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A COMPARATIVE RESERARCH ON THE IN VITRO ANTI SICKLING POTENTIAL IN VARIOUS ANTISICKLING DRUGS

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Abstract:

Objective: *Tellurite, thiocyanate and hydroxyurea are those chemical agents renowned to be used as antisickling drugs. Antisickling characteristics of these agents were comparatively investigated and studied in this research.****Methodology:*** *Collection of sickle blood was carried out from the patients attending Allied Hospital, Faisalabad (Haematology Department) in the time period of September, 2016 to October, 2017. Incubation of human sickle blood with drugs concentrations of both in vitro and in vivo was carried out. By employing standardized methods, particular features of sickling process and function of haemoglobin were measured.* ***Results:*** *Three drugs considerably manifested inhibition of sickling of deoxygenated sickle blood. Thiocyanate inhibited sickling maximum at (20 mM); whereas, hydroxyurea inhibited the same at 40mM. Tellurite inhibited the sickling maximum at (50 µM). Hydroxyurea and Thiocyanate prolonged sickle RBC life duration since it was suggested in the considerable reduction in osmotic fragility and haemolysis. However, these blood parameters were enhanced by tellurite. Prolongation of delay time of haemoglobin S (HbS) polymerization was caused by these three drugs. Oxygen affinity and solubility ratio of HbS was considerably enhanced by hydroxyurea and thiocyanate.****Conclusion:*** *Results from this study indicated that all these drugs posses tremendous antisickling potential in vitro. The most effective antisickling agent was thiocyanate. Tellurite stands at second position.*

Keywords: *RBC (red blood cells), Hydroxyurea, Potassium tellurite, Sickle blood, Anti sickling properties, Sodium thiocyanate.*

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INTRODUCTION:

The presence of anomalous haemoglobin in the red blood cell is a potential cause of sickle cell disease. This genetic blood disorder of haemoglobin is rampant in the Middle East and Africa [1]. The difference between abnormal haemoglobin S (HbS) and normal haemoglobin A (HbA) lies in the 6th amino acid of beta chain in which glutamic acid is replaced by valine. Such replacement places a non-polar residue is placed on the outside of HbS by such replacement. This leads to reduced solubility thus paving the way to polymer and sickling composition at low oxygen tensions [2]. Other factors responsible to sickle cell disease are functional anomalies of sickle RBC consist of decreased oxygen affinity and solubility, composition of irreversibly sickled cells (ISC), methaemoglobin formation, enhanced osmotic fragility and autohaemolysis.

Various earlier examined anti sickling agents created haemolysis of sickle RBC on efficient levels of doses. Therefore, they were clinically inappropriate [3]. Tellurite, thiocyanate and hydroxyurea are those chemical agents renowned to be used as antisickling drugs. Antisickling characteristics of these agents are comprehensively investigated in current times [4]. This study is undertaken to evaluate the antisickling actions of these three agents and further to monitor their impacts on RBC limitations to comprehend the action mechanisms.

MATERIALS AND METHODS:

Collection of sickle blood was carried out from the patients attending Allied Hospital, Faisalabad (Haematology Department) in the time period of September, 2016 to October, 2017. Consent of the patients was obtained. Ethical Committee of the concerned institution approved the study. Hopkins and Williams Ltd. (UK) was the source of potassium tellurite and sodium thiocyanate salts. British Drug House (Chemicals) Ltd. (UK) was the source of Hydroxyurea product.

Microscopic method which has been explained by Iyamu et al, was the procedure utilised [5]. Sample of blood (0.1mill litres) was pipette in Hemox buffer(1.0mililites) containing 5mM KCl, 30mM Tris, (135 mM) Sodium Chloride and pH 7.4. Addition of 0.1mililitre 45mM sodium dithionite and 0.1mililitre sample of drug ensured the deoxygenation. Temperature of 37 degree centigrade was maintained for 01 hours for incubation of mixture. Thereafter, collection of samples was carried out. It was instantly fixed with buffered saline containing 20mM NaH_2PO_4 and 130mM Sodium Chloride having two percent glutaraldehyde. Its mounting was executed on phase contrast microscope. Two observers checked it thoroughly in order to determine hundred cells count for normal and sickle cells. Those cells which were having wrinkle, star or elongated shape were considered abnormal cells. However, cells with the shape of a biconcave disc were regarded as normal cells. In order to make ISC count, for at least thirty minutes, the samples had been exposed to air prior to fixing in buffered saline.
(Sickle cell % = No of sickle cells multiplied by one hundred total number of cells counted)

Wolf procedure was adopted to measure osmotic fragility and auto haemolysis of sickle RBC [6]. For measuring HbS relative solubility ratio, the method of Chang et al [7] was used. Method of Schechter et al [8] was used for to measure delay time of HbS polymerization. In order to determine the percent of methaemoglobin and oxyhaemoglobin, method of Benesch et al [9] was used.

