Advanced Journal of Microbiology Research Vol. 2017
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Toxicological assessment of oral administration of some antisickling agents in rats

Oyewole, O. I.[1]* and Malomo, S. O.[2]

[1]Department of Biochemistry, College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown.
[2]Department of Biochemistry, University, Ilorin, Ilorin, Nigeria.

Accepted 29 July, 2017

This study investigated the toxicological effects of oral administration of hydroxyurea, tellurite and thiocyanate on some biochemical parameters in rats. The drugs were administered orally to rats daily at their therapeutic dose for 28 days after which some biochemical parameters were measured. All the drugs caused growth depression and significant elevation ($P < 0.05$) of serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate amino transferase (AST) while they also lowered serum albumin and total protein. Thiocyanate and tellurite significantly decreased activities of the three enzymes in the liver and kidney while hydroxyurea resulted in significant elevated activities. Tellurite significantly reduced serum concentrations of sodium ion and urea while it elevated serum potassium, creatinine and bilirubin. Hydroxyurea elevated serum urea and creatinine while it reduced bilirubin concentration. Thiocyanate significantly reduced ($P < 0.05$) serum bicarbonate and bilirubin. These results suggest that the drugs might be toxic at their therapeutic dose with thiocyanate exhibiting mildest toxicity followed by hydroxyurea.

Key words: Sickle cell disease, hydroxyurea, sodium thiocyanate, potassium tellurite

INTRODUCTION

Sickle cell disease is caused by a haemoglobin mutant, haemoglobin S (HbS) which result due to the replacement of glutamic acid by valine in the sixth position of the beta-chains. This change renders the HbS capable of polymerization in the deoxygenated state and eventual aggregation into elongated microtubular structures which distort the shape of the red blood cell to a sickle shape (Serjeant, 1992). Despite the fact that sickle cell disease has been studied extensively, there has not been a universally acceptable therapeutic agent for the treatment of this disease. Some of the antisickling agents investigated exhibit varying degrees of toxicity, and/or cause haemolysis of the sickle red blood cell at their effective dose levels and are therefore unsuitable for clinical use (Vichinsky, 2002; Redding-Lallinger, 2006).

Thiocyanate is used as an antithyroid drug and is about 100 times less toxic than cyanate. It is an ionic inhibitor that interferes with the concentration of iodine by the thyroid gland (Cooper, 1998). Hydroxyurea is a cytotoxic agent which has a specific effect on dividing cells. The major toxic symptom in animals surviving acute toxic dose is myelosuppression, which is rapidly reversed (Charache et al., 1996). It has been used in the treatment of chronic myelogenous leukaemia and other myeloproliferative disorders (Navarra and Preziosi, 1999). Animal studies suggested that tellurite ingestion might affect the conversion of squalene to cholesterol thereby interfering with neurotransmission via demyelination (Gerhardsson et al., 1986).

In vitro experiments and few case studies have revealed that thiocyanate and tellurite are able to prevent and reverse sickling in sickle cell patients and thereby help in ameliorating the painful crisis associated with the disease (Asakura et al., 1984; Haywood, 1987). These therapies are being given to patients in the absence of adequate toxicity data or any animal toxicity studies that describe their safety at their therapeutic dose. Hydroxy-urea has been approved for the management of this ailment but many uncertainties remain about its long- term benefits and adverse effects as varying degree of toxicity were reported in patients who received the drug (Steinberg et al., 2003). This study aims at investigating and comparing the toxicological effects of the therapeutic doses of these antisickling agents using experimental rats so as to determine their safety or otherwise.

*Corresponding author. E-mail: ioluoye@yahoo.com. Tel.: +23233757782.

