Analysis of hematological and biochemical parameters, to study the ecological relationship on selected species of vertebrates

Chuku LC and Uwakwe AA*

Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria.
uwadik@yahoo.com*

Abstract

Objectives: Hematological and biochemical analysis studies of ruminant vertebrates (cow, sheep and goat) will provide us an acceptable method for understanding the ecological relationship among the vertebrates.

Methods: Haematological/Biochemical studies were carried out on blood from the ruminants: Goat (*Capra hircus-L*), sheep (*Ovis Aries dolrchra*), cattle (*Bos Taurus*) and human (*Homosapiens*) as control.

Results: Electrophoretically, goat blood revealed three different Hb polymorphic types: HbAA (45%), HbAS (50%) and HbSS (5%). Only the 'O' Rh blood group was observed in all cases, and GST values of 4.05-6.30 I.U were obtained on the other hand. Cow Hb electrophoresis produced two patterns corresponding to the human HbAA and HbAS types. Only the 'O' Rh^- blood group was obtained in all cases and GST values of 2.85-5.02 I.U were obtained, while sheep blood produced only the HbAA-type, only the 'O' blood group was identified in all case. GST activity values were in the range of 3.05-4.52 IU.

Conclusion: These results could point to a definite haematological and biochemical similarity between man and ruminants and thus serves as a springboard for further genetic studies using lower vertebrate's as research models.

Keywords: Haemoglobin, glutathione-S-transferase, genotype, erythrocyte.

1. Introduction

Ruminants belong to the subphylum of mammalian.The characteristics feature of ruminants (as in other vertebrates) are the possession of vertebral column, or backbone, and a cranium which protects the central nervous system (brain and spinal cord) (Lawrence, 2000). And other major sense organs; the presence of bone and a neural crest of nerve cells that remain after the formation of the central nervous system.

Other distinctive features are the kidneys, with the nephron as the functional unit; a heart, red and white blood cells; a liver and a pancreas; specialized sense organs such as a complex eye, a lateral-line system, ears and a sense of smell; several unique endocrine organs, such as the pituitary and thyroid; and epidermis and dermis. The neural crest gives rise to widely diverse features including neurological cells, Schwann cells covering peripheral nerves etc. (Walter, 1997).

Blood is a fluid in which are suspended solid elements known as the blood cells or corpuscles. While 55% of blood is plasma (yellowish fluid), about 45% of the total blood volume consists of cells which are of three kinds: White blood cells (leucocytes), platelets (or thrombocytes) and the red blood cells (erythrocytes).

The plasma is very complex in composition. In it are dissolved or suspended a large variety of proteins, lipoproteins, nutrients, metabolites, waste products, inorganic ions and hormones. Erythrocytes are small biconcave discs. It is thinner in the middle then at the edges-somewhat like a ring doughnut without the hole. A mature erythrocyte has no nucleus, mitochondria, endoplasmic reticulum or other organelles.

The chief function of the red cells is respiratory – carrying oxygen from the lungs to the capillaries of the various tissues and to remove carbon dioxide from the tissue to the lungs. The ability to carry oxygen is dependent upon its content of a pigment called haemoglobin. It is this pigment that give blood its characteristic colour (Lehninger, 1982).

The leucocytes unlike the red cells posses a nucleus in all species but no hemoglobin or other coloring matter. The leucocytes fight against harmful substances that invade the body. Most of the cells are found colourless, they are of different sizes and their nuclei differ in shape. Some types of white blood cells kill bacteria by phagocytosis and digesting them.Other kinds produce antibody proteins that bind to bacteria, viruses, and other invaders in a bid to making them harmless. The blood platelets (Thrombocytes) are small bodies about a quarter of the diameter of a red blood cell. They do not possess any nucleus but their protoplasm contains distinct granules. The thrombocytes play an important role in blood coagulation (Best & Taylor, 1982).

This study investigated some aspects of the biochemistry and hematology of some ruminants (sheep, goat and cow) with a view

to obtaining results and information relevant to the provision of experimental models for the study of human genetic disorders as were the vertebrate evolutionary tree. The various aspects studied includes: Hb genotyping, Blood grouping and erythrocyte Glutathione-S-transferase assay.

