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PROTEIN KINASE C: AFTER THE COMBINATION OF METFORMIN AND HYPERBARIC OXYGEN THERAPY IN TYPE 2 DIABETES PATIENTS

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ABSTRAK

Metformin meningkatkan sensitivitas insulin tidak hanya tetapi juga fungsi endotel vaskular dan mengurangi kejadian kardiovaskular pada pasien dengan diabetes tipe 2. Terapi oksigen hiperbarik (HBO terapi), 2,4 ATA mengurangi glukosa darah secara langsung dengan menggunakan glukosa untuk menghasilkan energi (ATP), memiliki efek pada vasodilatasi dengan merangsang Synthase Oxid nitrat (NOS) dan mungkin meningkatkan sensitivitas insulin melalui aktivasi AMPK. Oleh karena itu, HBO dianggap sebagai pengobatan adjuvant pada DM tipe 2 di samping metformin. Protein kinase berperan dalam translokasi GLUT4 vesikel ke permukaan sel. Dalam studi ini, kami meneliti peran Protein kinase C dalam insulin yang diinduksi GLUT4 dalam meningkatkan pengurangan glukosa darah. Tujuan dari penelitian ini adalah untuk menemukan manajemen baru dalam optimalisasi terapi metformin pada pasien dengan diabetes tipe 2, terutama dalam mengurangi glukosa darah, dan meningkatkan konsentrasi PKC itu. Hal ini diyakini bahwa progresivity diabetes tergantung pada penampilan dan aktivitas PKC dan peningkatan efek sensitity insulin pada GLUT4. Ini adalah studi pra dan pasca klinis percobaan tanpa kontrol. Sepuluh koresponden laki-laki chosed secara acak. Glukosa darah dan konsentrasi PKC digunakan sebagai parameter, diperiksa sebelum, selama dan setelah perawatan kombinasi metformin dan HBO terapi diberikan. Glukosa darah berkurang secara signifikan setelah terapi. Titer PKC telah ada perubahan signifikan sebelum dan setelah pengobatan. Kesimpulan: Metformin dan terapi OHB mengurangi glukosa darah tapi tidak secara signifikan meningkatkan konsentrasi PKC itu. Cara spesifik metformin dan efek OHB terapi itu harus dieksplorasi lebih. Implikasi klinis dari terapi kombinasi tetap ditentukan.

ABSTRACT

Metformin improves not only insulin sensitivity but also vascular endothelial functions and reduces cardiovascular events in patients with type 2 diabetes. Hyperbaric oxygen therapy (HBO therapy), 2.4 ATA reduces directly blood glucose by using glucose to produce energy (ATP), has effect on vasodilatation by stimulating Nitric Oxid Synthase (NOS) and presumably improves Insulin sensitivity via AMPK activation. Therefore, HBO is considered as an adjuvant treatment in type 2 DM beside Metformin. Protein kinase plays a role in translocation of GLUT4 vesicles to cell surface. In this study, we investigated the role of Protein Kinase C in insulin-induced GLUT4 in improving glucose blood reduction. The aim of this study was to find a new management in metformin therapy optimalisation in patients with type 2 diabetes, especially in reducing blood glucose, and improving PKC's concentration. It is believed that the progresivity of diabetes depends on PKC appearance and activity and the improvement of insulin sensitity effects on GLUT4. This was a pre and post experimental clinical study without control. Ten male correspondents were chosed randomly. Blood glucose and PKC concentrations were used as the parameters, examined before, during and after combined treatment of Metformin and HBO therapy were given. Glucose blood reduced significantly after the therapy. PKC's titer had no significant change before and after treatment. Conclusion: Metformin and OHB therapy reduce blood glucose but not significantly increase PKC's concentration. The specific ways of Metformin and OHB therapy's effect should be explored more. The clinical implications of these combination therapies remain to be determined.

Keywords: *Metformin, OHB therapy, PKC, glucose blood*

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INTRODUCTION

Metformin, the Biguanides derivative, is the first line oral anti diabetic (American Diabetes Association 2006; PERKENI 2007) used for the treatment of type 2 diabetes. It is an effective hypoglycemic drug that also improves lipid profiles and reduces cardiovascular risk The Diabetes Prevention Program has recently shown that similar to diet and exercise, Metformin treatment reduces the risk of developing diabetes in glucose-intolerant individuals. Whereas most studies have shown that the glucose-lowering effects of Metformin

are secondary to a decrease in hepatic glucose production via its molecular site of action remains unclear, AMP-activated protein kinase (AMPK). In addition to its insulin sensitizing effects, Metformin has also been shown to have direct vascular effects (Bailey 2007).

