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Serum Prohepcidin and Iron Metabolism in Chloramphenicol-Induced Anaemia in Rats

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Abstract
Prohepcidin is a precursor of hepcidin, a peptide hormone synthesized in the liver and considered to be the master regulator of iron metabolism. Chloramphenicol, a bacteriostatic antimicrobial, has a broad spectrum of activity and known to cause reversible bone marrow suppression, aplastic anaemia amongst others. The study is aimed at investigating the regulatory mechanism of hepcidin in anaemia induced by chloramphenicol in wistar rats. The intervention of Telfairia occidentalis on anaemia was also undertaken. Serum prohepcidin was measured by an enzyme linked immunosorbent assay (ELISA) technique. Serum iron, ferritin, total iron binding capacity (TIBC) and haematological parameters were determined using standard methods. Results show that levels of serum prohepcidin, iron, ferritin, haemoglobin and haematocrit were significantly decreased (P <0.05) in chloramphenicol administered rats. Conversely, the total iron binding capacity was significantly increased (P<0.05) by chloramphenicol treatment when compared to control. Treatment with Telfairia occidentalis greatly enhanced the haemoglobin and haematocrit parameters, as well as the iron metabolism indicators. Humoral immunity of the rat models also received a boost with T. occidentalis ingestion. The implication of decreased levels of prohepcidin in iron deficiency anaemia by chloramphenicol and the role of T. occidentalis is discussed.

Keywords: Pro-hepcidin, Chloramphenicol, *Telfairia occidentalis,* Iron, Anaemia.

Introduction
Iron, a dietary requirement for human and other organisms, is an essential trace element. It is a key component of many cellular enzymes and plays vital roles in cellular processes[1]. In the blood, it is transported by the transport protein transferrin and stored as ferritin. The blood iron level and transferrin saturation are usual indicators of the body iron status[2]. Iron metabolism disorders could be in the form of iron deficiency or iron overload and include; anaemia, organ degenerative diseases such as liver, spleen and kidney, and enzyme dysfunctions[3]. Although iron excretion is important in maintaining iron balance, the absorption process in the proximal small intestine (duodenum) plays a more active role in regulating iron homeostasis[4].
Hepcidin, a small peptide hormone produced by the liver, has provided insight into the complexity of mammalian iron metabolism. It is said to be the master regulator of iron homeostasis in humans and other animals[5, 6]. The human hepcidin gene (locus 19q13) encodes a pre-propeptide of 84 amino acids. This compound is cleaved to give prohepcidin, a 60 amino acid molecule that is further cleaved to give hepcidin, a 25-amino acid form[7]. Hepcidin regulates (by inhibiting) iron transport across the gut mucosa and thus prevents excess dietary iron absorption. This action helps to maintain normal iron levels within the body. It also inhibits release of iron from macrophages that recycle used iron from senescent red cells. Hepcidin functions by controlling ferroportin activities, a transmembrane iron exporter which transports iron out of cells. It attaches to ferroportin and causes its internalization and destruction in the lysosome[8]. Iron overload and inflammation markedly influence hepcidin expression[6]. On the other hand, hepcidin expression is down regulated in response to anaemia and hypoxia[9]. Thus, hepcidin levels vary according to the body's need for iron.
The broad spectrum antibiotic, chloramphenicol, a bacteriostatic antimicrobial was first introduced into clinical practice in 1949 as chloramycetin[10]. However, chloramphenicol is no longer a first-line drug for any indication in developed countries because of its resistance and safety concerns. Use of chloramphenicol flourishes and remains a drug of abuse in developing countries such as Nigeria because it is inexpensive and readily available. Chloramphenicol causes serious bone marrow toxicity such as: bone marrow suppression; a direct effect of the drug which causes a reversible dose-related interference with iron metabolism. Chloramphenicol also causes mitochondrial stress and decreased ATP biosynthesis and accelerates cancer progression[11]. There is paucity of information on the effect of chloramphenicol on serum hepcidin level. Haematological biomarkers are useful for investigating iron bioavailability, and *T. occidentalis*[12,13] would be used to investigate iron repletion on chloramphenicol induced iron deficiency anaemia in rat models. Iron metabolism indices such as prohepcidin,

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iron and ferritin would be assessed with and without *T. occidentalis*. The haematinic effect of *T. occidentalis* would be compared against ferric hydroxide.

