

The Role of Glucose-6-phosphate Dehydrogenase in Coronary Artery Disease

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Background: Excess of oxygen-derived free radicals is known to predispose the vascular wall to development of atherosclerosis. In a variety of biochemical reactions involved in the elimination of these reactive oxygen species, glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme. However, the relationship between G6PD and atherosclerosis is not clear. In the present study we investigated the role of G6PD in human coronary atherosclerosis.

Methods: A total of 246 male participants receiving cardiac catheterization at our hospital from January 1998 to April 1999 were studied. Data of blood G6PD activity and the traditional risk factors for coronary artery disease were collected from each individual followed by analysis according to the severity of coronary artery disease.

Results: The frequency of G6PD deficiency in individuals having insignificant lesion (CAD (-)) was not statistically different from those with significant lesion (CAD (+); CAD(-) vs CAD (+), 6.3% vs 4.2%, $P>0.1$). Similar results were obtained in the analysis of CAD (+) subgroups, which were classified according to the number of the major coronary arterial branches involved (single-vessel disease (CAD1), 3.2%; double-vessel disease (CAD 2), 6.1%; triple-vessel disease (CAD 3), 5%; left main disease (LM), 0%; $P>0.1$). In addition, the levels of the G6PD enzyme activity of the individual groups and subgroups were similar to each other (CAD (-), 9.7 ± 2.7 ; CAD (+), 9.7 ± 2.2 ; CAD 1, 9.4 ± 1.8 ; CAD 2, 9.6 ± 2.5 ; CAD 3, 10.3 ± 2.7 ; LM, 9.5 ± 1.3 ; $P>0.1$).

Conclusion: From this study, we found that in males G6PD is not likely to play an important role in coronary atherosclerosis

Key Words: Glucose-6-phosphate-dehydrogenase, Human, Coronary artery disease, Male.

Introduction

Atherosclerosis is a leading cause of morbidity

and mortality in Taiwan and the other developed countries. This disease changes the normal structure of arterial wall long before manifestation of clinical symptoms, therefore, identification of mechanisms underlying its pathogenesis is important for early prevention of its initiation and progression. Evidence from a large number of studies in different fields indicates that atherosclerosis arises from interaction between a variety of factors, one of which involves for-

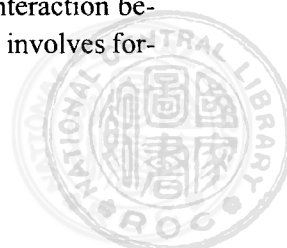
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mation of reactive oxygen species (known as oxidant stress) and its clearance. Under normal physiological conditions, oxidant stress in the vascular wall can be effectively eliminated by cooperation of antioxidants and their related enzymes. However in diseased vessels, the balance between oxidant stress and antioxidant defense is disturbed, which leads to accumulation of free radicals. This causes damage of cell structure and results in dysfunction or even death of cells. Elimination of oxygen-derived free radicals inside the cell involves several cascades of biochemical reactions, in which glucose-6-phosphate dehydrogenase (G6PD) plays a key role.

Glucose-6-phosphate dehydrogenase (G6PD) is an X chromosome-linked enzyme located in the cytoplasmic compartment that mediates production of the reduced form of the extramitochondrial nicotinate-adenosine-dinucleotide phosphate coenzyme (NADPH) by catalyzing the reaction from glucose-6-phosphate to 6-phospho-gluconate in the pentose phosphate pathway.¹ NADPH is in turn used by the cell to convert the oxidized glutathione (GSSG) to the reduced form (GSH), which has the ability to neutralize the toxic effects of hydrogen peroxide and organic peroxides.² Thus, inadequate G6PD activity influences the normal defense against oxidant stress. Such a defect has been confirmed to be associated with shortened survival of the cell. For example, G6PD deficiency increased the fragility of the erythrocytes and results in hemolytic anemia. In addition to red blood cells, other cell types, such as white blood cells and platelets,^{3,4} were also reported to have abnormal function. This indicates that G6PD is essential for maintaining the integrity of different cell types.