By following one-way analysis of variance (ANOVA), analysis of data was carried out by Duncan multiple range test. SPSS computer software package was used in analysing the data. Dissimilarities at P less than (0.05) regarded considerable.

RESULTS:

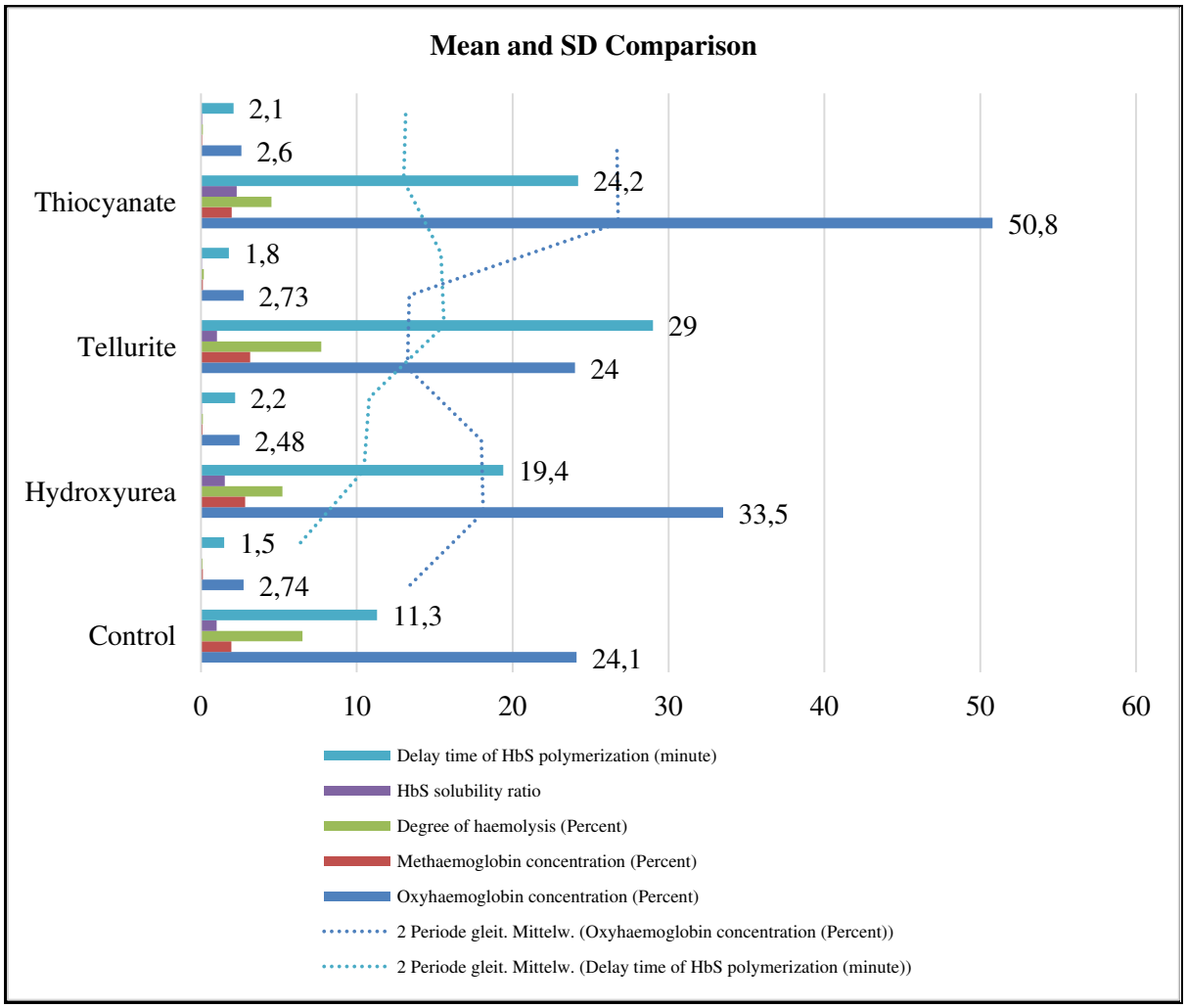
The best sickling inhibitory concentration for thiocyanate was 20mM whereas 40mM was in the case of hydroxyurea. 50µM was optimum sickling inhibitory concentration for tellurite. For the time period of 01 hour, these were the least concentrations to acquire optimum inhibition of sickling. Time based sickling inhibition of these 03 agents is demonstrated via Figure-One. After the time period of 02 hours, minimum sickle cells i.e. 38% was noted in blood incubated thiocyanate. Number of sickle cells came from tellurite and hydroxyurea were 50% and 63% respectively. After the incubatory time, 93% sickle blood was observed in untreated sample. As far as inhibitory impact of the antisickling agents on ISC formation is concerned after incubation time of two and half hour (As in Figure-two), ISC percentage found to be decreased from twenty eight percent in untreated blood to 19.50% in hydroxyurea treated blood, 16% in tellurite treated blood and 14.1% in thiocyanate treated blood.

