Assessment of C-Reactive Protein and Reactive Nitrogen Species in Diabetic Patients

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Abstract

Angiotensin receptor antagonistes named as sartans are clinically used for treatment of hypertension, congestive heart failure and in certain complications of diabetes mellitus. Recently the anti-inflammatory effects of these agents was reported. The aim of this study was to explore the effect of sartans on C- reactive protein and reactive nitrogen species in patients with diabetes. A total number of 98 diabetic patients (32 males and 66 females) were enrolled in this study. The patients were subgrouped into group 1 (n=40); diabetic patients on sartans and group 2 (n=58) without sartans therapy. Blood samples were collected for determination of the C-reactive protein, Nitric oxide and peroxynitrate. The results showed that hypertension was co-existed in 23 and 13 patients of group1 and 2 respectively. There was no significant difference between group 1 and 2 in positive C-reactive protein. Serum nitric oxide level was higher and serum peroxynitrite was lower in group 1 as compared with group 2, and the differences did not reach to the significance level. It was conclude that sartans act via three arms; reducing blood pressure by blocking angiotensin II receptor and elevating nitric oxide and variable effects on the inflammatory bio-markers.

تقييم بروتين التفاعل سي وتفاعلات النتروجين الخاصة في مرضى السكري

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الخلاصة

تسمى مضادات مستقبلات ألانجيوتينسين بـــ(سارتان) تستعمل سريريا في علاج أرتفـــاع ضـــغط الـــدم وقصور القلب الاحتقاني (حالة تتميز بالضعف وقصور التنفس الناتج عن دورة الدم، غير الكافية في الانســـجة المحيطة والرئتين) وتستخدم مضادات الانجيوتينسين في معالجة مضاعفات معينة في أمراض السكر. وقد تـــم الاكتشاف بأن المضادات لهذه العوامل المرضية قد تكونت أو تشكلت مؤخرا. أن الهدف من هذه الدراسة هو من أجل معرفة تأثير السارتان على بروتين التفاعل سي وتفاعلات النتروجين الخاصة في مرضى السكري. وقـــد سجل في هذه الدراسة العدد الكلي 98 مريض (32 ذكر و66 أنثى) توزعـــوا علـــى مجمـــوعتين، خضـــعت المجموعة 1 (العدد = 40) للعلاج بالسارتان، ولم تخضع المجموعة الثانية العدد = 58 للعـــلاج بالســـارتان. وكانت عينات الدم التي تم الحصول عليها لتحديد بروتينات التفاعل سي، أوكسيد النتريك وبيروكسي النتـــرات. وقد أظهرت نتائج الدراسة بأن مرضى أرتفاع الضغط قد وجود في 23 و13 مريض في مجموعة 1 و2 على التوالي. لم يلاحظ وجود فروق معنوية بين المجموعة 1 و2 في مايتعلق بعدد الحـــالات بتفاعـــل البروتينـــات الالتهابية (≤ 6 ملغرام/ لتر)، ومستوى أوكسيد النتريك في المصل كان أعلى وبيروكسي النترات كان أوطىء

في المجموعة 1 مقارنتا بالمجموعة 2، والأختلاف لم يصل الى المستوى المعنوي. وتوصلت الدراسة الحاليـــة الى أن السارتان يعمل عبر ثلاث أذرع: تقليل ضغط الدم بواسطة مستقبل الأنجيوتينسي 2 المحصورة وأرتفاع أوكسيد النتريك وتأثيرات متغايره في مؤشرات الالتهاب والعلامات الالتهابية.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. It is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to the development of nephropathy, neuropathy and retinopathy. It is classified on the basis of pathogenic process that lead to hyperglycemia into type 1 (T1D) and type 2 (T2D). There is no doubt that some elements of inflammatory process are predisposed to DM or associated with it or resulted from longstanding disease. C-reactive protein (CRP) is a non specific inflammatory marker belongs to acute phase reactant well correlated with insulin resistance and its measurement will be useful for detection of metabolic syndrome in T2D. Diabetic patients with high CRP levels are more prone to cardiovascular events including stroke (1). Moreover, Low-grade inflammation is linked to insulin resistance and is involved in the pathogenesis of type 2 diabetes mellitus (2). There is accumulating evidence supporting the key role of nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^{\cdot}$), in the pathogenesis of diabetes and diabetic complication (3, 4, 5). Recently, a group of medication termed "sartans" are prescribed to diabetic patients to counteract the cardiovascular complications. These drugs act by blocking angiotensin II receptors in vascular smooth muscle and thereby producing vasodilation. The anti-inflammatory property of "sartans" has been studied in a number of *in vitro* and animal studies. This study was aimed to investigate the anti-inflammatory effect of "sartans" in patients with DM *via* determining the serum levels of C-reactive protein, nitric oxide and peroxynitrite in two groups of diabetic patients; with and without "sartans" therapy.

Materials and Method

This study was conducted in the Department of Pharmacology, College of Medicine in cooperation with the Department of Biology, College of Science, Al-Mustansiriya University and the Laboratories of Al-Yarmouk Teaching Hospital in Baghdad, Iraq. Known cases of diabetes mellitus (32 males and 66 females) were randomly allocated from private clinic to be admitted in the study. Each patient was examined physically by specialist and all information related to this research were obtained. At the time of the entry, the fasting serum glucose ranged between 90 and 380 mg/dl. Venous blood was obtained from each patients and the sera separated by centrifugation. The sera were kept at -80°C for further analysis. Worked tests according to the procedure found in C-reactive protein kit latex, (Human Gesellschaft Für Biochemica und Diagnostica mbH, Germany), Nitric oxide ($NO^{\cdot}$) donating activity was determined as described by Newaz and co-workers (6) and Peroxynitrite ($ONOO^{\cdot}$) mediated nitration of phenol was measured in serum extracts as described by Beckman and Van Uffelen (7,8).

