Journal of Medical and Applied Biosciences
ISSN: 2277 - 0054
Volume 6, Number 1, 2014

Effects of Some Oral Hypoglycaemic Drugs on Erythrocyte Nicotinamide Adenine Dinucleotide Hydrogen Diaphorase (E.C.1.6.4.3) Activity of Wistar Alibino Rats (*Rattus rattus*)

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ABSTRACT

The in vivo effects of three oral hypoglycaemic drugs, daonil (a glubenclanude), diabenes (a sulphonyclurea) and glucophage (a metformun) on erythrocyte nicotinamide adenine dinocleotide hydrogen (NADH) activity of wistar albino rats *(Rattus rattus)* were monitored at drug concentrations of 0.00, 0.01, 0.02 and 0.03mg/200g body weight. The effects of the drugs were monitored for fourteen days at intervals of 1, 2, 6 and 14 day(s) following administration of each drug. Three rats were used per each drug concentration per time interval (days). NADH diaphorase activity was monitored at a pH 8.0 at 37°C. Daonil significantly ($P<0.05$) activated NADH-diaphorase activity in a concentration dependent manner with an optimal activation obtained at a concentration of 0.03mg/200g body weight and on the sixth day of drug administration. For instance, at drug concentration of 0.00, 0.01, 0.02 and 0.03mg/200g body weight and at 6^{th} day of administration; NADH diaphorase activity (iu/L) of 6.80 ± 0.65, 10.17 ± 0.69, 10.35 ± 0.97 and 11.44 ± 0.82 were obtained respectively. The increase in enzyme activity following drug administration was progressive with time duration (days).Maximum effect was obtained on the sixth day with a decline on the 14^{th} day. And 0.03mg/200g body weight, NADH activities (iu/L) of 6.70 ± 1.16, 14.74 ± 0.04, 19.50 ± 0.15 and 20.53 ± 0.57 were obtained on the sixth day. Comparatively, the activation of the erythrocyte enzyme by the drug (on the 6^{th} day of administration was in the order: Glucophage > Daonil: Diabenese had no significant effect. The implications of these findings to the functional integrity of erythrocytes are discussed in this work.

Keywords: Daonil, Diabenese, Glucophage, Erythrocyte, Nicotinamide Adeninedinucleotide Hydrogen, Hypoglycaemia.

INTRODUCTION

The red cell NADH dependent methaemoglobin reductase has also been referred to as NADH diaphorase or diaphorase I (Gibson, 1448; Breaking *et al* 1951), NADH

ferricynide reductase (Board, 1981) and NADH cytochrome – 65 reductase. This enzyme protects the erythrocyte from an accumulation of methaemoglobin. It does this by minimizing its rate of formation. Methaemoglobin is reduced to haemoglobin by NADH reductase. Methaemoglobin is haemoglobin in which the group (iron II) is oxidizide to iron III. As a result, it cannot function as an effective oxygen transporting protein. Small amounts of methaemoglobin are produced continually but the proportion of total haemoglobion that is present as methaemoglobin is maintained at about 1% by the action of an NADH dependent methaemoglobin reductase.

A methaemoglobin reductase concentration that is greater than 1% occurs if the rate of methaemoglobin formation exceeds its rate of reduction. Also methaemoglobin is not just incapable of binding oxygen, the oxidation of one or more of the heme iron atoms in the tetramer distort the tetramers structure. As a result, the remaining non-oxidized heme sub-units bind oxygen avidly and also release it less efficiently.

This shifts the oxygen dissociation curve to the left. Studies have shown that NADH methaemoglobin reductase reacts optimally over a broad pH range (pH 5.0 – pH 8.5). The deficiency of NADH methaemoglobin reductase is genetically transmitted as an autosomal recessive characteristic chromosome (Jaffe, 1959). Also biochemical variants of this enzyme have been reported (West *et al*, 1967).

NADH was speculated to be supplied by glyceraldehydes-3-phosphate dehydrogenase of the glycolytic pathway. However, it has been demonstrated in vitro that lactic dehydrogenase can also supply NADH when an excess of lactate is added to reverse the normal direction of the reaction. It was also suggested that NADH is only 1.5% as active as NADPH as an electron donor for the reduction of methaemoglobin by the purified diaphorase I. This erythrocyte diaphorase is lacking in the red cells of patients with high methaemoglobin level. The answer is NADH methemoglobin reductase which is also called NADH diaphorase II. (Neuwrit *et al,* 1977).

Oral hypoglycaemic drugs are pills or capsules which help in reducing the level of glucose in the blood (Kaln, 1993). The drugs are used only in the treatment of type II (non-insulin dependent) diabetes mellitus; a disorder involving resistance to secreted insulin.

