Original Article

Increase in activity of Na$^+$, K$^+$-ATPase by Porphyrin compounds as treatment for Dysnatremias caused by Diabetes Mellitus

Abdul Hai[1], Nadeem Kizilbash[2]

ABSTRACT

Objective: The aim of this study was to test the action of Porphyrin compounds, Tetraphenylporphine sulfonate (TPPS), 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) nitrosyl Chloride (FeNOTPPS), on Na+ , K+ -ATPase of cell membrane of erythrocytes.

Methods: Enzymatic assays, measuring the amount of inorganic phosphate produced, were used to estimate the activity of Na$^+$, K$^+$-ATPase.

Results: The results show that Porphyrin compounds exert an insulin-like effect on Na$^+$, K$^+$-ATPase. They act by increasing the activity of the membrane-bound enzyme.

Conclusion: All the three Porphyrin compounds increased the activity of erythrocyte Na$^+$, K$^+$-ATPase. The exact mechanism of action of these compounds is not clear.

KEY WORDS: Na$^+$, K$^+$-ATPase, Porphyrin derivatives, Diabetes Mellitus, Acetylcholinesterase, Dysnatremias.

List of Abbreviations: Acetylcholinesterase (AChE), Tetraphenylporphinesulfonate (TPPS), 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) nitrosyl Chloride (FeNOTPPS).

INTRODUCTION

The relationship between blood glucose level and the levels of Na$^+$ and K$^+$ ions is complex and depends on a number of factors.[1] Diabetes Mellitus

1. Abdul Hai,
2. Nadeem Kizilbash,
1,2: Department of Medical Laboratory Technology,
   Faculty of Applied Medical Sciences,
   Northern Border University,
   Arar, Saudi Arabia.

   Correspondence:

   Nadeem Kizilbash,
   Department of Medical Laboratory Technology,
   Faculty of Applied Medical Sciences,
   Northern Border University,
   P.O. Box 1321, Arar-91431,
   Saudi Arabia.
   E-mail: fsd707@gmail.com

   * Received for Publication: June 8, 2016
   * 1st Revision Received: June 13, 2016
   * 2nd Revision Received: August 23, 2016
   * Final Revision Received: * August 25, 2016

causes dysnatremias[2] via several mechanisms.[3,4] In Diabetes Mellitus, high glucose level increases serum osmolality which results in the loss of water from the cells and results in a reduction in serum Na$^+$ions level by dilution. In diabetic ketoacidosis, ketone bodies (β-hydroxybutyrate and acetoacetate) cause urinary electrolyte losses which can cause hypernatremia.[5]

Na$^+$, K$^+$-ATPase pumps Na$^+$ and K$^+$ ions across the cell membranes to maintain the trans-membrane gradients of these ions. Na$^+$ ions are pre-dominant in the extracellular fluid. Since Na$^+$ ions cannot enter the cells, they produce an osmotic pressure that results in the retention of water in the extracellular fluid. The osmolarity of the extracellular fluid is constant and the amount of Na$^+$ in the cells determines the volume of the extracellular fluid. The mechanism of action of Na$^+$, K$^+$-ATPase involves hydrolysis of ATP to produce a phosphorylated E1P-E2P state. It is

coupled to the transport of Na^+ and K^+ ions across the cell membrane. The phosphorylated enzyme intermediate is then hydrolyzed and an E2-E1 state is produced to complete the cycle.[6,7]

Erythrocytes are known to resemble liver cells. They possess specific receptors for insulin.[8] It is known that insulin deficiency during Diabetes Mellitus causes membrane alterations in erythrocytes.[9] A decreased activity of erythrocyte membrane Na^+, K^+-ATPase has been reported in Diabetics.[10] This decreased activity plays a major role in the development of cardiomyopathy.[11] and may also result in metabolic, cardiovascular, ocular, and neural complications.[12] Some studies have previously shown that Na^+, K^+-ATPase activity is decreased in erythrocytes during Diabetes Mellitus.[13] Diabetic patients show a 30% reduction in erythrocyte Na^+, K^+-ATPase activity as compared to controls. The decrease in ATPase activity can result in the development of Diabetic Neuropathy.