Results and Discussion

Information about the patients were presented in Table 1. In respect to the gender 55% of group1 were females compared with 75.8% in group 2. The mean of age patients in Group 1 was not significantly ($p>0.05$) higher than corresponding mean of patients in group 2. Co-morbid illnesses of diabetes mellitus and hypertension were reported in 57.5% (23 out of 40) and 22.4% (13 out of 58) in Groups 1 and 2 respectively. There was a significant between difference groups ($p<0.001$). The duration of diabetes mellitus in each group was approximately similar.

Table (1) The characteristics of the study

Gender	Group 1 (n=40)	Group 2 (n=58)	Total (98)
Male: Female	18:22	14:44	32:66
Age (year)	60.84±7.77	57.63±8.2	58.89±8.15
History of hypertension (No.)	23	13	36
Duration of diabetes (years) (range)	5-25	5-22	5-25

There was no significant difference between Group 1 and Group 2 regarding the age and the duration of diabetes mellitus. Therefore the results reported in this work are not biased or attributed to the age factor or duration of diabetes. High blood pressure (hypertension) that complicated or co-existed with diabetes mellitus is found in 36 out of 98 (37%) in this study. This results were agreed with others (9,10,11,12). All patients in Group 1 were on sartans antihypertensive agents while those patients in Group 2 were either on short term antihypertensive agents not related to the sartans remedies or not received any antihypertensive agents because they are normotensives. Qualitative C-reactive protein test revealed that 35% of patients in group 1 had positive test compared to 41.3% in group 2 (Table 2). There was no significant difference between ($p>0.05$) different groups.

Table (2) Assessment of bio-inflammatory marker

C-reactive protein (>6 mg/L)	Group 1	Group 2
Female	10/22	14/44
Male	04/18	10/14
Total	14/40 (35%)	24/58 (41.3%)

Several studies considered the role of inflammation in the etio-pathology of diabetes mellitus and ischemic heart disease (13, 14, 15). Therefore, significant high level CRP (i.e. $\geq$ 6 mg/L) is an expected finding in this study. Furthermore hypertensive patients also showed significant high CRP in this study as well as in other studies (16,17). Accordingly, the low number of cases who had high serum CRP level in Group 1 could be explained in terms of the antihypertensive effect of sartans i.e. sartans exert two pharmacological effects; antihypertensive and anti-inflammatory. In this respect, the study limitations include: Small number of patients that enrolled in this study and The semi-quantitative method that is used in the determination of CRP. Serum nitric oxide was not detected in 5 patients (2 female and 3 male) out of 40 (12.5%) in group 1 compared with 8 patients (6 female and 2 male) out of 58 (13.8%) in group 2 (Table 3). The difference did not reach to the significant level. The serum level of peroxynitrite in group 1 exceeded the level of group 2 by 1 µmol and the serum nitric oxide level in group 1 was higher than corresponding level of group 2 by 10.2 µmol (Table 4). There was no significant difference in the serum levels of reactive nitrogen species; peroxynitrite and nitric oxide between group 1 and 2.

Table (3) Assessment of reactive nitrogen species

Serum peroxynitrite(µmol)	Group 1	Group 2
Female	15.609±12.654(n=22)	16.723±13.866 (n=43)
Male	21.029±13.521(n=17)	19.038±8.122 (n=13)
Total	17.972±13.148(n=39)	16.951± 12.752(n=56)
Serum nitric oxide (µmol)		
Female	206.44±67.065(n=19)	156.314±102.143(n=37)
Male	140.217±76.959(n=14)	112.984±108 (n=9)
Total	158.020±71.950(n=33)	147.837±103.54 (n=46)

***The results are expressed as mean ± SD of number of patients.**

There is no doubt that reactive oxygen and nitrogen species are involved in pathogenesis of DM or its complications. Sartans improve the bioavailability of NO and reduced the level of ONOO. This finding may explain the other mechanism of sartan in reducing blood pressure in Group 1 since NO is a potent vasodilator (18).

Table (4) Comparison between group 1 and 2 in the serum levels of biochemical and immunological variables in respect to the inflammatory process assessed by determination of C-reactive protein

Total number	Group 1		Group 2	
	CRP (+ve)	CRP (-ve)	CRP (+ve)	CRP(-ve)
	14	26	24	34
Serum peroxy nitrite level (µM)	10.87±6.32† (n=14)	21.94±14.36 (n=25)	20.27±17.02 (n=23)	14.57±8.15 (n=33)
Serum nitric oxide level (µM)	136.7±58.73 (n=11)	168.69±76.73 (n=22)	110.67±104.3†† (n=18)	171.72±97.52 (n=28)
Non-detected (No.)	3/14 (21.4%)	2/24 (8.3%)	3/21 (14.3%)	5/33 (15.2%)

† $p < 0.01$ compared with corresponding negative CRP test in Group 1 and positive CRP tes in group 2, †† p=0.05 compared with negative CRP test in Group 2.

Table 4 shows that sartans significantly suppressed serum peroxynitrite when there is inflammation (demonstrated by high serum CRP) and failed to show such effect when there is no inflammation. The opposite effect was observed with serum nitric oxide, i.e. serum nitric oxide is elevated in Group I when there is an inflammatory reaction.

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