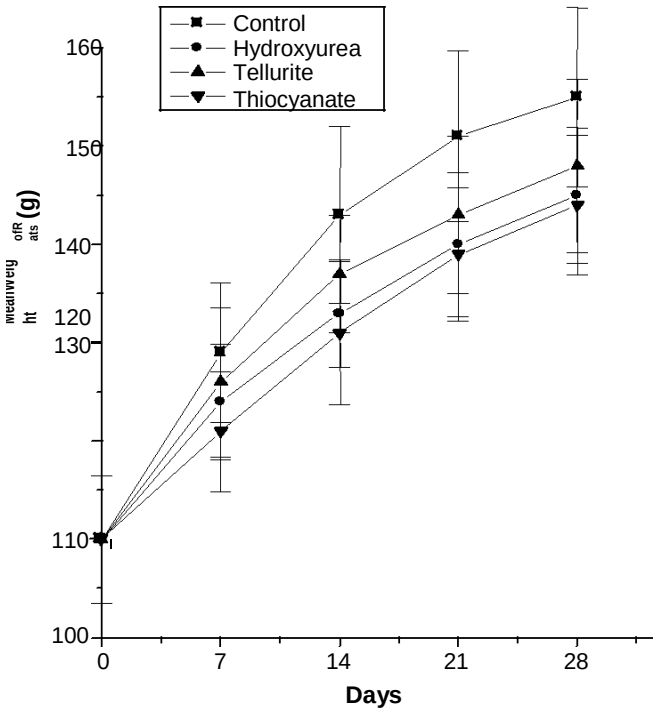

Figure 1. Growth pattern of rats administered with hydroxyurea, tellurite and thiocyanate for 28 Days**.**

MATERIALS AND METHODS

A total of twenty four male albino rats (*Rattus novergicus*) weighing between 110 – 120 g were obtained from the Animal House, Department of Physiology, University of Ibadan, Nigeria. They were kept in ventilated cages and allowed to acclimatize for two weeks prior to drug administration. Hydroxyurea is a product of British Drug House (Chemicals) Ltd., Poole England. Sodium thiocyanate and potassium tellurite salts were products of Hopkins and Williams Ltd. Essex, England.

Drug administration

The rats were randomly divided into four experimental groups (six in a group). Group A (control) were administered with distilled water, group B received 15 mg/kg body weight daily hydroxyurea, group C were administered with 0.1 mg/kg body weight daily potassium tellurite while group D received 10 mg/kg body weight daily sodium thiocyanate. These doses have been reported as the minimum required to achieve therapeutic effect in patients (Asakura et al., 1984; Richardson, 1991; Lima et al., 1997). The drugs were dissolved in distilled water and administered orally to rats by the use of canular daily for a period of 28 days.

Collection of blood samples from rats

Rats were sacrificed at the end of the experimental period and their blood collected in plain bottles. The blood was allowed to clot and then centrifuged at 3000 rpm for 10 min after which the clear supernatant (serum) was separated from the pellet and kept frozen till required.

Preparation of tissue homogenate

The animals were quickly dissected and the tissues (liver, kidney and heart) removed. Tissue homogenates were prepared by suspending the tissue in ice-cold 0.25 M sucrose solution to give a final volume of five times dilution and then homogenized using Teflon homogenizer. The homogenates were kept frozen overnight before analyses.

Measurement of biochemical parameters

Serum sodium and potassium ions were measured by flame photometry while calcium and bicarbonate ion concentrations were determined as described by Tietz et al. (1994). Inorganic phosphate was determined using the direct method of Fiske-Subbarow (1925) while the method of Veniamin and Vakirtzi- Lemonia (1970) which is based on the Fearon reaction was used to measure urea concentration in the serum.

Serum creatinine, total bilirubin and albumin concentrations were determined as described by Tietz et al. (1994). Protein concentration in serum and tissue homogenate was determined by the Biuret method of Gornall et al. (1949). Assay for ALP activity was by the method of Wright et al. (1972) while the method of Reitman and Frankel (1957) was used for the measurement of ALT and AST activities. All spectrophotometric measurements were done using the Jenway 6300 Spectrophotometer.

Statistical analysis

Data was analyzed using Duncan multiple range test following one-way analysis of variance (ANOVA) using SPSS 10.0 computer software package (SPSS Inc; Chicago, U.S.A). Differences at $P < 0.05$ were considered significant.

RESULTS

The growth pattern of animals administered with the drugs and that of the control is shown in Figure 1. The result indicates that the three drugs caused growth depression in rats starting from the second week to the end of the experimental period with thiocyanate having the highest effect.