However, the relationship between G6PD and atherosclerosis is not clear. Although as mentioned above they both have direct links with the issue of oxidant stress, to the best of our knowledge, no studies to date have explored the role of G6PD in atherosclerosis. To this end, the present study set out to evaluate the level of G6PD activity in male patients of coronary artery disease.

Methods

Study Patients

From January 1998 to April 1999, two hundred and forty six male patients receiving coronary catheterization in the Mackay Memorial Hospital were enrolled into this study. Data from each individual, including coronary angiogram, blood G6PD activity, and the traditional risk factors for coronary artery disease, were collected. The patients were divided into groups according to the severity of coronary artery disease, and the values of G6PD activity in each group were compared.

Coronary Angiography

The severity of coronary artery disease was classified into 4 categories as follows: single-vessel, double-vessel, triple-vessel, and left main disease according to the involvement of the left main artery and the 3 major branches, left anterior descending artery, left circumflex artery, and right coronary disease. When a stenotic lesion existed with a luminal diameter less than 50 % of the adjacent unaffected site, the lesion was defined to be significant and the artery to be involved. Patients with normal coronary angiogram or having insignificant lesion were assigned to have insignificant disease.

Assessment of G6PD Activity

Patients were kept at fasting state for at least 10 hours, and then 1 ml of blood was collected in the catheterization room from the femoral artery. G6PD activity was determined using the method measuring the amount of NADPH reduced from NADP in the presence of glucose-6-phosphate plus the hemolysate.¹ According to the standard of the Mackay laboratory, G6PD deficiency was defined to be present when the value of G6PD activity was less than 7.0 U/g Hb.

Stratification of Risk Factors

The presence of the traditional risk factors, including obesity, hypertension, hyperlipidemia, dia-



Table 1. Baseline Characteristic According to Severity of Coronary Angiogram

| | CAD (-) N=80 | CAD (+) N=166 | CAD 1 N=62 | CAD 2 N=49 | CAD 3 N=40 | LM N=15 |
|-------------|-----------------|------------------|---------------|---------------|---------------|------------|
| Age (years) | 60±9 | 60±10 | 58±12 | 62±10 | 61±9 | 64±9 |
| HTN | 40 (50%) | 91 (55%) | 38 (61%) | 24 (49%) | 23 (58%) | 6 (40%) |
| DM | 16 (20%) | 54 (33%) | 16 (26%) | 12 (24%) | 18 (45%) | 8 (53%)* |
| HL | 20 (25%) | 61 (37%) | 27 (44%) | 15 (31%) | 16 (40%) | 3 (20%) |
| Smoking | 46 (58%) | 105 (63%) | 39 (63%) | 30 (61%) | 26 (65%) | 10 (67%) |
| Obesity | 15 (19%) | 33 (20%) | 14 (23%) | 10 (20%) | 7 (18%) | 2 (13%) |
| FM | 0 (0%) | 4 (2%) | 2 (3%) | 1 (2%) | 1 (3%) | 0 (0%) |

HTN = Hypertension; DM = Diabetes mellitus; HL = Hyperlipidemia; FM = Family history * P<0.005

Table 2. G6PD Deficiency and Severity of Coronary Angiogram

| | CAD (-) N=80 | CAD (+) N=166 | CAD 1 N=62 | CAD 2 N=49 | CAD 3 N=40 | LM N=15 |
|------------------------|-----------------|------------------|---------------|---------------|---------------|------------|
| G6PD deficiency | 5 (6.3%) | 7 (4.2%) | 2 (3.2%) | 3 (6.1%) | 2 (5%) | 0 (0%) |
| G6PD activity (U/g Hb) | 9.7 ± 2.7 | 9.7 ± 2.2 | 9.4 ± 1.8 | 9.6 ± 2.5 | 10.3 ± 2.7 | 9.5 ± 1.3 |

betes mellitus, smoking and family history of premature coronary artery disease, were judged from a patient's blood biochemistry and the admission record.