2. Methods

2.1 Collection of blood and preparation of lysates

The blood of cow, sheep and goat were collected into potassium EDTA bottles. Red blood cells were separated from whole uncoagulated blood by addition of equal volume of saline (9.0g% of NaCl) and allowed to stand for 30 minutes. The cells were finally lysed by addition of two volumes of deionized water, exposing the haemoglobin.

2.2 Blood grouping

The ABO and Rhesus blood grouping system are based on agglutination formation and the procedure was indicated in the kit for human blood grouping (Sigma product).

2.3 Enzyme assay of Glutathione-S-Transferase

The reaction of 1-chloro 3,4-dinitrobenzene (CDNB) with the thiol group of glutathione is catalyzed by Glutathione-S-transferase. The CDNB- glutathione conjugate absorbs light at 340nm and the activity of the enzyme is therefore estimated by measuring the changes in optical density (O.D.) at this wavelength as described by Anosike *et al*.(1991).

2.4 Procedure for haemoglobin electrophoresis

Haemoglobin genotypes were determined as described by Anosike *et al*.(1991). With the Tris-EDTA-borate buffer of pH 8.9 already in the electrophoretic tank, the soaked cellulose acetate strips in tris-buffer was blotted dry. With the aid of an applicator, the prepared goat, cow and sheep haemolysate were applied on the cellulose acetate paper, using human HbAS as control.

The cellulose acetate paper containing the samples was then placed on the electrophoretic tank. A voltage of about 220volts was applied and allowed to run for about 2hrs. The power supply was turned off and the cellulose acetate strip was removed from the electrophoretic tank, stained for at least 10mins with the Ponceau –S-stain. The cellulose acetate strip was then removed and washed in three successive dishes of 5% acetic acid for 2mins. Haemoglobin bands were identified by comparison with known human standards on same cellulose acetate strip. The rate of migration for haemoglobins observed where in the order HbA>HbF>HbS>HbC. HbAA was identified as a double band or band running from HbA to HbS respectively.

3. Results

3.1 Haematological and biochemical analysis goat

3.1.1 Blood Group

Only blood group 'O' RhD^- was observed in all cases (table. 1).

Table 1. *Blood groups and rhesus factors in goat*

S.No	Blood group	Rhesus factors
Gi	'O'	RhD-
Gii	'O'	RhD-
Giii	'O'	RhD-
Giv	'O'	RhD-
Gv	'O'	RhD-
Gvi	'O'	RhD-
Gvii	'O'	RhD-
Gviii	'O'	RhD-
Gix	'O'	RhD-
Gx	'O'	RhD-

Table 2. *Genotype patterns and GST activity of goat blood*

S.No	Genotypes	GST (IU)
Gi	AA	4.90
Gii	AS	5.72
Giii	AS	5.34
Giv	AA	4.05
Gv	AA	6.30
Gvi	AS	5.22
Gvii	SS	6.65
Gviii	AA	5.76
Gix	AS	6.02
Gx	AS	5.56

3.1.2 Hb Electrophoresis

Hb electrophoresis of goat blood revealed almost equal (%) of HbAA – type (40%), HbAS-types (50%) and only 10% as HbSS-type (table. 2).

3.1.3 GST Activity

The GST activity of goat blood ranges from 4.05 - 6.65 I.U.

3.2 Haematological and biochemical analysis cow

3.2.1 Blood Groups

Only blood group "O" and Rh D^- was identified in all cases (table .3).

Table 3. *Blood groups in cow*

S.No	Blood group	Rhesus factors
Ci	'O'	RhD-
Cii	'O'	RhD-
Ciii	'O'	RhD-
Civ	'O'	RhD-
Cv	'O'	RhD-
Cvi	'O'	RhD-
Cvii	'O'	RhD-
Cviii	'O'	RhD-
Cix	'O'	RhD-
Cx	'O'	RhD-

3.2.2 Hb Electrophoresis

Hb electrophoresis of cow blood revealed the presence of HbAA-type and HbAS-type. No HbSS-type was identified (table .4).

