ISSN: 2278–294x (Print), ISSN: 2278- 2958 (Online)

Vol.1 No.2 (May 2012) 39

Indian Journal of Drugs and Diseases

Effects of some oral hypoglycemic drugs on Erythrocyte NADPH methaemoglobin reductase (E.C. 1.6.4.4) activity of albino rats (*Rattus rattus*)

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Abstract

The *invivo* effects of three oral hypoglycaemic drugs viz., daonil (a glIbenclamide), diabenese (a sulphonylurea) and glucophage (a metformin) on erythrocyte nicotinamide-adenine dinucleotide phosphate hydrogen (NADPH) methaemoglobin reductase activity of Wister 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) followed by administration of each drug. Three rats were used per each drug concentration per time interval (days). NADPH activity was measured at pH 8.0 and at 37^{o}C. Glucophage activated NADPH methaemoglobin reductase activity in a concentration dependent manner with optimal activity obtained at a concentration of 0.03mg/200g body weight and on the sixth day of drug administration. For instance, at drug concentrations of 0.00, 0.01, 0.02 and 0.03mg/200g body weight and at 6th day of administration, NADPH activity (IU/L) of 1.56 ± 0.04, 5.33± 0.20, 7.45 ± 0.53 and 12.49 ± 0.62 were obtained for glucophage . The increase in enzyme activity following drug administration was progressive with time duration (in days). Maximum effect was obtained on the sixth day, which declines on the 14th day. At the same concentrations, the activities of NADPH methaemoglobin reductase for daonil were: 1.56 ± 0.03, 0.92 ± 0.09, 0.94 ± 0.11, 1.17 ± 0.01 respectively. Thus, there was an inhibitory effect when daonil was administered ($P<0.05$) while for diabenese there was no significant effect at $P<0.05$. Thus, only glucophage displayed a significant increase in activity ($P<0.05$). The implications of these findings to the functional integrity of erythrocytes are discussed in this work.

Keywords: Hypoglycemia, NADPH, Erythrocyte, Daonil, Diabenese, Glucophage

Introduction

Nicotinamide adenine dinucleotide phosphate hydrogen methaemoglobin reductase (also known as NADPH diaphorase) is found in human liver, rats, pigeon and other sources. It is an NADPH dependent methaemoglobin reductase enzyme. (Kiese, 1944a).

According to Kiese (1944b), NADPH dependent activity occupies a separate active site on the same protein as NADH and funnels electrons through a common carrier system to methaemoglobin. In hereditary methaemoglobinaemia, the activity of NADPH is unaffected unlike that of NADH (Harris & Kellermeyer, 1974; Breaking *et al.,* 1951). NADPH dependent activity occupies a separate molecular entity (Scott, 1968). The NADPH activity requires methylene blue or other electron carrier when functioning as a physiologically significant methaemoglobin reductase (Beutler,1984; Gibson & Harrison, 1947).There are no known physiological substances in the erythrocyte functionally similar to methylene blue that can activate the reaction sequence and hence the suggestion

that the normal physiologic importance of this sequence is probably minimal (Dajami & Orten, 1958). This reaction sequence has been estimated to share 5-10% of the work of reducing methaemoglobin (Scott *et al.*, 1963). Erythrocyte NADPH dependent methaemoglobin reductase (NADPH diaphorase) has also been characterized as NADPH-flavin reductase.

Oral hypoglycaemic drugs are capsules or pills, which help in reducing the level of glucose in the blood (Kaln & Schechter, 1993). These 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 classes of hypoglycaemic agents and they are: the sulphorylureas (including arysulphorylureas) 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 and they include Glyburide (a micronase), Glipizide (Glucotrol), Tolazamide (tolinase), Tolbutamide (orinase), Acotohexamide (dymelor) and chloropropamide (diabenese). These drugs vary in their mode of excretion and duration of action. Chdloropropamide (diabenese) has a prolonged biologic action and increased potency/weight ratio (Jafe, 1959; Kaln & Schechter, 1993).

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

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

Materials and methods

The oral hypoclycaemic drugs, diabenese, glucophage and daonil 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. Louise, Missouri, USA.).

Animals

Wister Albino rats aged 12-14 weeks, weighing between 200-220g was maintained at the animal house, Department of Biochemistry, University of Port Harcourt, was used for the experiment. The animals were kept in cages (within a temperature of 25 ± 20^{o}C) were fed with standard laboratory chow obtained from Pfizer feeds Plc, Nigeria and water adlibitum. The animals were allowed to acclimatize for 2 weeks.

Experimental procedure

For the *invivo* test, 144 rats (with an average weight of 210.00 ± 10.0g) 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 the control. Each of the drugs was administered to the rats at four different concentrations of 0.00mg (control), 0.01mg, 0.02mg, 0.03mg per 200g body weight. The administration of the drugs to the rats was orally by intubations. The drugs at each of the concentration were administered to the rats at day one, two six and fourteen. Since water was used for the solubilization 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. Blood were collected by cardiac puncture into

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heparinized anticoagulant bottle and used for analysis as required.