There are two major classes of hypoglycaemic agents and they are;

- The sulphorylureas (including Arylsulforylureas) and
- The biguanides.

The sulphorylureas diminish hepatic glucose production, gluconeogenesis from alanine as well as delayed insulin release in response to glucose observed in patients with type II diabetes mellitus (Kaln, 1993). A number of sulphorylureas are available for the treatment of type II diabetes mellitus and they include; Glyburide (a mucronase), Glipizide (Glucotrol), Tolazamide (tolinase), Tolbutamide (orinase), Acotohexide (dymelor) and Chloropropamide (diabenase). These drugs vary in their mode of excretion and duration of action. Chloropropamide (diabenase) has a fairly prolonged biological action and increased potency/weight ratio (Kaln, 1993).

The biguanides are oral hypoglycaemic drugs which produces their effect by retarding the absorption of glucose from the gut. Their effects are also directed on oxidative phosphorylation (Ellenhorn, 1994). The biguanides have an extremely narrow therapeutic range and may cause toxic reactions such as acute lactic acidiosis in patients with renal disease. The biguanides include the following; Metformin (glucophage), Acarbose (glucobay), Glibenclamide (Daonil) and Phenformin (D.B.A dibotin).

Several works have been documented on possible side effects of these oral hypoglycaemic agents (Sulphorylureas and biguanides) on the human/animal systems (Fisher *et al,* 1986; Kaln, 1993, Mycek *et al* 2000), but not much is known of their possible effects on the erythrocytes.

MATERIALS AND METHODS

The oral hypoglycaemic drugs, diabenese, daonil and glucophage were obtained from Nigeria-German Drugs, Plc. (Lagos, Nigeria). Other chemicals used for the in vitro analysis were from BDH (Poole Dorset, U.K) and Sigma Chemical Company (St. Louis, Missouri, U.S.A).

Experimental Animals

Wistar albino rats age 12-14 weeks, weighing between 200-220g and derived from a colony maintained at the animal house unit of the Department of Biochemistry, University of Port Harcourt, were used for the experiment. The animals which were kept in cages (within a temperature of 25 ± 2°C) were fed with standard laboratory

chow (Pfizer Feeds Plc, Nigeria) and water *ad libitum*. The animals were allowed to acclimatize for two weeks.

Experimental Procedure

For the in vivo test, a total of 144 rats (with average weight of 210.15 ± 10.2g) were used. The rats were divided into three groups; diabenese group, daonil group and glucophage group. Each group had 48 test rats while 12 rats served as control. Each of the drugs was administered to the rats at four different concentrations of 0.00g/mg (control), 0.01mg, 0.02mg and 0.03mg per 200g body weight. The administration of the drugs to the rats was orally by intubations. The drugs at each of the concentrations were administered to the rats at day one, two, six and fourteen. Since water was used for the solubization of the drugs, the control rats were administered the equivalent volume (0.2ml) of water in each case.

On each of the day(s) interval and at three hours after the administration of the drug, three rats from each of the drug concentration groups were sacrificed after blood collection. Rats from the 0.00mg concentration group served as control. Blood from the rats were collected by cardiac puncture into heparinized anticoagulant bottle and used for analysis as required.

NADH Determination

The technique used for NADH assay is based on the method described by Board (1981). The assay mixture of 2ml contained 0.1m tri HCl with 0.5mM EDTA, pH 8.0, 0.2mM NADH, 0.2mM $K_3Fc\ (CN)_6$ and an aliquot (0.02ml) of haemolysate; A tris buffer NADH mixture was first incubated for 10 minutes at 30°C. The reaction was then initiated by the addition of 0.22ml of ferricyanide-haemolysate mixture (in the ratio 10:1) pre-mixed a minute before addition. The rate of decrease in optical density of the system at 340nm was measured for 10 minutes at 30 seconds intervals against ma blank containing the reaction mixture without haemolysate. The reaction was carried out at 30°C rather than the usual 37°C because the enzyme is unstable at higher temperature (Beutler, 1984).

Statistical Analysis

Results of Biochemical estimations were reported as mean ± SD and statistical analysis was performed using the students t-test of statistical significance at 95% confidence level ($P \leq 0.05$) (Brokes *et al*, 1979). Data were also analyzed by one-way analysis of variance (ANOVA) using SPSS/PC package and differences between means

were compared using Duncan's (1955) multiple range test.