Table 4. *Genotype patterns and GST activity of cow blood*

S.No	Genotypes	GST activity (IU)
Ci	AA	3.24
Cii	AA	3.33
Ciii	AA	3.54
Civ	AA	2.85
Cv	AS	4.86
Cvi	AS	4.95
Cvii	AA	3.02
Cviii	AA	2.97
Cix	AS	5.02
Cx	AA	2.91

3.2.3 GST Activity

The GST activity of cow blood ranged from 2.85 - 5.02 I.U

3.3 Haematological and biochemical analysis sheep

3.3.1 Blood group

All sheep blood samples tested had 'O' blood group and Rhesus factor D negative (table .5).

3.3.2 Hb electrophoresis

Electrophoresis of sheep blood revealed the presence of only the HbAA-type (table .6).

3.3.3 GST activity

The GST activity of sheep blood ranged from 3.05 - 4.52 I.U (table .6)

Table 5. *Blood groups in sheep*

S.No	Blood group	Rhesus facors
Si	'O'	RhD-
Sii	'O'	RhD-
Siii	'O'	RhD-
Siv	'O'	RhD-
Sv	'O'	RhD-
Svi	'O'	RhD-
Svii	'O'	RhD-
Sviii	'O'	RhD-
Six	'O'	RhD-
Sx	'O'	RhD-

Table 6. Genotype pattern and GST activity of sheep blood

S.No	Genotype	GST activity (IU)
Si	AA	3.99
Sii	AA	4.09
Siii	AA	3.15
Siv	AA	4.14
Sv	AA	3.78
Svi	AA	3.05
Svii	AA	3.76
Sviii	AA	4.52
Six	AA	4.32
Sx	AA	3.06

4. Discussion and conclusion

The phylogenic relationship in vertebrate animals has long been recognized (Lawrence, 2000). However, systematic haematological and biochemical studies geared towards a better understanding of the gains that underlie the ecological, biochemical and physiological relationship between lower and higher vertebrate animals remain rather unco-ordinated. For instance, one common function of blood is oxygen mobilization based on the common structure of the haemoglobin molecule, (Walter, 1997).

It would therefore, be a challenging prospect to tailor the blood of other vertebrates like these ruminants, to further serve man. If it were possible to discover the presence of certain genes with phenotypical expressions of interest in vertebrate it would then be envisaged that research models would be established using lower vertebrates, thereby minimizing moral and ethical problems. The study of sickle cell haemoglobinopathy is a good case in point, as some vertebrates have exhibited similar characteristics to human haemoglobin polymorphs.

The result of haemoglobin electrophoresis experiments carried out in this work, for instance, have demonstrated the presence of human heamoglobin A- type haemoglobins in goat, sheep and cow. Human haemoglobin AS- types were identified in goats and cow, it would be wrong however, to run into a quick conclusion at this stage about the exact similarity of these non-human haemoglobin variant to those of human. Such definite conclusions will only be feasible when other biochemical techniques such as the Globin sequencing are done.

Nevertheless, migration patterns of these haemoglobins are very revealing and obviously point to similarities in the charge properties of the haemoglobin polypeptides of man and these ruminants. The haemoglobin electrophoresis of the blood of goat, cow and sheep show some similarity to human haemoglobin of HbAA-type erythrocytes, and all has GST activity below 6.70 IU (table

7.2, 7.4 and 7.6) which compared favourably with a reported value of less thn 4.0 IU reported for human with HbAA erythrocytes (Carmagnole *et al*, 1981).

These results are further confirmation of the similarity in the human and non-human vertebrate established haemoglobin polymorphs. In an effort to further establish similarities in the blood tissues of vertebrates, the blood groups of these ruminants were determined using human antisera standards. The presence of only blood group 'O' whose significance is unclear was observed.

Also Rhesus factor (RhD^-) negative was observed in all. These results also point to obvious similarities in the erythrocyte antigenic determinants of the ruminants and those of humans. Following the results obtained, it is therefore pertinent to conclude that a relationship between GST activity and haemoglobin genotype in ruminants as is the case in humans was determined (Anosike *et al*, 1991). Also determined were the similarities in blood grouping of the ruminants and humans and the presence of human haemoglobin –type polymorphic genes in ruminants.

It is hoped that these findings will be used as a springboard towards the provision of experimental models for the study of human genetic disorder and also as a springboard for the better understanding of the vertebrate phylogeny.

5. References

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