Erythrocytes NADPH- methaemoglobin reductase determination

The assay method used for NADPH methaemoglobin reductase was based on the technique described by Board, (1981). The assay mixture of 2ml contained 0.1M Tris Hcl with 5mM EDTA, pH 8.0; 8Mm methylene blue and 0.1ml of the 1.20 haemolysate. The reaction was initiated with methylene blue after 10mins of incubation of the haemolysate NADPH-buffer mixture at 37^{o}C. Blank systems were made by decrease in the optical density (0.D) of the system was measured against that of the blank at 340nm at 30 seconds intervals for 10 minutes.

Statistical analysis

Results of biochemical estimations were reported as mean ± SD and statistical analysis were performed using the student t-test of statistical significance at 95% confidence level (P≤0.05) (Brookes *et al.*, 1979). Data were also analyzed by one-way analysis of Variance (ANOVA) using SPSS/PC package and difference between means werecompared using Duncan (1995) multiple range test.

Results

The *invivo* study showed that rat erythrocyte NADPH methaemoglobin reductase activity was significantly elevated in the presence of glucophage in a concentration dependent manner. The maximal in vivo effect of the drug (glucophage) on rat NADPH methaemoglobin reductase activity was obtained on the sixth day of the drug administration with a significance (P< 0.05) decline on the fourteenth day (Tables 1, 2 and 3).

Discussion

NADPH dependent methaemoglobin reductase is a methaemoglobin reducing agent although their role is complemented by NADH diaphorase in red blood cell methaemoglobin

Table 1. Invivo effect of glucophage on erythrocyte NADPH methaemoglobin reductase activity of rats at P.H 8.0 and 37^{o}C.

Glucophagea mg/200g Body weight	Day 1 x ± SD	Day 2 x ± SD	Day 6 x ± SD	Day 14 x ± SD
0.00	1.53±0.02 ^{a}	1.55±0.04 ^{a}	1.56±0.04 ^{a}	1.57± 0.05 ^{a}
0.01	1.66± 0.04 ^{a}	2.85^{b}± 0.02 ^{e}	5.33± 0.20 ^{c}	1.89±0.07 ^{d}
0.02	1.81±0.02 ^{d}	3.50± 0.03 ^{e}	7.45± 0.53 ^{f}	2.28±0.01 ^{b}
0.03	2.50± 0.53 ^{b}	6.66± 0.82 ^{g}	12.48± 0.62 ^{b}	1.96± 0.03 ^{d}

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

Table 2. Invivo effect of Diabenese on erythrocyte NADPH methaemoglobin reductase activity of rats at P.H 8.0 at 37^{o}C.

Diabeneseam mg /200g /B.W	Day 1x ± SD	Day 2x ± SD	Day 6 x ± SD	Day 14x ± SD
0.00	1.53± 0.02 ^{a}	1. 55± 0.04 ^{a}	1.56± 0.04 ^{a}	1.57± 0.05 ^{a}
0.01	1.55± 0.01 ^{a}	1.66± 0.04 ^{b}	1.57± 0.04 ^{a}	1.63± 0.08 ^{a}
0.02	1.56± 0.03 ^{a}	1.62± 0.01 ^{b}	1.61± 0.04 ^{b}	1.72± 0.11 ^{e}
0.03	1.63± 0.05 ^{b}	1.58± 0.01 ^{c}	1.60± 0.03 ^{b}	1.56± 0.01 ^{e}

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

Table 3. Invivo effect of Daonil on erythrocyre NADPH methaemoglobin reductase activity of rats at pH 8.0 and 37^{o}C.

Daonila mg/200g/B.W	Day 1 X ± SD	Day 2 X ± SD	Day 6 X ± SD	Day 14 X ± SD
0.00	1.54± 0.04 ^{a}	1.55± 0.04 ^{a}	1.56± 0.03 ^{a}	1.56± 0.42 ^{a}
0.01	1.49± 0.01 ^{b}	1.44± 0.02 ^{e}	0.92± 0.09 ^{d}	1.50± 0.01 ^{a}
0.02	1.48± 0.14 ^{b}	1.45± 0.06 ^{b}	0.94± 0.11 ^{d}	1.13± 0.01 ^{e}
0.03	1.47± 0.01 ^{b}	1.36± 0.04 ^{f}	1.17± 0.01 ^{s}	1.09± 0.05 ^{e}

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

reduction. The study shows that daonil inhibited the activity of NADPH methaemoglobin reductase while its action was activated by glucophage. Diabanese had no significant effect. The result thus, shows that daonil or its metabolite could be acting as a red cell nucleophile and competitively inhibits the NADPH diaphprase activity. The reverse could be the case for glucophage, which activated the enzyme.

The present study is important because an increase in the rate of oxidation of baemoglobin leads to the accumulation of methaemoglobin which, forms granules. This leads to an increase in the rate at which it isdestroyed by the spleen. Thus, the patient becomes anaemic (Robert & Mc-Gilvery, 1979; Newwrit & Ponka, 1977). According to Ellenhorn and Barceloux, (1994), drugs even when taken in the right doses exhibits a number of deleterious effects in addition to the desired clinical effect. As a result, in conclusion that rat erythrocyte NADPH methaemoglobin reductase studied in this work was involved in the oxidation/reduction of the oral hypoglycaemic drugs, diabenese, daonil and glucophage.

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 in this work.

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