Materials and Methods

Experimental Animals and Treatment

Male Wister rats were procured and housed in the animal house of the Anatomy Department, School of Basic Medical Sciences, University of Benin, Benin City. Animals were allowed to acclimatize for two weeks before experimentation.

Sixty (60) rats, 12-13 weeks old, weighing between 140-160g and divided into two groups, were used. Group one was the control group comprising thirty (30) healthy Wister rats. These were sub-divided into 3 groups of ten (10) rats per sub group. The first sub group (n=10) was given normal rat food and water freely and no other treatment. The second sub-group (n=10) was given *Telfairia occidentalis* leaf extract (Ugu) (50mg/ml) for 21 days in addition to food and water. The third sub-group (n=10) was given ferric hydroxide (trade name Orofer) (50mg/5ml) for 21 days, as well as normal feeding with food and water. Ferric hydroxide (Orofer) was bought at a reputable Pharmacy in Benin City. Treatments were administered through orogastric tube.

The second group of rats (n=30) were administered Chloramphenicol (Clofencol) through orogastric feeding for 21 days at a dose of 86mg/kg body weight as previously described by Ebaid et al[14]. Chloramphenicol, a prescription drug, was bought at a reputable Pharmacy in Benin City. The three (3) sub-groups of group two (2) were as follows: Sub-group 1 (n=10): chloramphenicol only, sub-group 2 (n=10): chloramphenicol and *Telfairia occidentalis* (50mg/ml), and sub-group 3: chloramphenicol and ferric hydroxide (orofer) 50mg/5ml. All sub-groups were freely given normal food and water. The weights of the experimental animals were taken before and after treatments.

Methods

Haemoglobin, haematocrit and red blood cell count: The rats were killed at the indicated time by anaesthetizing with chloroform. Blood samples were collected by cardiac puncture, into EDTA containers and analyzed using ERMA INC hematology analyzer (Model PCE-210).

Blood for serum was collected into non-anticoagulant specimen containers, centrifuged and sera were preserved at -70°C.

Telfairia occidentalis Leaf Extract: The leaves of *Telfairia occidentalis*, bought from the local market were ground (800g/800ml distilled water). Filtration was done and the filtrate freeze-dried and stored.

Assay for Iron, Prohepcidin and Ferritin: The serum iron levels were determined using atomic absorption spectrophotometer (AAS) (SALAAR 969 Unicam series). Serum prohepcidin level was measured by commercially available ELISA kit (DRG Diagnostics, GmbH, Germany) and serum rat ferritin (Alpco Diagnostics, 26 Keewydin Drive Salem, NH03079).

Statistical Analysis: Experimental data were expressed as Mean±SEM. Statistical difference between groups was analyzed using ANOVA followed by Student-Newman-Keul post test. P-value < 0.05 was considered to be significant. Statistical analysis was performed using GraphPad InStat3 software.

Results

After treatment with chloramphenicol, the rats showed symptoms of haemolytic anaemia. The results indicated decreased haemoglobin and haematocrit levels. Chloramphenicol had significant effects on serum prohepcidin, iron and ferritin levels revealing a decrease in these iron metabolism parameters.

In Figure 1, serum prohepcidin levels of control rats were compared with chloramphenicol-treated. There was a significant decrease ($P<0.05$) in chloramphenicol-treated rats, but the decrease was not significant in chloramphenicol+Ugu-treated rats. Serum prohepcidin level was significantly increased in rats fed ferric hydroxide ($P<0.05$), but not for rats fed *T.occidentalis*, chloramphenicol+Ugu and chloramphenicol+Orofer. In Figure 2, Serum ferritin levels for control rats were compared against chloramphenicol-treated rat sub-groups. Serum ferritin levels were elevated for rats fed Ugu and Orofer and decreased in chloramphenicol-treated rats. but not for chloramphenicol+Ugu and chloramphenicol+Orofer-treated rats were unaltered. Serum iron level was significantly reduced ($P<0.05$) for chloramphenicol-treated rats. It was increased in all the other sub-groups. except for chloramphenicol+Ugu-treated sub-group (Figure 3). Figure 4 shows that TIBC level was significantly decreased in rats fed Ugu and Orofer while an increase in chloramphenicol-treated rats was observed.