Obesity was defined when the patient's body mass index exceeded 27.8 kg/m².⁵ The criteria for hypertension followed the guideline of JNC VI, that is, either the diastolic blood pressure was greater than 90 mmHg, the systolic blood pressure was greater than 140 mmHg, or the patients were currently taking antihypertensive drugs. The criteria for hyperlipidemia was according to the guideline from the National Cholesterol Education Program (NCEP); hyperlipidemia was defined to exist when total serum cholesterol concentration was greater than 6 mmol/L (240 mg/dL), the low density lipoprotein component was greater than 4.2 mmol/l (160 mg/dl), or the patients were currently taking lipid-lowering agents. Diabetes mellitus was diagnosed when patients had symptoms of diabetes with a casual plasma glucose concentration greater than 200 mg/dl, or with either fasting plasma glucose concentration greater than 126 mg/dl or post-prandial two-hour venous plasma glucose exceeding 200 mg/dl.⁶ Family history of premature coronary artery disease was defined to be

present if the patient's father or other male first-degree relative had myocardial infarction or sudden death before 55 years of age, or before 65 years of age in the mother or other female first-degree relative.⁷

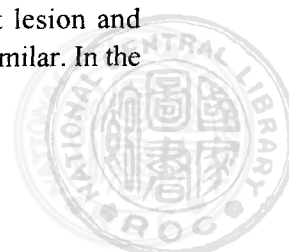
Statistical Analysis

Continuous data were expressed as mean ± SD and categorical variables as percentages. Data from each group were compared by chi-square test, t test or ANOVA test. When the p value < 0.05, it was considered statistically significant.

Results

Two hundred and forty six male patients, aged from 26 to 81 years (60 ± 10), were recruited into this study. The baseline characteristics according to the finding of the coronary angiogram are listed in Table 1. The profile of G6PD in each group is listed in Table 2.

As shown in Table 1, the age distribution between individuals having insignificant lesion and those with significant lesion was quite similar. In the



significant lesion group, though patients with single-vessel lesions were younger and those with left main artery lesions were older, the difference was not significant. Considering the distribution of risk factors, compared to the insignificant lesion group, each factor was more frequently seen in the significant lesion group. For diabetes, the frequency was elevated as more of the major coronary branches were involved. The highest frequency was found in those with left main artery disease ($P < 0.005$).

As far as G6PD was concerned (Table 2), the frequency of G6PD deficiency was higher in the significant lesion group, though not statistically significant. When the activity of G6PD was analyzed, we still could not find any relationship between the activity of G6PD and presence or severity of coronary artery disease.

Discussion

This study demonstrates that coronary atherosclerosis, either its presence or severity, is independent of G6PD activity in men. This result is based on the gold standard diagnostic tool, coronary angiography, performed in men of middle to old age. The distribution of traditional risk factors, more frequently seen in the stenotic lesion group, is consistent with those of large scale epidemiological studies.^{8,9} Thus, the participants in this study represent typical cases encountered in daily practice. Such a finding clarifies the relationship between G6PD activity and coronary artery disease.

Theoretically, G6PD may influence the development of coronary artery disease in several ways. For example, insufficient content of GSH due to inadequate G6PD activity may exist in vascular cells and cause overload of oxidant stress, and abnormal function of platelets associated with deficiency of this enzyme may favor thrombus formation. Both have potential impacts on this vascular disease. However, opposite to this hypothesis, Cocco et al. reported that, based on the mortality analysis, G6PD-deficient individuals were protected from ischemic heart disease.¹⁰ But, in contrast to the pres-