Protein kinase C (PKC) comprises a multigene family that encodes at least 12 distinct isoforms differing in catalytic and regulatory properties (Concorelli 2001). These PKC isoforms can be divided into the following three subgroups based on cofactor requirements: conventional PKCs (α, β, and γ), which are dependent on Ca2+ and diacylglycerol (DAG) for activity; novel PKCs (δ, ε, η, and υ), which are not dependent on Ca2+ but are activated by DAG; and atypical PKCs (ζ, λ, and ι), which are not dependent on Ca2+ and are not stimulated by DAG.

PKC plays a pivotal role in controlling numerous cellular functions, including glucose transport. Indeed, current evidence indicates that DAG-dependent PKCs may control Insulin dependent glucose transport in several different cell types (Dipiro 2009). More recent work also indicates that different PKC isoforms have an important role in signaling insulin action on glucose transport. More recent work also indicates that different PKC isoforms have an important role in signaling insulin action on glucose transport. It is presumable that Metformin improves PKC activity via the action of Insulin on glucose transport (Shen et al 2001). In this study, our aims is finding the effect of combination Metformin and HBO therapy in PKC concentration.

Despite the long history and success of metformin as a treatment for type 2 diabetes, there is a cumulative incidence at 5 years study in UK, that the failure of Metformin monotherapy is 21% out of 4360 patients with type 2 diabetes. Even, to solve the problem of Metformin monotherapy failure, patients were given combination of with either other oral anti diabetics drugs, such as the Thiazolidinediones derivative – Rosiglitazone; the Sulphonylureas derivative – Glibenclamide or Insulin analogues. The progress of diabetes complication such cardiovascular events were still happened.

In this current study, Hyperbaric Oxygen (HBO) Therapy, used 100 percent oxygen at 2.4 Atmosphere Absolute (ATA), is considered as an adjuvant treatment in patients with type 2 diabetes and Metformin monotherapy failure, especially in improving PKC activity.

MATERIALS AND METHODS

The one group pre – post without control design. A total of 10 Indonesian men with type 2 diabetes were selected among 20 subjects recruited in the context of a study on Metformin monotherapy failure at the Department of Internal Medicine, Naval Hospital Dr.Ramelan Surabaya to represent as subjects in this study. The age ranged from 50 to 65 years and type 2 diabetes on the basis of more than 7% in HbA1 according to American Diabetes Association criteria. All subjects fulfilled the following inclusion criteria: 1) absence of any acute or chronic inflammatory diseases as determined by a leukocyte count or clinical signs of infection, 2) no medical history of hypertension (i.e., systolic blood pressure was less than140 mmHg and diastolic blood pressure was less than 85 mmHg, 3) no clinical evidence of either cardiovascular or peripheral artery disease, 4) no thyroid dysfunction, 5) no contra indications for both Metformin or HBO therapy, 6) do diet for diabetes, 7) no Insulin therapy and 8) no anti scavenger (i.e., Ca2+ supplement, Vitamin E) within last 6 months. The study was approved by the ethics committee of the Medical Faculty University of Airlangga. All subjects gave written informed consent before taking part in the study.

Ten subjects were given Metformin 500 mg 4 times a day and HBO therapy 2.4 ATA 10 sessions within 10 days with 2 days without HBO therapy. One HBO session took around 2 hours therapy with 3 activities, 1) 10 minutes adaptation for the change of the atmosphere pressure at the sea level, 2) breath with 100 percent Oxygen, 3 times 30 minutes with 5 minutes interval, 3) 10 minutes adaptation for back to the normal atmosphere pressure. All based line blood sample were collected three times within the treatment of HBO periods for measuring blood glucose, PKC concentration and hepar – renal function. First measurement was taken before the subjects entered HBO chamber (pre test). Second measurement was taken after the HBO therapy day 5th (durantee test) and the last measurement was taken after the subjects come out from HBO chamber day 10th (post test).

Assays, measures of blood glucose and liver – kidneys function. Samples were using serum, that took from 30 minutes clotting whole blood then centrifuged for 15 minutes at approximately 1000 x g, then homogenized and measured with "fortex mixer" equipments. PKC ELISA development and measurement of PKC serum concentrations.