Haemoglobin (Hgb) and haematocrit (HCT) values significantly decreased ($P<0.05$) in chloramphenicol-treated rats. However, co-treatment of chloramphenicol with Ugu and Orofer separately restored these levels Figures 5 & 6).

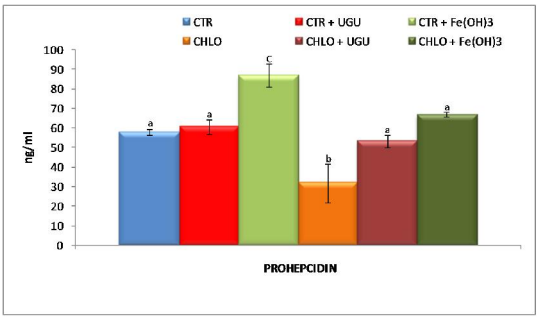

Figure 1: Serum level of Prohepcidin for Control rats, Controls+Ugu, Controls+Orofer, Chloramphenicol-treated rats, Chloramphenicol+Ugu, and Chloramphenicol+ Orofer-treated rats. Different superscript letters differ significantly from each other at P<0.05.

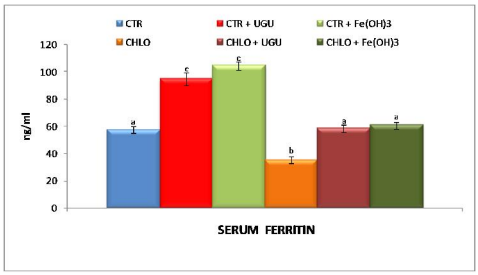

Figure 2: Serum Ferritin level for Control rats, Controls+Ugu, Controls+Orofer, Chloramphenicol-treated rats, Chloramphenicol+Ugu-treated and Chloramphenicol+ Orofer-treated rats respectively. Different superscript letters differ significantly from each other at P<0.05.

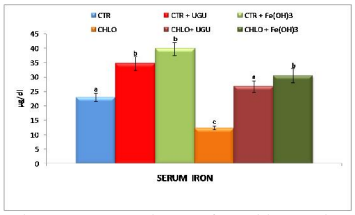

Figure 3.Serum iron level for Control rats, Controls+Ugu, Controls+Orofer, Chloramphenicol-treated rats, Chloramphenicol+Ugu-treated and Chloramphenicol+Orofer-treated rats respectively. Different superscript letters differ significantly from each other at P<0.05.

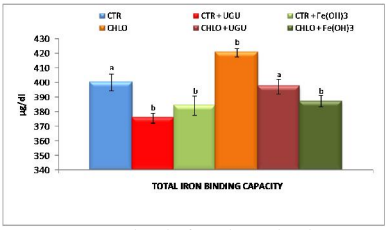

Figure 4.Serum level of total iron binding capacity (TIBC) for control rats, Controls+Ugu, Controls+Orofer, Chloramphenicol-treated rats, Chloramphenicol+Ugu-treated and Chloramphenicol+ orofer-treated rats respectively. Different superscript letters differ significantly from each other at P<0.05.

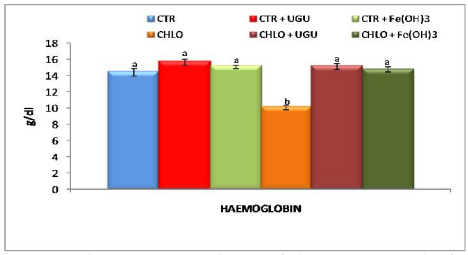

Figure 5. Haemoglobin (Hgb) level for Controls rats, Control rats fed Ugu, Controls fed Orofer, Chloramphenicol-treated rats Chloramphenicol+ Ugu-treated, and Chloramphenicol+ Orofer-treated rats respectively. Different superscript letters differ significantly from each other at P<0.05.