The concentration of measured parameters in the serum of experimental animals is shown in Table 1. Tellurite induced a significant decrease ($P < 0.05$) in sodium ion while it elevated potassium ion compared with the control. Thiocyanate and hydroxyurea had no significant effect on serum sodium and potassium ions. Thiocyanate significantly lowered serum bicarbonate while hydroxylurea and tellurite had no significant effect on this serum parameter. There was no significant effect of the three drugs on serum calcium and phosphate following their administration for 28 days.

Table 1 also shows that hydroxyurea significantly increased ($P < 0.05$) serum urea while tellurite significantly reduced it compared with the control. Rats administered with thiocyanate did not show change in serum urea concentration. Tellurite and hydroxyurea caused a significant elevation ($P < 0.05$) in serum creatinine while thiocyanate had no effect on this serum parameter.

Hydroxyurea and thiocyanate significantly reduced serum bili-

Table 1. Serum Parameters in Rats Administered with Hydroxyurea, Tellurite and Thiocyanate for 28 days.

Parameters	Control	Hydroxyurea	Tellurite	Thiocyanate
Na+ (mmol/L)	145.0±1.4 ^{a}	144.0±1.7 ^{a}	130.4±1.5 ^{b}	146.0±2.0 ^{a}
K^{+} (mmol/L)	4.10±0.16 ^{a}	4.13±1.5 ^{a}	4.71±0.12 ^{b}	4.20±0.14 ^{a}
Ca^{2+} (mmol/L)	2.25±0.02 ^{a}	2.22±0.03 ^{a}	2.27±0.02 ^{a}	2.22±0.02 ^{a}
HCO^{3-} (mmol/L)	23.00±0.35 ^{a}	23.00±0.41 ^{a}	23.20±0.43 ^{a}	20.40±0.48 ^{b}
PO_4^{3-} (mg/dl)	2.12±0.09 ^{a}	2.17±0.07 ^{a}	2.10±0.08 ^{a}	2.14±0.06 ^{a}
Urea (mmol/L)	5.26±0.04 ^{a}	5.80±0.08 ^{b}	4.70±0.05 ^{c}	5.30±0.08 ^{a}
Creatinine (mg/dl)	44.40±1.34 ^{a}	49.30±1.10 ^{b}	50.60±1.56 ^{b}	45.20±1.20 ^{a}
Bilirubin (mol/L)	16.30±0.61 ^{a}	13.90±0.46 ^{b}	19.70±0.68 ^{c}	14.20±0.23 ^{b}
Albumin (g/L)	42.50±0.42 ^{a}	37.00±0.19 ^{b}	36.90±0.29 ^{b}	36.70±0.38 ^{b}
Protein (mg/ml)	69.30±0.52 ^{a}	66.50±0.16 ^{b}	66.70±0.34 ^{b}	66.30±0.27 ^{b}
ALP (IU/mg protein)	19.50±0.22 ^{a}	21.00±0.31 ^{b}	21.20±0.25 ^{b}	20.90±0.61 ^{b}
ALT (IU/mg protein)	36.30±1.20 ^{a}	41.40±0.58 ^{b}	40.10±1.30 ^{b}	40.00±1.40 ^{b}
AST (IU/mg protein)	34.10±0.43 ^{a}	37.20±0.80 ^{b}	36.80±0.92 ^{b}	36.50±0.76 ^{b}

Values are Mean ± SD, n = 6. Values with different alphabetical superscripts along a row are significantly different at $p < 0.05$.

Table 2. Enzyme Activities in Tissues of Rats Administered with Hydroxyurea, Tellurite and Thiocyanate for 28 days (IU/mg protein).