The gene-encoding human PKC was amplified from the human pPKC cDNA library by PCR, standard E. coli–expressed PKC was used as an immunogen for

generating human PKC – specific monoclonal and polyclonal Abs. For making recombinant PKC proteins in HEK293 cells and E. coli, the portion of the gene encompassing presumed mature polypeptides, Leu21 through Lys414, was amplified and digested with appropriate restriction enzymes and then cloned into pPKC (USCN Life Science Inc.,Wuhan, China). Standards and samples are pipetted into the wells and any pPKC present is bound by the immobilized antibody. An enzyme-linked polyclonal antibody specific for p-PKC is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of p-PKC bound in the initial step. The color development is stopped and the intensity of the color is measured.

Data are shown as means SE, unless stated otherwise. Before statistical analysis, normally distributed parameters were logarithmically approximate a normal distribution (Kolmogorov-Smirnov test). The following statistical tests were used: paired Student's t test or paired samples test. Statistical analysis was performed using SPSS version 14.0 (Chicago, IL). P values _0.05 were considered to be statistically significant.

RESULTS

Development and validation of PKC ELISA. The current ELISA system was made possible by a human PKC – specific monoclonal antibody. We were able to observe a certain range of serum PKC levels from all the sera we tested. PKC serum concentrations in response to combination Metformin and 10 sessions of HBO therapy. A total of 10 Indonesian men and women completed a combination Metformin and HBO therapy program. The treatment effect was confirmed by a significant improvement in reducing blood glucose in all subjects (table and figure1.1), on the other hand, the treatment was not significant improvement in PKC concentration (Table and Figure 1).

Table 1. Paired t-Test for Fasting Blood Glucose (FBG)

	Paired t-Test	Sig.	Mean
Pair 1	FBG_I - FBG_II (pre – durantee HBO)	.000	55.2
Pair 2	FBG_II - FBG_III (durantee – post HBO)	.011	26.7
Pair 3	FBG_I - FBG_III (pre – post HBO)	.000	81.5

Sig. = significant < 0,05 ; Resource: SPSS version 14.0

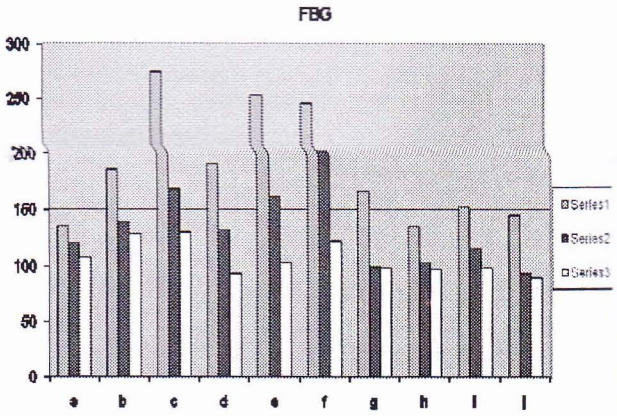

Fig. 1. The chart of reducing (fasting) blood glucose. Series 1 = 1st measurement; Series 2 = 2nd measurement; Series 3 = 3rd measurement

Table 2 : Paired t-Test for PKC concentration

	Paired t-Test	Sig.	Mean
Pair 1	PKC_I - PKC _II (pre – durantee HBO)	.598	-0.185
Pair 2	PKC _II - PKC _III (durantee – post HBO)	.235	1.131
Pair 3	PKC _I - PKC _III (pre – post HBO)	.234	.946

Sig. = significant < 0,05 ; Resource: SPSS version 14.0

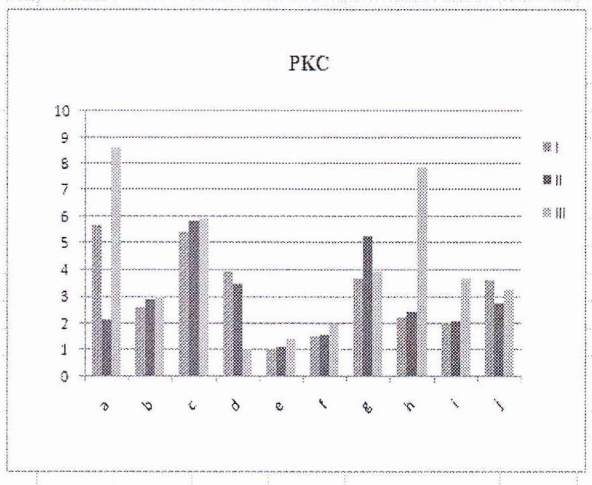

Fig. 2 : The chart of improvement PKC concentration. I = 1st measurement; II= 2nd measurement; Series III = 3rd measurement

Hepar and Renal functions. Hepar function was evaluated by SGOT and SGPT during combination Metformin and HBO therapy were given. It needed to evaluate because of Metformin's mainly work was in hepar. Metformin therapy was considered to stop if hepar function increased (Goodman and Gilman's 2006). Result of hepar function was no significant raised (Table and Figure 3).

