Measurement of the Total Antioxidant Activity of Human Aqueous Humor

To the Editor:
Oxidative stress has been implicated in the etiology of a large number of human long-term degenerative diseases including cataract (1). The free radicals that damage cellular macromolecules, producing oxidative stress, are scavenged in the human body by a range of antioxidant enzymes and small molecule antioxidants. The pivotal role that micronutrients such as vitamins C and E and flavonoids play in human antioxidant defenses has generated a great deal of interest in assays that will provide a rapid diagnostic tool to measure the total antioxidant activity of clinical samples such as plasma and other biological fluids.

One such colorimetric assay, the Total Antioxidant Activity (TAA) assay (2), is based on the antioxidant potential of pure compounds and biological fluids to both quench and inhibit the formation of a colored radical cation produced by the action of metmyoglobin and hydrogen peroxide on 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid; ABTS). This assay has been used to measure the antioxidant capacity of a wide range of dietary antioxidants, such as phenolics (3) and biological fluids (4, 5). Recently, however, a study using a proprietary kit to measure TAA on a centrifugal analyzer has raised doubts about the suitability of this assay for serum/plasma and, by implication, other biological fluids (6). In the light of this study, we have investigated the suitability of the assay to measure the total antioxidant activity of human aqueous humor as part of a larger study into cataract development.

Aqueous humor was collected from cataract patients attending the Ophthalmology Clinic of the West Norwich Hospital for surgery. The procedure was approved by the Norwich and District Ethics Committee. We used the manual spectrophotometric assay to determine the TAA of experimental samples and a reference antioxidant, the water soluble analog of vitamin E, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The kinetics of the quenching of the ABTS radical cation was followed at 734 nm, using a DU-70 UV/visible spectrophotometer (Beckman Instruments) with a temperature-controlled cuvette holder. TAA values were calculated from a Trolox standard curve that showed a linear relationship ($r^2 = 0.997$) between concentration (0–2.5 mM) and the length of the lag phase before the production of the ABTS radical cation.

Our results (Fig. 1.) show that the production of the ABTS radical cation in the presence of human aqueous humor follows lag phase kinetics consistent with this fluid acting as a sacrificial antioxidant. The kinetics are comparable with the published data on Trolox and ascorbate (6) and have been confirmed by ourselves. Our results also show that the assay responds in a predictable manner to two- and fourfold dilution of the aqueous humor, i.e., the lag phase decreases by two- and fourfold, respectively. This is in contrast to human serum that, in agreement with the published data (6), we find does not behave in such a predictable manner when diluted.

In the above study (6), the authors highlighted two main shortcomings of the TAA assay for determining the total antioxidant status in serum and other biological fluids. The kinetics of ABTS radical cation generation in the presence of serum were different from those of the calibrator (Trolox) used in the standard curve, and the assay did not respond in a predictable manner to dilution of the serum. They suggested that these shortcomings were primarily due to the interaction of the protein human serum albumin, the primary antioxidant in serum (4), with the ABTS radical cation. In contrast, human aqueous humor contains practically no protein, and one of its principal components is ascorbate (7). In our study, we have found that the kinetics of ABTS radical cation production in the presence of human aqueous humor were identical to the kinetics of both the standard curve calibrator, Trolox, and one of the principal components of that fluid, ascorbate. We also found that the assay responded in a predictable manner to dilution of the aqueous humor.

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References
Cardiac Troponin I in Myocardial Contusion

To the Editor:

Myocardial contusion (MC) is defined as cellular damage that results from nonpenetrating chest trauma. MC is the most common cause of morbidity and mortality in patients who have had chest trauma. Diagnosis of MC is laborious, and chest pain, dyspnea, and nonspecific electrocardiograph changes are commonly present in patients with chest injury. Although transesophageal echocardiography (TEE) can provide high quality images for diagnosis (1, 2), noninvasive diagnosis of this lesion has been always problematic. Cardiac enzyme activity is not commonly used for MC diagnosis. In recent years, the development of assays using new, highly cardiac-specific proteins has opened new frontiers for the diagnosis of myocardial cell damage. Cardiac troponin I (cTnI) is a small filament-associated regulatory protein of muscle, uniquely specific for the heart (3). cTnI is not expressed by developing or diseased human skeletal muscles, even when the skeletal muscle creatine kinase MB isoenzyme (CK-MB) concentration is increased (4).

We measured cTnI and CK-MB mass by immunoassay (Stratus, Dade International) in 28 consecutive patients (23 men and 5 women; ages 43.8 ± 20.5 years, mean ± SD) admitted for chest trauma; patients with previous histories of cardiac diseases were excluded.

After admission, patients underwent a TEE, and the images were interpreted by an expert echocardiographer who was unaware of the biochemical results. The presence of a segmental wall-motion abnormality, which resolved on a subsequent echocardiogram, or a fixed segmental asynergy associated with the development of new Q waves on the electrocardiogram were used as the prospective criteria for diagnosing MC. Kinetics of the right ventricular antero-apical wall and the left ventricular apex were particularly examined (5). Serial blood samples were obtained from patients 6, 12, 24, 48, and 96 h after chest injury. Five patients showed evidence of MC (18%) by TEE imaging; among these, temporary regional dysfunction was documented in four patients, whereas only one patient showed a fixed regional asynergy associated with the appearance of new Q waves. In all five patients, serum cTnI and CK-MB were increased, with medians and ranges that were 2.4 μg/L (1.2–5.5 μg/L) and 14.5 μg/L (6.9–24.8 μg/L), respectively. No myocardial lesions were found by TEE investigation in the remaining 23 patients. Within this group, cTnI was undetectable (<0.4 μg/L) in 16 patients; in the other patients, the median cTnI concentration was 0.8 μg/L (0.5–1.1 μg/L). The CK-MB concentration was within the reference range (<6 μg/L) in 10 of these 23 patients but...