Porphyrins are heterocyclic aromatic compounds. The parent porphyrin is called porphin, and substituted porphines are called porphyrins. We have previously evaluated Porphyrins in the context of photodynamic therapy (PDT) since they strongly absorb visible light in the blue region.[14] In addition, we have also shown that these compounds serve as inhibitors for Acetylcholinesterase enzyme in erythrocytes and nerve tissue.[15,16] This study was conducted to investigate the three Porphyrin derivatives: Tetraphenylporphinesulfonate (TPPS), 5,10,15,20-Tetrakis(4sulfonatophenyl)porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis (4sulfonatophenyl) porphyrinato Iron(III) nitrosyl Chloride (FeNOTPPS), as candidate compounds for increasing the Na^+, K^+-ATPase activity in erythrocytes. The use of these compounds can be useful for the prevention of dysnatremias in Diabetes Mellitus.

METHODS

Synthesis of Porphyrin compounds: The three Porphyrin compounds, TPPS, FeTPPS and FeNOTPPS, were synthesized by use of established procedures.[17] Commercially obtained pyrrole, benzaldehyde and acetone were distilled before use. A stock solution (2.5 M) of BF_3-ether was prepared in CH_2Cl_2. ^{1}H NMR were recorded in $CDCl_3$ using a 400 MHz NMR spectrometer. The UV-Vis. spectra were recorded on a UV-Vis. spectrophotometer.

Collection of blood samples and isolation of erythrocyte membranes: Blood samples were obtained from 15 volunteers. The exclusion criteria were that none of the subjects suffered from arthritis, hypertension or Diabetes Mellitus. The samples were centrifuged at 4°C for 10 min at 100 g. The erythrocyte membranes were isolated by established procedures.[18]

Determination of protein content: The erythrocyte membranes were assayed for protein content by the method of Lowry et al.[19]

Determination of Na^+, K^+-ATPase activity: The enzymatic activity of Na^+, K^+-ATPase was measured using published protocols.[20] The amount of inorganic phosphate produced was estimated by the method of Fiske and Subbarow.[21]

In vitro experiments of Porphyrin compounds with Na^+, K^+-ATPase: The effect of Porphyrin compounds on Na^+, K^+-ATPase activity was investigated using published protocols.[20] A stock solution (10 mM) of Porphyrin compounds in 5% ethanol was used. This solution was diluted further with water before use. The *in vitro* effect of Porphyrin compounds was monitored by incubating the reaction mixture for 60 min at 37°C.

Statistical analysis: Statistical analysis was performed by the use of MedCalc® program version 8.1.0.0.[22] The enzyme activity data was expressed as mean ± standard deviation. *P*-value was considered significant if it was less than 0.05.

Ethical Considerations: The Vice Dean (Academics), Faculty of Applied Medical Sciences, Northern Border University reviewed the ethical considerations for this project and found them to have been satisfied.

RESULTS

Treatment of erythrocyte Na^+, K^+-ATPase with the three Porphyrin compounds resulted in an increase in activity of the enzyme significantly (Table-I). The observation of increased Na^+, K^+-ATPase activity in response to Tetraphenylporphinesulfonate (TPPS), 5,10,15,20-Tetrakis(4sulfonatophenyl)porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis (4sulfonatophenyl) porphyrinato Iron(III) nitrosyl Chloride (FeNOTPPS) pre-treatment is interesting since insulin also causes an increase in Na^+-K^+-ATPase activity in erythrocytes of Diabetics. Further work is needed to explain the effect of Porphyrin compounds on erythrocyte Na^+, K^+-ATPase and also on other membrane bound enzymes.

DISCUSSION

The results show that all the three Porphyrin compounds caused an increase in the activity of erythrocyte Na^+, K^+-ATPase (Table-I). The

Table-I: Erythrocyte membrane Na+-K+-ATPase activity in human erythrocytes before and after in vitro treatment with synthetic compounds: TPPS, FeTPPS and FeTPPSNO.