Enzymes	Control	Hydroxyurea	Tellurite	Thiocyanate
Liver				
ALP	357±17 ^{a}	398±21 ^{b}	296±10 ^{c}	318±13 ^{d}
ALT	3082±48 ^{a}	3524±66 ^{b}	2361±36 ^{c}	2643±44 ^{d}
AST	2078±43 ^{a}	2340±52 ^{b}	1672±29 ^{c}	1748±31 ^{d}
Kidney				
ALP	284±11 ^{a}	334±18 ^{b}	212±08 ^{c}	245±10 ^{d}
ALT	3352±60 ^{a}	3745±68 ^{b}	2688±31 ^{c}	2964±53 ^{d}
AST	2433±42 ^{a}	2678±50 ^{b}	1825±29 ^{c}	2013±34 ^{d}
Heart				
ALP	316±10 ^{a}	322 ±04 ^{a}	312 ±12 ^{a}	320 ±05 ^{a}
ALT	2061±22 ^{a}	2070 ±20 ^{a}	2046 ±36 ^{a}	2072 ±24 ^{a}
AST	3245±28 ^{a}	3220 ±33 ^{a}	3324±14 ^{a}	3214 ±52 ^{a}

Values are Mean ± SD, n = 6. Values with different alphabetical superscripts along a row are significantly different at $p < 0.05$.

rubin concentration while tellurite on the other hand significantly increased this serum metabolite. There was significant decrease ($P < 0.05$) in serum total protein and albumin accompanied with significant elevation in serum concentration of ALP, ALT and AST in all the experimental groups compared with the control as seen in Table 1.

Table 2 shows the levels of ALP, ALT and AST in the tissues of the experimental rats at the end of the experimental period. There was significant decrease ($P < 0.05$) in activities of ALP, ALT and AST in the liver and kidney of rats following administration of thiocyanate and tellurite with tellurite having the highest effect. Hydroxy-urea on the other hand significantly increased the activities of all the enzymes in the kidney and liver of rats. None of the drugs affected the enzyme activities of the heart.

DISCUSSION

Growth pattern of experimental rats

The significant growth depression caused by the anti-sickling agents might be due to loss of appetite or mal-absorption. Thiocyanate has been reported to induce loss of appetite (Cooper, 1998) while hydroxyurea and tellurite were reported to cause gastrointestinal irritation and may therefore hinder intestinal absorption of food (Gerhardsson et al., 1986; Ferster et al., 1996).

Serum electrolytes

The significant decrease in sodium ion and elevation of potassium ion induced by tellurite suggests an inhibitory action of tellurite on monovalent cation transport in the tissues of the rats. The reduction in serum sodium ion might be beneficial to sickle cell patients because it has been demonstrated that inducement of hyponatremia in sickle cell patient could be used in the prevention and treatment of crisis (Rosa et al., 1980). The increase in serum potassium (hyperkalemia) by tellurite might occur due to erythrocyte destruction caused by the drug. Hyperkalemia has been reported to arise in states characterized by excess destruction of cells with redistribution of K^+ from the intracellular to the extracellular compartment as in massive haemolysis (Guyton and Hall, 2000).

The observed reduction in serum bicarbonate by thiocyanate suggests an inhibitory effect of the drug on acid-base balance mechanism in the blood of the rats. Thiocyanate has been reported to be an inhibitor of carbonic anhydrase, an enzyme responsible for convert-ing carbon dioxide to carbonic acid and finally to hydrogen carbonate in the blood (Cooper, 1998). This inhibition might be responsible for the observed decrease in bicarbonate production.

Serum metabolites

Rats administered with hydroxyurea had significantly increaseed ($P < 0.05$) serum urea. This might be due to conversion of hydroxyurea to urea or as a result of impairment of renal function caused by hydroxyurea. Serum urea has been reported to increase in acute and chronic intrinsic renal disease and also when there is decreased effective circulating blood volume with decreased renal perfusion (Cameron and Greger, 1998). The decrease in serum urea concentration by tellurite might be as a result of tubular necrosis or liver disease which caused decreased urea synthesis in the liver. Another possibility is that urea might have been converted to other products such as hydroxyurea which are undetectable by the direct method of urea determination used in this study.