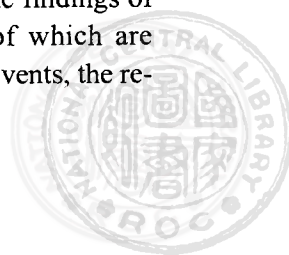
ent study, no information was given about the anatomy of the coronary artery. In the present study, we studied males only, since the female has a pair of X chromosomes and thus duplicate G6PD genes; if the two alleles are not identical, enzyme activity can fluctuate, which may influence the result. Our data showed that as a whole, the frequency of G6PD-deficient carriers is less than 5%, which is similar to the previous reports in Taiwan (2.6 to 5.47%).^{11,12,13} Since the number of carriers was limited, we then analyzed the enzyme activities and compared them between subgroups of different severity of the disease. The result did not support the existence of any link.

Although the negative association seems not in line with the oxidant stress concept, its interpretation requires careful consideration of the protecting strategies against reactive oxygen species in different cell types. In the body, elimination of the noxious effects of oxygen-derived free radicals can be achieved by several mechanisms. For example, the origin of oxygen-derived free radicals, superoxide anion, is detoxicated with antioxidants, such as vitamins C and E, and superoxide anion is converted to peroxide (by superoxide dismutase)¹⁴ and subsequently destroyed by either catalase or reduces glutathione (GSH). Since reaction with GSH is not the only way to clear peroxide, this may explain why the vascular cells were not affected in the present study. On the other hand, the red blood cells are reported to solely depend on the pentose phosphate pathway to generate NADPH.¹ Association of G6PD deficiency with the cell damage suggests that compensatory mechanisms do not function well in erythrocytes.

In conclusion, the present study shows that the level of G6PD activity is not likely to be linked to the genesis and progression of coronary atherosclerosis. The precise mechanisms protecting the vascular wall from the influence of different G6PD activity remain to be defined.

Study Limitations

Our study must be interpreted in light of certain limitations. Because it was based on the findings of coronary angiography, the severity of which are known to not faithfully reflect clinical events, the re-



sults should be carefully applied to the outcomes of coronary artery disease.

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葡萄糖-6-磷酸脫氫酶在冠狀動脈疾病所扮演的角色

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背景： 病兆動脈壁所產生過多的氧化自由基可造成動脈硬化。而氧化自由基能藉由生化反應消除，其中葡萄糖-6-磷酸脫氫酶為關鍵催化劑。然而葡萄糖-6-磷酸脫氫酶與動脈硬化的關係仍未知曉，因此本篇研究將評估男性冠狀動脈疾病嚴重程度與葡萄糖-6-磷酸脫氫酶活性的關係。

方法： 分析從民國 88 年元月至 89 年四月於馬偕醫院接受心導管檢查的 246 位男性病人，搜集每位患者接受心導管檢查，血液葡萄糖-6-磷酸脫氫酶活性，及傳統冠狀動脈危險因子的資料。我們比較冠狀動脈疾病的嚴重程度與葡萄糖-6-磷酸脫氫酶活性的關係。

結果： 我們發現缺乏葡萄糖-6-磷酸脫氫酶的頻率在冠狀動脈血管正常[CAD(-)]及不正常[CAD(+)]的族群或冠狀動脈疾病次族群間沒有差別。[CAD(-): CAD(+), 6.3%: 4.2%, $P > 0.1$ ；單一血管疾病(CAD 1), 3.2%；二條血管疾病(CAD 2), 6.1%；三條血管疾病(CAD 3), 5%；左主枝血管疾病(LM), 0%; $P > 0.1$]。除此之外，葡萄糖-6-磷酸脫氫酶活性的值在各族群及次族群間亦有類似的結果[CAD(-): 9.7 ± 2.7 , CAD (+): 9.7 ± 2.2 , CAD 1: 9.4 ± 1.8 , CAD 2: 9.6 ± 2.5 , CAD 3: 10.3 ± 2.7 , LM: 9.5 ± 1.3 ; $P > 0.1$]。

