Mutual Interference of Hemoglobin Glycation and Carbamylation

Thesis

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Abbreviations

2hPG	2 hours plasma glucose
5HMF	5-hydroxymethylfurfural
ADA	American Diabetes Association
ARF	Acute renal failure
ACE	Angiotensin converting enzyme
BUN	Blood urea nitrogen
CarHb	Carbamylated hemoglobin
CDA	Canadian Diabetes Association
CO_2	Carbon dioxide
CO	Carbon monoxide
CVD	Cardiovascular disease
CHF	Chronic heart failure
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disorder
DM	Diabetes mellitus
DKA	Diabetic ketoacidosis
ESRD	End-stage renal disease
EPO	Erythropoiten
ECF	Extracellular fluid
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
GFR	Glomerular filtration rate
GHb	Glycated hemoglobin
GH	Growth hormone

HSP	Heat shock protein
Hb	Hemoglobin
HbA1c	Hemoglobin A1c
HPLC	High performance liquid chromatography
IDDM	Insulin Dependent Diabetes Mellitus
IGF-1	Insulin-like growth factor-1
IFNγ	Interferon gamma
IL-1β	Interleukin-1β
kDa	kilo Dalton
LBM	Lean body mass
MetS	Metabolic syndrome
NO	Nitric oxide
NKHS	Non-ketotic hyper-osmolar state
OGTT	Oral glucose tolerance test
O_2	Oxygen
PTMs	Post translational modifications
RBCs	Red blood cells
RAAS	Renin-angiotensin-aldosterone system
$NaHCO_3$	Sodium bicarbonate
TNFα	tumor necrosis factor α
T1DM	Type 1 diabetes mellitus
UKM	Urea kinetic modeling

INTRODUCTION

Diabetic nephropathy is a major complication of diabetes mellitus. Many studies have shown that in many regions of the world, including Egypt, diabetic nephropathy is the main cause of end-stage renal disease ***(Ritz et al., 1999; Lee, 2003; Afifi et al., 2004)***.

The high blood glucose concentration in patients with diabetes mellitus leads to abnormally high incidence of protein glycation. Post-translational modification of hemoglobin by non-enzymatic glycation occurs in the circulating red blood cells. Determination of glycated hemoglobin in diabetic patients is currently acknowledged as the most reliable indicator for assessment of retrospective glycemic control and the planning of clinical management ***(Bernstein, 1987; Shojania et al., 2006; Guerin et al., 2007; Nicholls et al., 2008; Mannucci et al., 2009; Selvarai et al., 2009)***.

Non-enzymatic carbamylation of hemoglobin is another type of post-translational modification, caused by blood urea. Impaired kidney function has always been associated with increase in blood urea concentration and carbamylation of proteins ***(Hörkkö et al., 1992; Hörkkö et al., 1994; Balion et***

al., 1998; Berlyne, 1998). It has been documented that carbamylation contributes to uremic toxicity and that carbamylated proteins are implicated in the atherogenesis of chronic renal failure ***(Hörkkö et al., 1994; Roxborough and Young, 1995)***.

Carbamylated hemoglobin is the most simple, useful and reproducible index for measuring the carbamylation reaction in vivo. Its level reflects the intensity of uremic toxicity and it has a role in the clinical management of patients with renal failure similar to the way glycated hemoglobin is used in the monitoring of diabetic patients ***(Flückiger et al., 1981; Oimomi et al., 1984; Davenport et al., 1993; Frazao et al., 1995; Davenport et al., 1996; Han et al., 1997; Tarif et al., 1997)***.

Since both carbamylation and glycation reactions involve the free amino groups of the protein, especially the terminal valine residues in the case of hemoglobin, the possible interference of the two reactions should be considered ***(Hammouda and Mady, 2001; Szymezak et al., 2009)***. Would the kinetics of carbamylation remain the same in the presence of high blood glucose concentration, and would the glycation kinetics be the same in presence of high blood urea concentration? In this study, we tried to find an answer for the previous question. This is particularly important when

managing diabetic patients with chronic renal failure as in these patients; two related problems are monitored with two parameters that might be affected by each other.

AIM OF THE WORK

The aim of this work is to quantify the effect of hemoglobin glycation on the measured carbamylated hemoglobin and the effect of hemoglobin carbamylation on the measured glycated hemoglobin in hemoglobin obtained from normal subjects and incubated with varying concentrations of glucose and urea.

DIABETES MELLITUS (DM)

Diabetes is a metabolic disorder characterized by resistance to the action of insulin and/or insufficient insulin secretion ***(Longo et al., 2002)***.

The estimated number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in year 2025 and the prevalence in the world is expected to rise from 4% to 5.4% by the year 2025 ***(Ltief et al., 2003)***. It is estimated that by the year 2030, Egypt will have at least 8.6 million adults with diabetes. Diabetes is the eleventh most important cause of premature mortality in Egypt, and is responsible for 2.4% of all years of life lost. Similarly, diabetes is the sixth most important cause of disability burden in Egypt ***(Shaw et al., 2010)***.

Etiologic classification of diabetes mellitus: ***(ADA, 2011)***.

I. Type 1 diabetes which results from β-cell destruction, usually leading to absolute insulin deficiency. It includes immune-mediated and idiopathic diabetes.

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a