The observed elevation of serum creatinine caused by tellurite and hydroxyurea suggests renal function impairment, which might result from intrinsic renal lesions, decreased perfusion of the kidney, obstruction of lower urinary tract or due to deranged metabolic processes caused by the two drugs (Cameron and Greger, 1998). The reduction in serum bilirubin by hydroxyurea and thiocyanate is an indication that the two drugs prevented haemolysis of red blood cell in contrast to tellurite which caused red blood cell haemolysis. *In vitro* studies demonstrated that tellurite (Te^{4+}) ions can penetrate the erythrocyte membrane and, in the presence of reduced glutathione, form telluride (Te^{2+}) which causes irreversible membrane damage and hence haemolysis (Kurantsin-Mills et al., 1988).

Serum total protein and albumin

The significant decrease ($P < 0.05$) in serum total protein and albumin in all the three experimental groups compared with the control might be due to decreased synthesis, increased loss, increased catabolism, malnutrition, malabsorption or liver disease caused by the drugs (Guyton and Hall, 2000). It has been suggested that gross changes in the activities of some plasma proteins might result due to thiocyanate ingestion (Haywood, 1987). Decrease in albumin has been observed in serum of patients with tissue inflammation and damages (Gabay and Kushner, 1999), which suggest that the tissues of the experimental rats might have been damaged with administration of the drugs.

Serum enzymes

The three drugs caused significant increase in serum concentration of ALP, ALT and AST probably as a result of release of the enzymes from some tissues indicating tissue damage. Elevation of serum ALP activity has been attributed to increased osteoblastic activity such as in hyperparathyroidism, osteomalacia, neoplasm and heaptobiliary diseases (Guyton and Hall, 2000). Increase in serum ALT and AST activity has been reported in conditions involving necrosis of hepatocytes, myocardial cells, erythrocyte and skeletal muscle cells (Macfarlane et al., 2000).

Tissue enzymes

The significant decrease in activities of ALP, ALT and AST in the liver and kidney of rats following administration of thiocyanate and tellurite might be due to inactivation of the enzymes by the drugs or their metabolites, which could also inhibit the synthesis of the enzymes. The reduction might also be due to some stress imposed on the liver and kidney resulting from the metabolism and excretion of the drugs (Meditext, 1997). It is also possible that the drugs caused membrane damage which allowed the enzymes to leak through the cell membrane out of the cell. It has been suggested that chemical compounds might enter into reaction with key molecules in membranes (enzymes inclusive) and then inactivate or denature them (Cotran et al., 1989).

Hydroxyurea on the other hand cause elevation of these enzymes in the liver and kidney of the rats probably due to its stimulatory action on the enzymes in these tissues. This might arise due to stabilizing effect of the drug on the membrane, which makes the localized environment in the membrane more conducive to enhance the actions of the enzymes. The increase might also be due to the response of the cellular system to offset the stress introduced by exposure to the drugs. Increased activity of various enzymes was reported in some tissues under various conditions of stress (Malomo et al., 1995).

Conclusion

Administration of the drugs at their therapeutic dose caused growth depression and derangement in cellular activities as indicated in significant alterations in the levels of biochemical parameters in the serum and tissues of the rats. This study suggests that the drugs might be toxic at their therapeutic dose especially when one considers the fact that the patient may have to take the drugs for life. It appears that thiocyanate exhibits mildest toxicity followed by hydroxyurea, considering the extent of alteration in biochemical parameters caused by the drugs.

REFERENCES

Asakura T, Shibutani Y, Reilly MP, Demeio RH (1984). Antisickling effect of tellurite: a potent membrane-acting agent *in vitro*. Blood 64: 305-307.

Cameron JS, Greger R (1998). Renal function and testing of function. (Davidson AM, Cameron JS, Grunfeld JP, Kerr DNS, Rits E, Winearl GC eds.) Oxford Textbook of Clinical Nephrology. pp. 36-39.

Charache S, Barton FB, Moore RD, Terrin ML, Steinberg MH, Dover GJ, Ballas SK, McMahon RP, Castro O, Orringer EP (1996). Hydroxyurea in sickle cell anaemia: clinical utility of a myelosuppressive switching agent. Medicine 75: 300-326.

Cooper DS (1998). Antithyroid drugs for the treatment of hyperthyroidism caused by Graves' disease. Endocrinol. Metab. Clin. North Am. 27: 225-247.