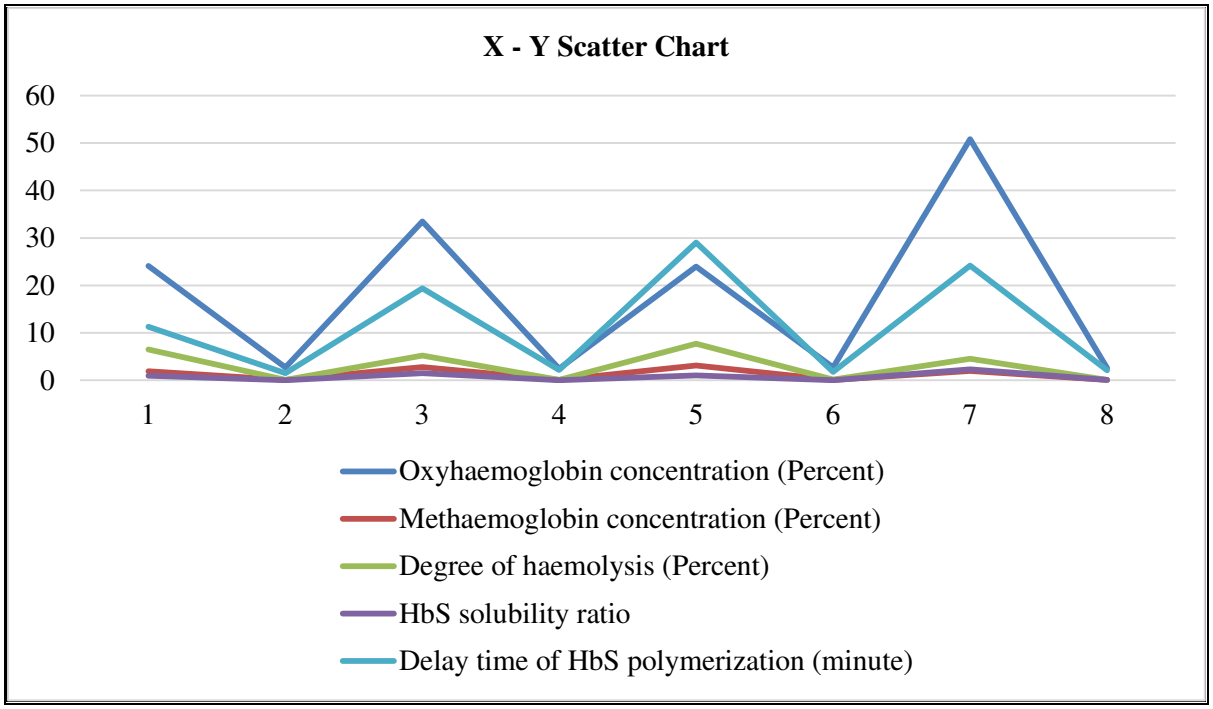
(As demonstrated in Figure-three) in the presence of tellurite, osmotic fragility curve of sickle blood red cell was tilted to left hand side. However, it tilted to right hand side in case of hydroxyurea and thiocyanate. Table one has shown the impact of drugs on various RBC indices. 18.60% red cell haemolysis increase was noted on case of Tellurite but it was decreased 19.6% and 30.7% in case of hydroxyurea and thiocyanate respectively. When

contrasted with the control, (P less than 00.05%) oxyhaemoglobin was increased by 39.0% and 111% in case of hydroxyurea and thiocyanate respectively. It is pertinent to mention here that oxyhaemoglobin content was remained uninfluenced by tellurite treatment. Additionally, hydroxyurea and tellurite considerably provoked (P less than 00.05) production of methaemoglobin by 45% and 61.50 respectively contrasted with the control. Thiocyanate increased (P less than 0.05) HbS solubility by 130% and 54% in case of hydroxyurea contrasted with control. However, HbS solubility was remained unchanged with tellurite. These three drugs prolonged the delay time HbS polymerisation i.e. 71.7% with hydroxyurea, 156% with tellurite and 114%, thiocyanate) contrasted with control as shown in the given table.