		*Enzyme Activity	P-value
Control Solution		0.010 ± 0.001	0.02
Compound	Concentration (M)		
TPPS	10^{-10}	0.011 ± 0.001	0.002
	10^{-9}	0.013 ± 0.003	0.05
	10^{-8}	0.018 ± 0.002	0.02
	10^{-7}	0.022 ± 0.002	0.03
	10^{-6}	0.027 ± 0.003	0.04
	10^{-5}	0.033 ± 0.007	0.002
	10^{-4}	0.036 ± 0.003	0.02
	10^{-3}	0.039 ± 0.003	0.03
FeTPPS	10^{-10}	0.011 ± 0.002	0.04
	10^{-9}	0.012 ± 0.002	0.002
	10^{-8}	0.015 ± 0.002	0.02
	10^{-7}	0.018 ± 0.002	0.03
	10^{-6}	0.024 ± 0.003	0.002
	10^{-5}	0.028 ± 0.005	0.05
	10^{-4}	0.032 ± 0.004	0.02
	10^{-3}	0.036 ± 0.003	0.03
FeNOTPPS	10^{-10}	0.011 ± 0.001	0.05
	10^{-9}	0.011 ± 0.001	0.002
	10^{-8}	0.014 ± 0.003	0.05
	10^{-7}	0.016 ± 0.002	0.02
	10^{-6}	0.021 ± 0.003	0.04
	10^{-5}	0.025 ± 0.002	0.002
	10^{-4}	0.028 ± 0.003	0.02
	10^{-3}	0.033 ± 0.004	0.04

*Each value is the mean of at least 3-4 independent experiments. Values are expressed as mean +/- standard deviation. Na+, K+-ATPase activity was measured as described earlier.[11]

mechanism underlying the non-neuronal effects of Porphyrin compounds is unclear. It is possible that these lipophilic compounds insert into the plasma membrane of erythrocytes and alter their membrane fluidity. It has previously been reported that erythrocytes exhibit different properties in Diabetic patients.[12,23] These cells have decreased deformability,[24] increased membrane viscosity[25] and increased erythrocyte aggregation.[26] The decreased Na^+, K^+-ATPase activity observed in the erythrocyte membrane of Diabetics leads to an increase in Na^+ and Ca^{+2} ions. The decrease in plasma Na^+ level and decreased activity of erythrocyte membrane Na^+, K^+-ATPase can disturb ion homeostasis of cells. ROS-induced oxidation damages the erythrocyte membrane Na^+, K^+-ATPase and results in an increase in the efflux of K^+ from the cells that results in cell lysis.

The intra-cellular increase in Na^+ and Ca^{+2} ions that can decrease the deformability of erythrocytes in Diabetics.[12] The diabetes-induced decrease in Na^+, K^+-ATPase activity may also be due to hyperglycemia that increases glycosylation of the β subunit of Na^+, K^+-ATPase[27] or there can be a defect in myo-inositol metabolism that changes the lipid content of the cell membranes.[28] However, a direct effect of hyperglycemia on this enzyme's activity has never been proven. In type 1 diabetic patients, erythrocyte membrane Na^+, K^+-ATPase activity is not related to the plasma glucose, HbA_{1c}, plasma triglyceride, or total cholesterol levels. In type 2 diabetes, this enzyme's activity is less reduced than in type 1 diabetes despite similar HbA_{1c} values.[29] A similar observation has been made for Na^+, K^+-ATPase activity in the sciatic nerve in type 2 Diabetic BBZDR rats compared to type 1 Diabetic BB rats.[30]

CONCLUSION

The three Porphyrin compounds, TPPS, FeTPPS and FeNOTPPS, increased the activity of erythrocyte Na^+, K^+-ATPase. Although, Porphyrin compounds seem to mimic insulin in their effect on Na^+, K^+-ATPase, it is difficult to predict the mechanism of action. It is possible that these lipophilic compounds bind to the plasma membrane of erythrocytes and change the membrane fluidity. The findings being reported here prove the importance of the non-neuronal effects of Porphyrin compounds.

