Antibody Inhibition Procedure for Myocardial (CK-MB) isoenzyme Determination by a Modified Program on the Technicon RA-1000 Analyzer, K. Itiaba, R. Sanfaçon,1 M.-W. Hsiung, and J. C. Crawhall (Div. Clinical Biochemistry, McGill University Clinic, Royal Victoria Hospital, Montreal, Quebec, Canada H3A 1A1)

The method of choice for the determination of the CK-MB isoenzyme is the antibody-inhibition procedure (1–2). CK-MB is the usual isoenzyme found in human serum. Its release is accepted as one of the best indicators of myocardial necrosis (3). The Technicon RA-1000 analyzer is easy to operate and well-suited for enzymatic assays.

Here we compare the antibody-inhibition procedure for CK-MB in the RA-1000 with the manual-ultraviolet method used in the Unicam SP-800 spectrophotometer. The same reagent was used for both procedures (Boehringer Mannheim, Canada) and, for ideal comparison, antibody and substrate concentrations were also made the same. Sample/antibody concentrations were also similar and identical dilutions were made whenever the total CK exceeded 1000 U/L (i.e., allowing maximum inhibition of CK-MM isoenzyme activities at all times).

We determined total CK in the RA-1000 (procedure unmodified). The RA-1000 program for CK-MB isoenzyme was used with blank correction and the initial steps of sample and first-reagent additions were replaced by sham operations (4% and 70% minimum acceptable values, respectively). The required antibody-inhibition period of 10 min was satisfied with incubation periods of 6 min outside and about 4 min within the RA-1000 instrument (1A delay of 30 s and A2 delay of 3 min 45 s).

We first added the sample (20 µL) and the first reagent (500 µL) manually to the RA-1000 cuvettes, and then incubated the mixture for the specified time. We performed the manual-ultraviolet procedure, as described elsewhere (2), with the antibody-incubation step taking place in a temperature-controlled cuvette compartment.

We tested reproducibility at enzyme activities between 8 and 150 U/L and found CVs of 1.5% to 9.4%; inter-day CVs for the BMC commercial control were also assessed and found to be ≤3%. We found good correlation (r = 0.89) between the two procedures (Figure 1).

This semi-automated procedure simplifies estimation of CK-MB activity and also increases the reliability and speed as compared with the manual-ultraviolet method. We have used the procedure extensively in the emergency and intensive-care areas during the past three years with satisfactory results where rapid confirmation of myocardial infarction was essential.

References

Relationship between Serum Cholinesterase Activity and Duration of Succinylcholine Action in Subjects with the “Usual” Phenotype for the Enzyme, Doğan Yücel,1 Sadık Top,1 Özcan Erdemli,2 and Hamdi Öğüş3 (1 Clin. Biochem. Lab. and 2 Dept. of Anaesthesia and Reanimation, Türkiye Yüksek İhtisas Hastanesi, Ankara, Turkey; 3 Dept. of Biochemistry of Hacettepe University, Ankara, Turkey)

Measurement of serum cholinesterase (ChE, EC 3.1.1.8) is important in predicting whether prolonged apnea may take place after administration of the widely used anesthetic, succinylcholine (SCh), a short-acting muscle relaxant. Although decreases in this activity owing to an inherited enzyme abnormality or an acquired condition are associated with most cases of prolonged apnea, subjects with the “usual” (most common) ChE phenotype also may encounter SCh apnea (1–3).

We determined the reference interval for ChE activity in 150 healthy blood donors (75 women, 75 men), measuring ChE activity manually by a Selected Method (4), with propionylthiocholine as substrate. Because the frequency distribution of ChE activity in these subjects was not normal (gaussian) but log-normal (the histograms were positively skewed in both groups), we calculated the reference intervals by using the logarithms of the measurements; the reference intervals for women and men were 4.61–11.0 kU/L and 5.3–11.3 kU/L, respectively. This difference between groups was statistically significant, but not strongly so (0.025 < P < 0.05). Combining the data for the two groups gave a reference interval of 4.94–11.23 kU/L or about 5.0–11.0 kU/L.