RESULTS

The in vivo study showed that rat erythrocyte NADH diaphorase activity was significantly elevated in the presence of the oral hypoglycaemic drugs, daonil, glucophage and diabenese. The maximal in vivo effect of the drugs on rat erythrocyte NADH diaphorase activity was obtained on the sixth day of drug administration with a significant ($P<0.05$) decline on the fourteenth day (Tables 1, 2 and 3). Comparatively, the effect of the three drugs on the enzyme was in the order:

Table I: In vivo Effect of Daonil on Erythrocyte NADH Diaphorase Activity of Rat at pH 8.0 and 30°C NADH (iu/L)

Daonil (mg/200g) Body Weight	Day 1 X ± SD	Day 2 X ± SD	Day 6 X ± SD	Day 14 X ± SD
0.00	6.13^{a} ± 1.24	6.191 ± 0.81	6.70^{a} ± 0.65	6.91^{b} ± 0.42
0.01	6.66^{a} ± 0.83	7.83^{b} ± 0.59	10.17^{e} ± 0.69	10.35^{e} ±0.96
0.02	7.00^{b} ± 0.02	8.50^{c} ± 0.01	10.35^{e} ± 0.97	11.01^{f} ± 1.41
0.03	7.43^{b} ± 0.01	9.41^{d} ± 0.14	11.44^{f} ± 0.82	7.04^{b} ± 1.03

Values with the same superscript letters are not statistically significant at 95% confidence level ($P<0.05$).

Table 2: In vivo Effect of Diabenese on Erythrocyte NADH Diaphorase Activity of Rat at pH 8.0 and 30°C

Diabenes (mg/200g) Body Weight	Day 1 X ± SD	Day 2 X ± SD	Day 6 X ± SD	Day 14 X ± SD
0.00	6.13^{a} ± 1.24	5.70^{a} ±0.81	6.70^{b} ±1.16	6.91^{b} ±0.42
0.01	7.00^{b} ± 1.40	6.91^{b} ±0.09	7.40^{c} ± 0.01	7.39^{e} ±0.22
0.02	6.95^{b} ±1.04	8.33^{d} ± 0.54	7.34^{e} ± 0.45	8.91^{d} ± 0.62
0.03	7.09b ± 0.05	9.41d ± 0.14	7.71c ± 0.98	10.66f ± 0.52

Values with the same superscript letters are not statistically significant at 95% confidence level ($P<0.05$).

Table 3: In vivo effect of Glucophage on erythrocyte NADH diaphorase activity of rat at pH 8.0 and 30°C

Glucophage (mg/200g) Body weight	Day I X ± SD	Day 2 X ± SD	Day 6 X ± SD	Day 14 X ± SD
0.00	6.13^{a} ± 1.24	5.70^{a} ± 0.81	6.70^{a} ± 1.16	6.91^{b} ± 0.42
0.01	8.18^{b} ± 0.23	12.06^{e} ± 0.69	14.74^{f} ± 0.04	17.57^{g} ± 0.08
0.02	9.07^{b} ± 0.06	14.71^{f} ± 0.60	19.50^{h} ± 0.15	17.87^{g} ± 0.09
0.03	10.56^{b} ± 0.67	18.20^{g} ± 0.43	20.55^{h} ± 0.57	17.92^{g} ± 0.15

Values with the same superscript letters are not statistically significant at 95% confidence level.

DISCUSSION

NADH methaemoglobin reductase is also known as NADH diaphorase (Board, 1981). It is a key enzyme involved in methaemoglobin reduction. The result of this work has shown that NADH diaphorase activity was enhanced following the administration of glucophage and daonil at concentration dependent manner. The effect of glucophage on NADH diaphorase activity was greater than that of daonil for the various concentrations. Also the effect was significantly different than that of the control.

On the otherhand, diabenese had no significant effect on the erythrocytes enzyme activity. The activation of the enzyme by daonil and glucophage confirms the work of Chasseaud, (1979) in which he proposed that erythrocyte enzyme functions to detoxify red cell xenobiotics. The increase in the rate of oxidation of haemoglobin leads to the accumulation of methaemoglobion which forms granules. This leads to an increase in the rate of its destruction by the spleen. As a result, the patient becomes anaemic (Robert Mc Gilvery, 1979). Thus NADH diaphorase helps in curbing this effect as has been demonstrated by this work. This observation perfectly agrees with the suggested role of erythrocyte NADH diaphorase in Xenobiotics detoxication (Robert Mc Gilvery, 1979).

ACKNOWLEDGEMENT

The authors are grateful to the Animal House Unit of the Department of Biochemistry, University of Port Harcourt, Nigeria for the provision of the experimental animals used for this work.

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Reference to this paper should be made as follows: Uzuegbu, U.E. and Onuoha, S.C. (2014), Effects of Some Oral Hypoglycaemic Drugs on Erythrocyte Nicotinamide Adenine Dinucleotide Hydrogen Diaphorase (E.C.1.6.4.3) Activity of Wistar. *J. of Medical and Applied Biosciences,* Vol. 6, No. 1, Pp. 115 – 122.