ACKNOWLEDGEMENT

REFERENCES

1. Shah D, Nandakumar S, Jaishankar GB, Chilakala S, Wang K, Kumaraguru U. Pre-Term Exposure Patterns in Neonatal Intensive Care Unit Alters Immunological Outcome in Neonates. J Allergy Therapy. 2012;2011. doi: 10.4172/2155-6121.1000106

2. Elisaf MS, Tsatsoulis AA, Katopodis KP, Siamopoulos KC. Acid-base and electrolyte disturbances in patients with diabetic ketoacidosis. Diabetes Res Clin Pract. 1996;34(1):23-27. doi:1016/S0168-8227(96)01332-0

3. Liamis G, Rodenburg EM, Hofman A, Zietse R, Stricker BH, Hoorn EJ. Electrolyte disorders in community subjects: prevalence and risk factors. Am J Med. 2013;126(3):256-263. doi: 10.1016/j.amjmed.2012.06.037.

4. Liamis G, Tsimihodimos V, Doumas M, Spyrou A, Bairaktari E, Elisaf M. Clinical and laboratory characteristics of hypernatraemia in an internal medicine clinic. Nephrol Dial Transplant. 2008;23(1):136-143. doi: 10.1093/ndt/gfm376

5. Hajek AS, Joist JH, Baker RK, Jarett LE, Daughaday WH. Demonstration and partial characterization of insulin receptors in human platelets. J Clin Invest. 1979;63(5):1060. doi: 10.1172/JCI109375

6. Skou JC. The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochimica et biophysica acta. 1957;23:394-401. doi:10.1016/0006-3002(57)90343-8

7. Finotti P, Verbaro R. Identification and partial purification of a (Na-K) ATPase stimulating serine protease from plasma of insulin-dependent diabetics. Clinica Chimica Acta. 1987;170(2):121-34. doi:10.1016/0009-8981(87)90120-3

8. Watala C. Altered structural and dynamic properties of blood cell membranes in diabetes mellitus. Diabetic Med. 1993;10(1):13-20. doi: 10.1111/j.1464-5491.1993.tb01990.x

9. Agarwal VR, Rastogi AK, Sahib MK, Sagar P. In vitro insulin action on different ATPases of erythrocyte membranes in normal and diabetic rats. Acta Diabetologia Latina. 1985;22(2):111-118. doi: 10.1007/bf02590784

10. Spinale FG, Clayton C, Tanaka R, Fulbright BM, Mukherjee R, Schulte BA, et a. Myocardial Na+, K+-ATPase in tachycardia induced cardiomyopathy. J Mol Cell Cardiol. 1992;24(3):277-294. doi: 10.1016/0022-2828(92)93165-g

11. Greene DA, Lattimer SA, Sima AA. Are disturbances of sorbitol, phosphoinositide, and Na+-K+-ATPase regulation involved in pathogenesis of diabetic neuropathy? Diabetes. 1988;37(6):688-693. doi: 10.2337/diab.37.6.688

12. Bonilla SI, Goecke IA, Bozzo SA, Alvo MI, Michea LU, Marusic ET. Effect of chronic renal failure on Na, K-ATPase alpha 1 and alpha 2 mRNA transcription in rat skeletal muscle. J Clin Invest. 1991;88(6):2137. doi: 10.1172/jci115544

13. Rahmani-Jourdheuil D, Mourayre Y, Vague P, Boyer J, Juhan-Vague I. In vivo insulin effect on ATPase activities in erythrocyte membrane from insulin-dependent diabetics. Diabetes. 1987;36(9):991-5. doi: 10.2337/diab.36.9.991

14. Hai A, Kizilbash NA, Zaidi SH, Alruwaili J. Binding interactions of porphyrin derivatives with Ca²⁺ ATPase of sarcoplasmic reticulum (SERCA1a). Bioinformation. 2013;9(8):409. doi: 10.6026/97320630009409