Table 3 : Paired Samples Test for SGOT and SGPT

	Paired Samples Test	Sig.	Mean
Pair 1	SGOT_I – SGOT_II (pre – durantee HBO)	.061	1.40
Pair 2	SGOT_II – SGOT_III (durantee – post HBO)	.015	1.20
Pair 3	SGOT_I – SGOT_III (pre – post HBO)	.893	.10
Pair 4	SGPT_I – SGPT_II (pre – durantee HBO)	.541	.30
Pair 5	SGPT_II – SGPT_III (durantee – post HBO)	.560	.30
Pair 6	SGPT_I – SGPT_III (pre – post HBO)	.443	.60

Sig. = significant < 0,05 ; Resource: SPSS version 14.0

Renal function was evaluated by BUN and Creatinine Serum during combination Metformin and HBO therapy were given. It needed to evaluate because of Metformin's mainly excretion was in renal. Metformin therapy was considered to stop if there is impaired in renal function (Goodman and Gilman's 2006). Result of renal function was no significant raised (Table and Figure 4).

Table 4 : Paired t-Test for BUN dan Creatinine Serum (SK)

	Paired t-Test	Sig.	Mean
Pair 1	BUN_I – BUN_II (pre – durantee HBO)	.771	.771
Pair 2	BUN_II – BUN_III (durantee – post HBO)	.479	.479
Pair 3	BUN_I – BUN_III (pre – post HBO)	.364	.364
Pair 4	SK_I – SK_II (pre – durantee HBO)	1.00	1.00
Pair 5	SK_II – SK_III (durantee – post HBO)	1.00	1.00
Pair 6	SK_I – SK_III (pre – post HBO)	1.00	1.00

