Letters to the Editor

not affected by hemolysis and are suggested when hemolysis is suspected.

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References


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High Glucose Upregulates C-Reactive Protein Synthesis in Macrophages

To the Editor:

C-reactive protein (CRP)1 released from hepatocytes during the acute-phase response is a diagnostically sensitive systemic marker for inflammation; CRP also demonstrates substantial proinflammatory effects (1). Although CRP might have an important role in the pathogenesis and prediction of coronary heart diseases (2), the factors influencing its concentration are not yet well understood. Recent histologic investigations have demonstrated that CRP is present in the human arterial intima of atherosclerotic lesions (3) and is located in macrophages of the arterial plaque. In addition, macrophages have been shown to produce CRP mRNA (4).

Diabetes mellitus is associated with premature and accelerated atherosclerosis (5), and hyperglycemia accelerates atherosclerosis by inducing vascular dysfunction and an increased inflammatory burden. Our goal was to analyze the possible modulation of CRP production in macrophages after their exposure to high glucose concentrations.

J-774A.1 macrophage-like cells (ATCC) were plated in DMEM with 100 000 U/L penicillin, 100 mg/L streptomycin, and 2 mmol/L glutamine (P/S/G), and used within 7 days. THP-1 human monocyte cells were maintained in RPMI-1640 medium with P/S/G, and macrophage induction was achieved by incubation with phorbol myristate acetate. Cells were incubated with either 5–40 mmol/L glucose for 18 h or with 22 mmol/L mannitol (an osmotic control that did not appreciably affect CRP cellular production).

We extracted cellular RNA with MasterPure™ RNA Purification Kit (Epicentre Biotechnologies) and prepared cDNA with the Verso™ cDNA Kit (Thermo Scientific) according the manufacturers’ instructions. CRP production was measured by quantitative real-time PCR by means of Rotor-Gene 6000 (Corbett Life Science/Qiagen) amplification with ABSolute Blue QPCR ROX Mix (Thermo Scientific) and primers and probes for the CRP2 (C-reactive protein,

1 Nonstandard abbreviations: CRP, C-reactive protein; P/S/G, 100 000 U/L penicillin, 100 mg/L streptomycin, and 2 mmol/L glutamine.

2 Human genes: CRP, C-reactive protein, pentraxin-related; ACTB, actin, beta.
pentraxin-related) and ACTB (actin, beta) genes (PrimerDesign) (6).

CRP in sections on slides was stained with antihuman CRP antibody (Sigma–Aldrich) and subsequently counterstained with secondary antibody (antimouse fluorescein isothiocyanate–conjugated IgG; Sigma–Aldrich) (7). All control sections were processed without primary antibody. The slides were photographed with the aid of a fluorescence digital microscope camera [Zeiss Axioskop 2 plus microscope; Image-Pro Plus 6.0 (Media Cybernetics)]. Light intensity and contrast were calibrated with an appropriate control section. Image-Pro Plus 6.0 software (Media Cybernetics) was used to quantify CRP on the slides, and CRP was measured by ELISA (Mouse CRP 96-well ELISA; Life Diagnostics) as recommended by the manufacturer. Lactate dehydrogenase was measured to assess cell viability, and incubation with 5–40 mmol/L glucose did not affect cell viability. Analysis of variance was used for statistical analyses. Results are expressed as the mean (SD).

THP-1 macrophages incubated with 30 or 40 mmol/L glucose exhibited increased production of CRP mRNA [61% and 109%, respectively; mean ratio of CRP mRNA to β-actin mRNA, 1.144 (0.08) and 1.487 (0.09), respectively], compared with cells incubated with only 5 mmol/L glucose [ratio of CRP mRNA to β-actin mRNA, 0.71 (0.03)]. J-774A.1 mouse macrophages incubated with 40 mmol/L glucose increased CRP mRNA production by 313% [ratio of CRP mRNA to β-actin mRNA, 1.28 (0.05)], compared with cells incubated with only 5 mmol/L glucose [ratio of CRP mRNA to β-actin mRNA, 0.31 (0.01)].