結論： 由本篇研究顯示，我們發現葡萄糖-6-磷酸脫氫酶在男性中，對於動脈硬化似乎並不扮演著重要的角色。

關鍵詞： 葡萄糖-6-磷酸脫氫酶；冠狀動脈疾病；人類；男性。



Comment

Glucose-6-phosphate Dehydrogenase Level and Coronary Artery Disease

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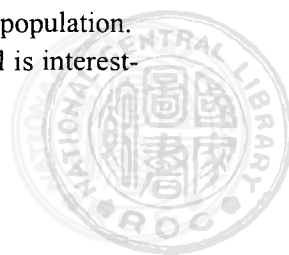
The over-production of reactive oxygen species or oxygen-derived free radicals (known as oxidative stress) is hazardous to various forms of cell. In the vascular wall, this phenomenon can lead to the damage of endothelial cells and cause the peroxidation and penetration of LDL into the intima layer, which thus produce the cascade of atherogenesis. Under normal physiological condition, the oxidative stress can be effectively removed by anti-oxidants and other related scavenger enzymes. For example, the superoxide anion can be detoxified by several anti-oxidants i.e. vitamin C and E, or converted to peroxides by superoxide dismutase and subsequently destroyed by catalase or reduced form glutathione (GSH).

In recent years, several interventional studies supported the hypothesis that anti-oxidant therapy could prevent or delay the development of atherosclerosis, although there are still some conflicting results.¹ Ascorbic acid has been reported to be able to reverse endothelial vasomotor dysfunction in the brachial circulation of patients with coronary artery disease, suggesting that oxidative stress contributes to endothelial dysfunction in atherosclerosis that may respond to anti-oxidant therapy.² Vitamin E has also been reported with some anti-oxidation effect on LDL and thus play a role in the prevention of atherosclerosis. However, recent clinical trials could not support its value in short-term preventing the progress of atherosclerosis.³ Therefore it is too early to suggest the usage of those substances for coronary artery disease prevention.⁴

The oxidative stress and anti-oxidant balance is

disturbed in atherosclerosis. Many investigators continue pursuing new evidences of the role of oxidative stress in the atherogenesis. In this issue of Journal, Peng MC et al presented an intriguing study on the association between glucose-6-phosphate dehydrogenase (G6PD) level and activity and coronary atherosclerosis.⁵ As we know, G6PD is a cytoplasmic enzyme mediating the production of NADPH by catalyzing glucose-6-phosphate to 6-phosphate-glucanate in the pentose phosphate pathway. NADPH can facilitate the conversion of oxidized glutathione (GSSG) to its reduced form (GSH), which is able to neutralize the toxic effects of hydrogen peroxide or other organic peroxides. Therefore it was hypothesized that the G6PD level or activity might be lower in patients with coronary artery disease (CAD). In Peng's report, 166 patients with angiographically proved CAD were recruited. Their fasting plasma G6PD level and activity were assayed and compared to 88 non-CAD controls. Although CAD group had significant traditional risk factors, they did not show significantly lower G6PD level or activity as compared to the control group. Thus, they made a conclusion that G6PD might not be an important risk factor in the coronary atherosclerosis. In a similar study, Yegin A et al found that the activity of G6PD was not different between CAD and non-CAD patients, but the activity of selenium-dependant glutathione peroxidase and erythrocyte selenium level were decreased in 37 angiographically proved CAD. The activity also paralleled the severity of CAD.⁶ However, the data needs to be confirmed in a large population.

Peng's study in this issue of Journal is interest-



ing, but has some limitations. First, the CAD group showed strong traditional risk factors which might masked the effect of G6PD on atherogenesis, since G6PD might just play a minor role. If their CAD group could be classified into subgroups with high or low risk factors, the significant of G6PD might become marked in the low risk subgroup. Second, the statistics is weak. Univariate or multivariate regression analysis are needed to clarify the relation between G6PD and CAD. Third, as in Yegin 's study, more markers needs to be evaluated in their CAD group.

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