Modification of simple and specific test for measuring lipid peroxides in plasma

In our paper, we used and recommended an iodometric (spectrophotometric) test to measure lipid peroxides in human plasma samples. The use of a commercially available colour reagent (CHO-iodide; Merck, Germany) made the technique very simple and easy to use.

Not long after the paper had been published, we and others have had difficulties performing the test as it was described. Basically, the saline treated (control) sample resulted in higher absorbance than the colour reagent (pont (test) sample. This was due to some as yet unidentified changes in the CHO-iodide reagent. The colour reagent presently available somehow quenches the original absorbance of plasma due to yellowish carotenoids. All our efforts to identify and eliminate this interference or change the protocol while measuring the formed I₂ at 360 nm have failed.

We therefore adopted a technique, which measures iodine in the presence of starch as a blue colour iodine-starch complex at 560 nm, where the natural yellowish colour of plasma does not interfere with the measurement. This modification has been in use for six months with different batches of CHO-iodide and produced reliable estimate of the overall oxidative capacity of plasma.

Reagents:
CHO-iodide (Merck, Germany). Butylated hydroxy toluene (BHT): 0.572 mg/ml in ethanol. Sodium thiosulphate solution from Sigma, No. S-2630: A 1% solution is made freshly in 6.7 mM sodium chloride (39.2 mg/100 ml) by immersing into boiling water for a few minutes. After centrifugation (3 minutes at 12 000 rpm, Eppendorf centrifuge), the clear supernatant fluid is used the same day.

Procedure: Blood is taken into EDTA (1.5 mg/ml), centrifuged, and the plasma samples kept at 4°C for no longer than two days. In a small phial, place 200 µl plasma, 1.0 ml colour reagent (CHOD), 10 µl BHT and 200 µl starch solution. Mix and transfer into a disposable cuvette and read the absorbance immediately at 560 nm against distilled water. Place the cuvette immediately into dark and then read the absorbance again at 60 minutes. The difference in the absorbance represents the peroxide content of plasma. Direct calculation by using molar absorptivity is difficult due to the variation of the absorption with the source of starch. However, for one batch, the system can be calibrated by hydrogen peroxide, according to the stoichiometry of the reaction:

\[
\begin{align*}
(1) \quad H_2O_2 + 2I^- + 2H^+ & \rightarrow I_2 + H_2O \\
(2) \quad I_2 + I^- & \rightarrow 2I^-
\end{align*}
\]

Absorption changes are linear between the amounts of peroxide added (0.2–1 nmol) and the I₂ produced.

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Parathyroid hormone related protein (PTHrP) in hypercalcaemia of lymphoproliferative disease

Bolo-Deoku et al have shown that PTHrP was not implicated in a case of hypercalcaemia in Hodgkin's disease, thus expanding the very limited literature on PTHrP in lymphoproliferative disorders reported. A case of hypercalcaemia in chronic lymphocytic leukaemia (CLL) where the same conclusion was reached.

Eight years after presentation, a patient with type IV C - B-CLL developed severe acute low back pain due to vertebral body collapse. Generalised loss of bone density was apparent radiologically, but no discreet osteolytic lesions were found. Corrected serum calcium concentration was 2.94 mmol/l (range 2.26–2.60), phosphate activity 1.31 mmol/l (range 0.8–1.4), alkaline phosphatase 84 IU/l (range 30–150), and renal function was normal. Vigorous intravenous hydration and intensified treatment with corticosteroids failed to control the hypercalcaemia and a peak corrected serum calcium of 3.29 mmol/l was reached before effective treatment with pamidronate sodium was started. Prolymphocytoid transformation and myeloma were excluded and there was no evidence of coincidental malignancy. Serum 25-hydroxycholecalciferol was 11.8 ng/ml (range 8–50), PTH 1.7 pg/ml (range 0.9–5.4), and PTH-related protein (PTHrP) was less than 0.23 pmol/l (normal).

While lymphomatous tumour necrosis factor β, interleukins 1 and 6, and prosta- glandins have a well defined role in the bone disease of myelomatosis, the mechanisms underlying dysfunctional osteoclast activity in other lymphoproliferative disorders are unknown. Although larger studies must be performed, it seems unlikely that PTHrP has an important role.

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