenated sickle cell erythrocytes and the formation of irreversibly sickled formation of sickled erythrocytes are related to abnormal properties of their membranes.

MATERIALS AND METHODS

Allopurinol: This was produced by Dizpharm and purchased from a reputable pharmacy.Allopurinol Preparation: 1000mg of allopurinol was dissolved in 10ml of distilled water, and the drug concentration of 100mg/ml was used.

Blood collection: The samples of blood were collected from patients attending sickle cell clinic at General Hospital Owerri. The HbSS conditions were confirmed by using Haemoglobin Electrophoresis. The total of 40 HbSS patients were selected for the tests. Blood were collected in heparin containers and were used within 6hours after collection.

Sickling test: This was carried out using the method described by Cheesbrough[11]. The test was prepared and observed under the microscope for 100mg allopurinol concentrations. Normal saline was used as control test.

Uric acid estimation: The uric acid concentration was determined by a commercially prepared reagent by Randox. Uric acid is converted by Uricase to allantion and hydrogen peroxide which under catalytic influence of peroxides oxidizes 3,5-Dichloro-2-hydroxybenzensulfonic acid and 4-aminophenazone to form a red violet quinoneimine compound.

Statistical analysis: The control and experimental tests carried out were analyzed by statistical analysis using SPSS software. Mean and standard deviation were used. The results significance was measured by t-test.

RESULTS

Table 1

Sickling rate and uric acid level of HbSS treated with allopurinol and normal saline (control).

Parameter	Normal saline	Allopurinol
Sickling rate (%)	22.52±1.3	41.30±2.16*
Uric acid (mg/dl)	5.23±1.48	2.91±1.25*

*Significantly different from control at P<0.05

The sickling rate of HbSS treated with normal saline was 22.52 ± 1.35% which was significantly increased (P <0.05) in allopurinol treated HbSS erythrocyte (41.30±2.16%) when compared. The level of uric acid in normal saline treated plasma of HbSS was significantly higher (5.23±1.48 mgldl) when compared with allopurinol treated plasma

HbSS (2.91±1.25mg/dl). This showed that uric acid level was reduced while sickling rate was increased with allopurinol treatment.

DISCUSSIONS

Allopurinol is a Xanthine Oxidase inhibitor used in the prevention of attacks of gout and is well tolerated. It is safe to use in patients with renal impairment and urate stones[10]. Gout is a disease hallmarked by the elevated levels of uric acid in the blood stream[7].

From this study, some increases were observed for sickling rate of HbSS erythrocytes with the endogenous allopurinol treatment. Indeed, this demonstration suggests that the results observed with this phenomenon was really a reflection of allopurinol action on sickle cell disease subjects. This action of allopurinol could be that it induces dehydration. When the red blood cells are dehydrated sickling sets in. Also, the mechanism of action could be that the drug allupurinol induces the increase in intracellular calcium and loss of potassium. Alterations in electrolyte and calcium contribute maximally in sickling. Hence the red blood cell becomes dehydrated leading to the sickling of the red blood cell. This is consistent with the work of Uwakwe[2] in which the sickling rate of HbSS erythrocyte treated with caffeine was increased.

Furthermore, it was observed that the plasma uric acid level in HbSS treated erythrocyte was decreased when compared with the control. The decrease in uric acid level could be attributed to the action of Xanthine oxidase inhibitor and hence blocks the synthesis of uric acid. Uric acid are high in the sickle cell disease patients[12]. Hence , the decrease in uric acid could reduce its antioxidant capacity and antisickling property.

These results suggest that allopurinol increases sickling rate and decreases uric acid level of sickle cell erythrocytes. Hence, the use of allopurinol could have some detrimental effect in sickle cell anaemia

REFERENCES

1. Switzer, J.A., Hess, D.C., Nichols, F.I. and Adams, R.J. Pathophysiology and treatment of stroke in sickle cell disease Lancet Neurol 2006; 6.501-512.
2. Uwakwe, A.A, Onwuegbuke, C. and Nwinuka, M.N. Effect of Caffeine on the Polymerization of HbS and sickling rate, Osmotic fragility of HbS and sickling rate. Nig. J. Biochem. Mol. Biol 2002; 1:27-32.
3. Mmeremiukwu, M. Sickle cell disease. Clin Evid. 2005; 14:15 -28.
4. Dunlop, R.J and Benneth, K.C. Pain Management for Sickle cell Disease. Cochrane Database Syst. Rev. 2006;19(2) 246-248.
5. Wilson, O.D., Nwauche, C.A., and Ejele OA.Splenic changes in sickle cell anaemia Niger J. Med 2006;15 (1): 20-3.
6. Hebbel, J.W. Beyond Haemoglobin Polymerization. The red blood cell membrance and sickle cell disease. Blood 1991;77, 214 – 237.
7. Nduka,N.Clinical Biochemistry for Student of Pathology.Longman Nigeria.1999; p147
8. Dalbeth,N and Stamp,L. Allopurinol dosing in renal impairment. Walking the tighrobe between adequate urate lowering and adverse events. Semin Dial 2007; 20(5):391-39.
9. Pacher, P. Nivorozhkin, A and Szabo, C. Therapeutic effect of xanthine oxidase inhibitors. Renaissance half a century after the discovery of allopurinol. Pharmacol Rev 2006; 58(1): 87-114
10. Zahran, A.M; Azab, K.S and Abbady, M.I. Modulatory role of allopurinol on xanthine oxidoreductase status in irradiated rats. Egypt J. Rad. Sci applic. 2006;199(2):373-358.
11. Cheesbrough, M. District Laboratory Practice in tropical countries part 2 Cambridge University press.2000; 334 – 340.
12. Spector, T. Inhibition of urate production by allopurinol. Biochem pharmacol 1997;26(5):355-358