15. Kizilbash N, Alrowaili M. Molecular Docking to Test for Efficacy of Porphyrin Compounds to Cure Alzheimer's Disease. J Biotechnol Biomater. 2015;5(3):1. doi:10.4172/2155-952X.1000199

16. Hai A, Kizilbash NA, Zaidi SH, Alruwaili J. Porphyrin derivatives as inhibitors for acetylcholinesterase from Drosophila melanogaster. Bioinformation. 2013;9(12):645. doi: 10.6026/97320630009645

17. Fleischer EB, Palmer JM, Srivastava TS, Chatterjee A. Thermodyamic and kinetic properties of an iron-porphyrin system. J Am Chem Soc. 1971;93(13):3162-3167. doi: 10.1021/ja00742a012

18. Marchesi VT, Palade GE. The localization of Mg-Na-K-activated adenosine triphosphatase on red cell ghost membranes. J Cell Biol. 1967;35(2):385-404. doi: 10.1083/jcb.35.2.385

19. Peterson GL. Review of the Folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. Analytical Biochem. 1979;100(2):201-220. doi: 10.1016/0003-2697(79)90222-7

20. Suhail M, Rizvi SI. Red cell membrane (Na⁺ K⁺)-ATPase in diabetes mellitus. Biochem Biophy Res Commun. 1987;146(1):179-186. doi: 10.1016/0006-291x(87)90708-x

21. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J Biol Chem. 1925;66(2):375-400. doi: 10.1016/0003-2697(68)90255-8

22. Schoonjans FR, Zalata A, Depuydt CE, Comhaire FH. MedCalc: a new computer program for medical statistics. Comput Methods Programs Biomed. 1995;48(3):257-62. doi:10.1016/0169-2607(95)01703-8

23. Juhan I, Buonocore M, Jouve R, Vague PH, Moulin JP, Vialettes B. Abnormalities of erythrocyte deformability and platelet aggregation in insulin-dependent diabetics corrected by insulin in vivo and in vitro. Lancet. 1982;319(8271):535-537. doi: 10.1016/S0140-6736(82)92045-1

24. McMillan DE. Deterioration of the microcirculation in diabetes. Diabetes. 1975;24(10):944-957. doi: 10.2337/diab.24.10.944

25. Baba Y, Kai M, Kamada T, Setoyama S, Otsuji S. Higher levels of erythrocyte membrane microviscosity in diabetes. Diabetes. 1979;28(12):1138-1140. doi: 10.2337/diab.28.12.1138

26. McMillan DE, Utterback NG, La Puma J. Reduced erythrocyte deformability in diabetes. Diabetes. 1978;27(9):895-901. doi: 10.2337/diab.27.9.895

27. Jha S, Dryer SE. The β1 subunit of Na+/K+-ATPase interacts with BKCa channels and affects their steady-state expression on the cell surface. FEBS Letters. 2009;583(19):3109-3114. doi: 10.1016/j.febslet.2009.08.039

28. Yorek MA, Dunlap JA, Ginsberg BH. Effect of Increased Glucose Levels on Na⁺/K⁺-Pump Activity in Cultured Neuroblastoma Cells. J Neurochem. 1988;51(2):605-610. doi: 10.1111/j.1471-4159.1988.tb01081.x

29. Raccah D, Fabreguettes C, Azulay JP, Vague P. Erythrocyte Na⁺-K⁺-ATPase activity, metabolic control, and neuropathy in IDDM patients. Diabetes Care. 1996;19(6):564-568. doi: 10.2337/diacare.19.6.564

30. Sima AA, Zhang W, Xu G, Sugimoto K, Guberski D, Yorek MA. A comparison of diabetic polyneuropathy in type II diabetic BBZDR/Wor rats and in type I diabetic BB/Wor rats. Diabetologia. 2000;43(6):786-793. doi: 10.1007/s001250051376