When macrophages were incubated with increasing glucose concentrations (5–40 mmol/L) for 18 h and analyzed by immunohistochemistry for CRP protein content, the intensity of fluorescein isothiocyanate staining of the cells was proportional to the detected CRP concentration. Cells incubated with 20, 30, or 40 mmol/L glucose exhibited increased cellular CRP concentrations (19%, 48%, and 103%, respectively), compared with control cells incubated with 5 mmol/L glucose (Fig. 1). Measured CRP concentrations in media harvested from incubated macrophages treated with 40 mmol/L glucose were significantly higher (by 2.5-fold, 8.8 μg/L) than the concentrations in media harvested from incubated macrophages treated with 5 mmol/L glucose (3.5 μg/L).

We hypothesize that CRP secreted by arterial macrophages could be up-regulated under atherogenic conditions such as diabetes and could therefore actively accelerate inflammatory processes in atherosclerotic lesions. Macrophages exposed to high glucose concentrations modulate the regulation of cellular CRP expression and CRP protein biosynthesis and secretion. Therefore, proinflammatory effects mediated by CRP in the arterial wall could be triggered by CRP secreted locally by macrophages, in addition to the effect of circulating, hepatic-derived CRP. Use of pharmacologic therapy to modulate CRP synthesis at the site of the atherosclerotic lesion possibly could reduce the inflammatory cascade markedly during atherosclerosis development and hence help prevent coronary heart disease.

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To the Editor:

The US Food and Drug Administration (FDA)\(^1\) has issued a second alert to warn of serious errors with certain blood glucose–monitoring strips that use glucose dehydrogenase pyrroloquinoline quinine (GDH-PQQ) methods (1). Various nonglucose sugars, including maltose, galactose, and xylose, increase the glucose concentrations reported by these methods. Nonglucose sugars are present in a variety of pharmaceutical preparations (1), including Extranéal (icodextrin) peritoneal dialysis solutions (2) and various immunoglobulin preparations (3). At least 13 deaths associated with erroneous meter measurements of glucose in patients receiving such pharmaceutical preparations have been reported to the FDA.

A 76-year-old man at our institution had 3 blood glucose readings that unexpectedly were >22.2 mmol/L (>400 mg/dL) on a point-of-care (POC) testing meter that used a GDH-PQQ method (Roche Accu-Chek Inform). By contrast, glucose measurements made with a hexokinase method (Abbott Archit) at about the same times were in the range of 7.2–11.2 mmol/L (129–202 mg/dL), suggesting the possibility of a positive interference in the POC method. The patient’s medication list contained none of the pharmaceutical preparations listed in the FDA alert as producing interference in the POC testing method and none that were known to contain nonglucose sugars.

In a search for other sources of interfering compounds that might contribute to this patient’s high POC glucose readings, we investigated his dietary intake. The patient had been receiving Nepro\(^2\)/Carb Steady\(^2\) (NPS), an Abbott Nutrition product that contains, among other ingredients, maltitol and Fibersol\(^3\), a modified maltodextrin. Neither maltitol nor Fibersol was listed in the FDA alert or in the product insert as an interference in the GDH-PQQ method, but consideration of their chemical structures and the method of Fibersol manufacture led us to investigate their possible interference in the GDH-PQQ method.

We obtained a bottle of NPS solution from hospital stores, maltitol from Sigma-Aldrich, and Fibersol from Matsutani America. The latter 2 compounds were dissolved in whole blood from a healthy donor to make stock solutions of 5 g/L, similar to the stated concentration of Fibersol in NPS (approximately 6.25 g/L). Increasing volumes of the resulting maltitol or Fibersol solutions, or of NPS, were added to separate portions of whole blood from the same donor. In all steps that involved dissolving solids or mixing liquids, samples were rocked on a Nutator mixer (BD) for 5 min to ensure homogeneity. Glucose was measured in samples of whole blood by use of the Roche meter and in the separated plasma by the hexokinase method.

Both NPS and Fibersol produced results like those seen in the patient (Fig. 1). As expected, NPS and Fibersol had little effect on the results of the hexokinase method, but each increased the glucose concentration reported by the POC

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1 Nonstandard abbreviations: FDA, US Food and Drug Administration; GDH-PQQ, glucose dehydrogenase pyrroloquinoline quinine; POC, point of care; NPS, Nepro\(^2\)/with Carb Steady\(^2\).

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