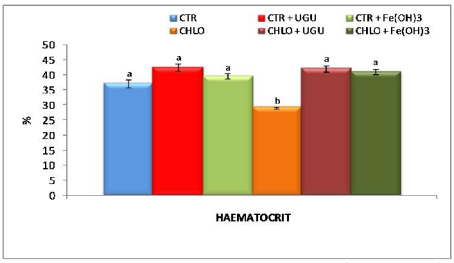

Figure 6: Haematocrit concentration for Control rats, Controls fed Ugu, Controls fed Orofer, Chloramphenicol-treated rats, Chloramphenicol+ Ugu-treated and Chloramphenicol+ Orofer-treated rats. Different superscript letters differ significantly from each other at P<0.05.

Discussion

This study is an approach to determine whether chloramphenicol has any side effect on the activity of iron regulator, serum hepcidin, serum ferritin and total iron binding capacity (TIBC) levels. It is also an attempt to find whether *Telfairia occidentalis* (ugu) can ameliorate the side effect of chloramphenicol. Serum prohepcidin was analyzed in this study. It is presently not practicable to analyze serum hepcidin in rat using enzyme linked immunosorbent assay (ELISA) technique. Literature has reported significant positive correlation between serum prohepcidin and hepcidin levels[15,16].

Chloramphenicol had significant effects on the parameters measured: serum prohepcidin level, serum iron, serum ferritin and TIBC levels. Results obtained revealed there were decreases in the measured values of serum prohepcidin level and serum iron levels. The findings corroborate the hypothesis that low iron concentration negatively regulates hepcidin expression[17]. Hepcidin regulates iron absorption into the blood stream by affecting the iron protein transporter, ferroportin. Binding of hepcidin to ferroportin causes its internalization and eventual lysosomal degradation of both proteins[18]. This prevents absorbed iron from entering the blood stream, but is stored in the ferritin. Hepcidin also inhibits release of iron from senescent red blood cells in the macrophages[19]. Iron which is an essential trace element for most life is also potentially toxic. The regulatory role of hepcidin thus, strives to maintain iron homeostasis.

Serum ferritin concentrations were decreased in the present study. Our findings tend to lead credence to the reports of Young *et al.*[20] which indicates that serum iron levels in mice depends on diet intake, while human subjects can obtain iron from iron stores in the body. Thus iron absorption in the rat models could be dependent on serum iron levels, rather than on iron turnover or storage, as in the case of humans. Decreased serum iron levels obtained in the present study would not encourage storage of iron as ferritin.

In the duodenal enterocytes, hepcidin regulates iron absorption by causing the reduction of divalent metal ion transporter-1 (DMT-1) expression[4]. This would lead to sequestration of cellular iron and reduction of serum iron level. The low serum iron levels observed in this study implied low iron transport in the blood stream. Consequently, total iron binding capacity would be unsaturated. Data from this study indicated high TIBC levels for chloramphenicol treated rats.

Administration of *Telfairia occidentalis* (ugu) improved the haematological indices of the rat models. The present report also indicated that *T. occidentalis* had both iron replenishing and protective effects against anaemia induced side effect of chloramphenicol on the rat models. Data from literature have attributed some haematological indices to the vegetable[12]. Both spleen and duodenum (small intestine) are important tissues (organs) involved in iron regulation[19]. This study has emphasized the notion that abuse of chloramphenicol may have negative implications in iron homeostasis. Hypothetically, chloramphenicol administration can impair hepcidin production via its effect on iron metabolism. We therefore, advocate strict regulation of prescription drugs such as chloramphenicol in view of health implications of doing otherwise.

In conclusion, the present study demonstrated that chloramphenicol ingestion could lead to decreased serum levels of prohepcidin, iron and ferritin. *Telfairia occidentalis* has been shown to be a good haematological biomarker for investigating iron bioavailability. Further studies are necessary to clarify the role of chloramphenicol on serum hepcidin with regard to iron deficiency anaemia.

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