Sig. = significant < 0,05 ; Resource: SPSS version 14.0

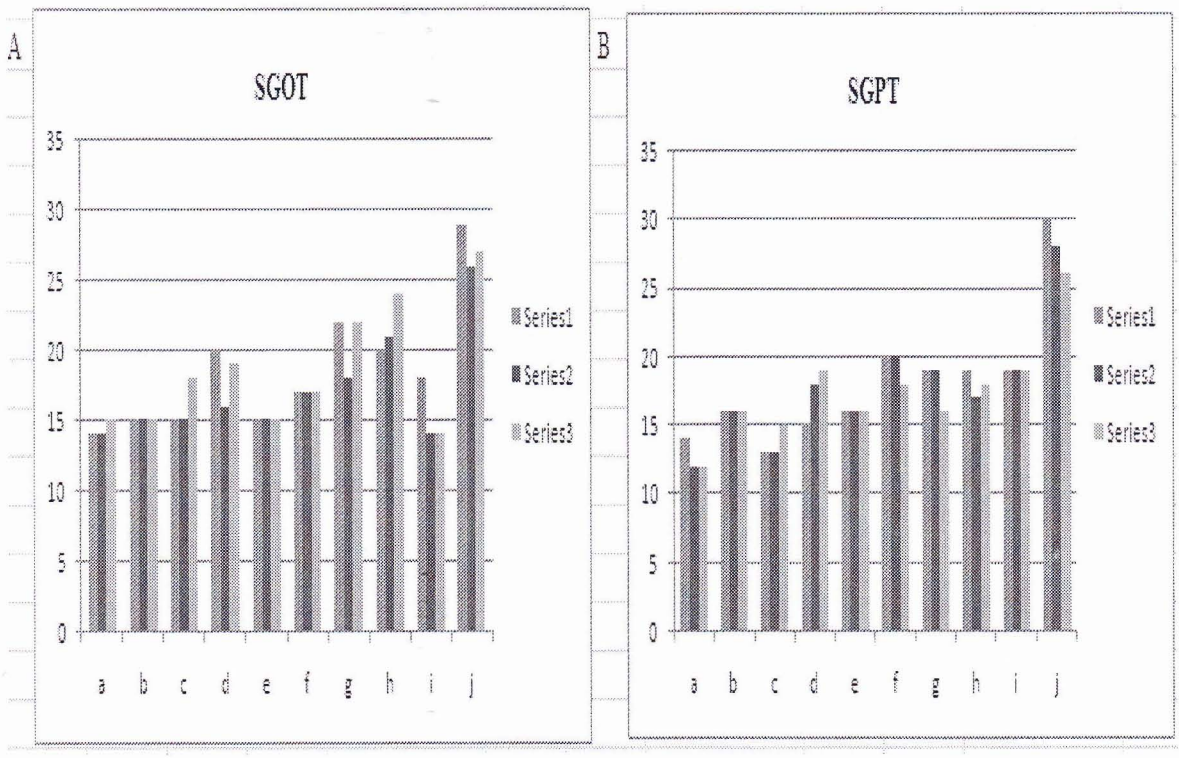

Fig. 3 : Chart (A) result of SGOT evaluation; (B) result of SGPT evaluation. series 1 = 1st measurement of SGOT and SGPT, series 2 = 2nd measurement of SGOT and SGPT, series 3 = 3rd measurement of SGOT and SGPT

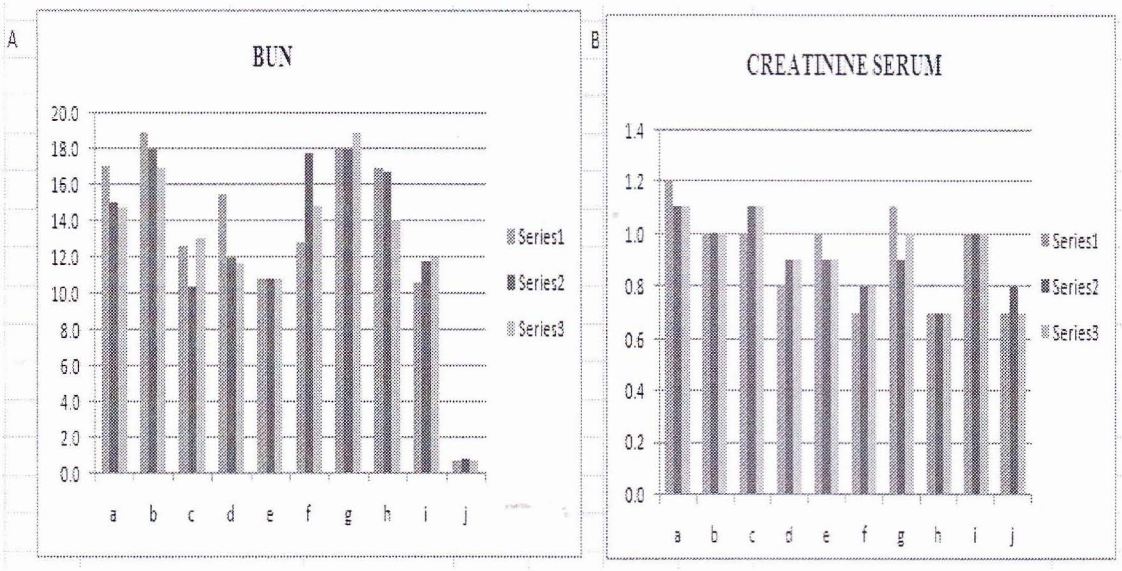

Fig. 4 : Chart (A) result of BUN evaluation; (B) result of SK evaluatiob. series 1 = 1st measurement of BUN and SK, series 2 = 2nd measurement of BUN and SK, series 3 = 3rd measurement of BUN and SK

DISCUSSION

Previous studies in other laboratories have shown that medium-level improvement of classic PKCs, including PKC-α and -β, may improve insulin responses by inducing early steps in the insulin signal transduction system. insulin receptor autophosphorylation, phosphorylation of IRS-1 and IRS-2, and phosphatidylinositol (PI) 3-kinase activation occur normally so that blood glucose reached nearly "normoglycaemia". In our study, PKC concentration was not significant increased after giving combination of Metformin and HBO therapy but the subjects' blood glucose were nearly "normo-glycemia". It presumable Metformin and HBO therapy had synergism effect in reducing hyperglycemia condition. HBO therapy has effect as same as exercise so that able to activate AMPK, thus glucose was able to use as ATP resource. However, specific mechanism of action combination Metfomin and HBO therapy remains to be determined.

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