Table: Some Red Blood Cell Indices of Human Sickle Blood Incubated with Hydroxyurea, Tellurite and Thiocyanate

Indices	Control		Hydroxyurea		Tellurite		Thiocyanate	
	Mean	(±) SD	Mean	(±) SD	Mean	(±) SD	Mean	(±) SD
Oxyhemoglobin concentration (Percent)	24.1	2.74	33.5	2.48	24	2.73	50.8	2.6
Methemoglobin concentration (Percent)	1.95	0.12	2.83	0.1	3.15	0.13	1.97	0.09
Degree of hemolysis (Percent)	6.52	0.1	5.24	0.13	7.73	0.18	4.52	0.12
HbS solubility ratio	1	0.035	1.54	0.058	1.02	0.061	2.3	0.095
Delay time of HbS polymerization (minute)	11.3	1.5	19.4	2.2	29	1.8	24.2	2.1





DISCUSSION:

After incubation with the drugs, there was an improvement in the abnormal shape of sickle erythrocyte. Inhibition related to sickling was observed to be time and dose based. When observed at the end of incubatory period, it was Thiocyanate that surpassed other drugs in its effectiveness in vitro as minimum number of sickle cells were seen when blood incubation was done with Thiocyanate. The direct covalent modification effect on haemoglobin can be a likely reason behind this. Reaction of amino terminal valine residue of haemoglobin and thiocyanate takes place which paves the way to carbamylation [10]. After carbamylation of haemoglobin S, its "R" form is ensured that is unaffected by sickling.

Tellurite mode of action was indicated to have the capacity to create tremendous RBC swelling thus paving the way to cell hydration [11]. On the other hand, hydroxyurea was indicated in vitro to react with haemoglobin to formulate nitrosyl haemoglobin, a change that may inhibit RBC sickling [12]. Existence of irreversibly sickled cells is believed to be due to conditions e.g. dehydration in some proportion of the red blood cell, permanent membrane damage, ATP depletion, calcium ion accumulation and repeated sickling-unsickling [13].

Autohaemolysis and Osmotic Fragility:

With the tellurite, level of osmotic fragility and haemolysis of sickle RBC was enhanced. However, the same parameters were decreased in case of hydroxyurea and thiocyanate. According to these findings, Tellurite causes RBC to become more fragile and thus susceptible to stimulate haemolytic anaemia in sickle cell patients. Life span of red blood cell was increased in the presence of hydroxyurea and thiocyanate. Researches in vitro have exhibited that erythrocyte membrane can be penetrated by tellurite (Te4+) ions, thus in the company of decreased glutathione, formulate telluride (Te2+), hence leading towards haemolysis and membrane damage [11]. Greatest effect of inhibition on osmotic fragility and auto haemolysis was found with Thiocyanate as it carbamylates haemoglobin, a change which can enhance the life span of RBC [14].

Oxyhaemoglobin Content and HbS Solubility:

When sickle blood patients were treated with hydroxyurea and thiocyanate, a considerable increase in HbS solubility and oxyhaemoglobin is a valid proof that direct connection exists between haemoglobin and these two drugs. When deoxygenation of haemoglobin is carried out, polymerisation and sickling of HbS are advisable. Rodgers has pointed out a solution to the problems of sickle cell patient i.e. the avoidance of occurrence of sickling and polymerisation by increasing oxygen affinity of the haemoglobin to such an extent that the proportion of deoxy HbS at tissue-oxygen tension is very low [15]. Compounds which change haemoglobin and enhance solubility and oxygen affinity would assist in decreasing the extent of sickling [16].

Methaemoglobin Formation:

The stimulation of methaemoglobin production in sickle blood which is created due to hydroxyurea and tellurite suggested a non-productive impact of

these drugs for the patients of sickle cell. It may be due to characterization of sickle blood which is noted by greater production of MetHb thus is believed to decrease the life span of sickle RBC and may cause health risks [19, 20].

Delay Time of HbS Polymerization:
Three drugs which created considerable delay time of HbS polymerization have suggested their utility as anti-sickling agents. To assess the utility of a proposed anti-sickling agent, the most effective tool suggested was calculation of delay time.

CONCLUSION:

By concluding, it has been observed that these three drugs are antisickling agents. They have shown a tremendous capacity to stop sickling of deoxygenated sickle RBC in vitro. In vitro, hydroxyurea and thiocyanate were found to have enhanced life span of red cells. On the other hand, RBC osmotic fragility and stimulation of haemolytic anaemia in sickle cell patients were seen with tellurite. Interaction of hydroxyurea and thiocyanate with sickle RBC is direct since it was suggested by greater solubility and oxygen affinity of sickle RBC in case of these two drugs. Efficiency of thiocyanate is unequivocal amongst all three drugs. Tellurite stands at second position in terms of its efficiency.

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