R, Kumar V, Robins S (1989). Robin's pathological basis of disease. 4th ed. W.B Saunders Co. Harcourt. pp 234-245.

Ferster A, Vermyelen C, Cornu G, Burse M, Corazza F, Devalck C, Fondu P, Toppet M, Sariban E (1996). Hydroxyurea for the treatment of severe sickle cell anaemia: a paediatric clinical trial. Blood 88: 1960-1964.

Fiske CH, Subbarow Y (1925). Colorimetric determination of phosphorus J. Biol. Chem. 66: 375-378.

Gabay C, Kushner J (1999). Acute-phase proteins and other systemic response to inflammation. N. Engl. J. Med. 340: 448-455.

Gerhardsson L, Glover JR, Nordberg GF, Vouk V (1986). Tellurium. In: Handbook of the toxicology of metals. (Friberg L, Nordberg GF, Vouk VB eds) 2nd ed. Amsterdam: Elsevier Science Publishers. pp. 532-548.

Gornall AC, Bardwawill CJ, David MM (1949). Determination of serum protein by means of the biuret reaction. J. Biol. Chem. 177: 751-756.

Guyton AC, Hall JE (2000). A textbook of Medical Physiology 10th ed. W.B. Saunders Co. Philadelphia. pp. 382-401.

Haywood LJ (1987). Thiocyanate in sickle cell anaemia. J. Natl. Med. Assoc. 79(10): 1032-1037.

Kurantsin-Mills J, Klug RK, Lessin LS (1988). Irreversible erythrocyte volume expansion induced by tellurite. Br. J. Haem. 70: 369-374.

Lima CS, Arruda VR, Costa FF, Saad ST (1997). Minimal doses of hydroxyurea for sickle cell disease. Braz. J. Med. Biol. Res. 30: 933-940.

Macfarlane I, Bomford A, Sherwood RA (2000). Liver diseases and Laboratory Medicine. ACB Ventures Publications London. pp. 67-72.

Malomo SO, Daramola AS, Balogun EA (1995). Some serum and tissue enzymes changes in mice infected with plasmodium yoelii nigeriensis before and after administration of halofantrine hydrochloride. Nig. J. Biochem. Mol. Biol. 10: 71-77.

Meditext (1997). In: Tomes Plus Environmental Health and Safety Series 1 (32) Colorado Micromedex Inc. pp. 87-95.

Navarra P, Preziosi P (1999). Hydroxyurea: New insight on an old drug. Crit. Rev. Oncol. Haem. 29: 249-255.

Redding-Lallinger R (2006). Questions in the management of sickle cell. J. Paed. 149: 595-597.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloaceytate aminotransferase. Am. J. Clin. Pathol. 28: 56-63.

Richardson L (1991). Haematological analysis of sickle cell patients in a clinical trial with potassium thiocyanate (unpublished paper presented at the 1991 Sickle cell disease Association of America, SCDAA).

Rosa RM, Bierer BE, Thomas R (1980). A study of induced hyponatremia in the prevention and treatment of sickle cell crisis. N. Engl. J. Med. 303: 1135-1143.

Serjeant GR (1992). Sickle cell disease 2nd ed. Oxford University Press. pp. 521-532.

Steinberg MH, Barton F, Castro O (2003). Effect of hydroxyurea on mortality and morbidity in adult sickle cell anaemia: risks and benefits up to 9 years of treatment. JAMA. 289: 1645-1651.

Tietz NW, Pruden EL, Siggaard-Andersen O (1994). In: Tietz textbook of Clinical Chemistry (Burtis CA and Ashwell ER eds.) WB Saunders Company London. pp. 1354-1374.

Veniamin MP, Vakirtzi-Lemonia C (1970). Chemical bases of the carbamidodiacetyl micro method for estimation of urea, citruline and carbamyl derivatives. Clin. Chem. 16: 3-6.

Vichinsky EP (2002). New therapies in sickle cell disease. Lancet 360 (9333): 629-631.

Wright PJ, Plummer DT, Leathwood PT (1972). Enzyme in rat urine. Alkaline phosphatase. Enzymologia. 42: 317-327.