The decision (cutoff) value of ChE activity for SCh apnea was calculated as log X = 3 log SD, or 4.0 kU/L, whereas Dietz et al. (4) used X = 2.5 SD for this value. The ranges for the dibucaine and fluoride numbers for the “usual” phenotype were 78–90 and 75–82, respectively.

SCh, 1 mg/kg, was given intravenously to our surgical patients in the gastroenterology and urology operating room of our hospital, and the duration of action (the time from cessation of administering SCh to recurrence of spontaneous

Fig. 1. Correlation between CK-MB activities by the manual-ultraviolet and Technicon RA-1000 semi-automated procedures

Linear regression equation: y = 1.03x; r = 0.89

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HPLC Determination of Etofenamate and Flufenamic Acid in Biological Material, G. Dannhardt, S. Laufer, and M. Lehr (Institut für Pharmazeutische Chemie der Johann Wolfgang Goethe-Universität, Georg-Voigt-Str. 14, 6000 Frankfurt a. M. 11, F.R.G.)

Etofenamate is a derivative of N-phenylantranilic acid (see formula) and has been selected as an anti-inflammatory agent for percutaneous therapy. Hydrolysis of etofenamate leads to the corresponding flufenamic acid.

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 R = \text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OH}: \text{etofenamate} \\
 R = \text{OH}: \text{flufenamic acid}
\]

Etofenamate has been quantified by gas chromatography (1) or by fluorimetry after thin-layer chromatography (2). Here we describe a simple HPLC method for determining etofenamate and its metabolite flufenamic acid in plasma and in tissues.

**Plasma.** To 1 mL of blood add 5 μL of an equimolar solution of etofenamate and flufenamic acid (used concentrations see below) in dimethyl sulfoxide. Next add 5 mL of an equimolar mixture of acetonitrile/methanol and centri-fuge (15 min, 4000 × g). Resuspend the supernate in 5 mL of the acetonitrile/methanol solvent and re-centrifuge. Combine the supernates, dilute them with 100 mL of water, then pass them through an octadecyl reversed-phase extraction column (6 mL; Baker no. 70206) that has been previously washed successively with 10 mL of methanol, 5 mL of water, and 5 mL of a 1 g/L EDTA solution. After washing the column with two 5-mL portions of water, elute the adsorbed substances with 3 mL of methanol. Add 5 mL of water to the eluate and inject 2 mL into a 250 × 4 mm column packed with Nucleosil 7 C18 (Macherey & Nagel, Düren, F.R.G.).

Under the conditions used (flow rate 1 mL/min, detection 286 nm) no transformation of etofenamate to flufenamic

Fig. 1. Correlation of ChE activity with (top) the ratio of duration of action to activity, and (bottom) the logarithm of the ratio of duration of action to activity.

respiration) was recorded. The ChE activities and phenotypes of all patients were determined. The samples with dibucaine and fluoride numbers less than 78 and 75, respectively, were discarded, so that only the subjects with UU phenotype (143 patients) were considered. Of these subjects, 33 patients (23%) had ChE activities <5.0 kU/L, and 22 (15.4%) had less than the cutoff value, 4.0 kU/L, owing to an acquired condition such as metastatic or primary carcinomas of the hepatobiliary, gastrointestinal, and (or) urinary system; gastric or duodenal ulcers; or malnutrition and malabsorption syndromes.

In these 143 patients we found a weak negative correlation between ChE activity (x) and the duration of action (y) of Sch: \( r = -0.55, y = -1.81x + 26.58 \). In contrast, as shown in Figure 1, the correlation between activity and the logarithm of the ratio of duration of action to activity was more significant: \( r = -0.78, y = -0.13x + 1.16 \).

References