

Title: A PLATELET - HALOTHANE BIOASSAY FOR MALIGNANT HYPERTHERMIA

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Introduction. A relatively non-invasive screening technique has been developed to assess possible adverse effects of halothane anesthesia in malignant hyperthermia susceptible (MHS) patients and their relatives who are at risk for malignant hyperthermia (MH). This test uses platelets to study the changes in adenosine triphosphate (ATP), adenosine diphosphate (ADP), hypoxanthine (HYPX) and adenosine monophosphate (AMP) after in vitro exposure to halothane.

Methods. 1) A heparinized blood sample was taken from each of 23 patients having a clinical history of MH (8 of whom had a positive muscle biopsy), 10 patients having muscle or metabolic disease without having MH (i.e. myotonic dystrophy, osteogenesis imperfecta, hyperthermia, hyperkalemia, acidemia) and 25 normal patients. 2) Ten ml of blood is centrifuged slowly to obtain platelet-rich plasma (PRP). The PRP is divided into aliquots of 0.4 ml each, one of which is used as a control. Five microliters (ul) of halothane is added to the other aliquot and both tubes are capped and incubated at 37° C for 20 minutes. The tubes are then centrifuged at high speed to form a platelet pellet. The platelet-poor plasma is decanted and 0.1 ml of cold 6% perchloric acid (PCA) is added to the platelet pellet. Adenine is added as an internal standard and the PCA extract is analyzed by high performance liquid chromatography (HPLC). 3) HPLC analysis is performed using an isocratic system and a detector set on 254 nm. Ten - 20 ul of the PCA extract is injected into a C-18 microbondapak column and phosphate buffer is pumped through a steel jacketed column at 2 ml/min (3-6 ml/min for a radial compression module). The buffer is aqueous 0.1 M KH₂PO₄ or 0.1M NH₄H₂PO₄. (the latter being recommended) without any pH adjustment for either buffer. The nucleotides are separated and emerge in the order ATP, uric acid (UA), HYPX, xanthine (XA), AMP and adenine (Figure 1) with their peak heights being proportional to their concentrations. 4) R is an index derived from the classical energy charge of Atkinson (1) but modified to reflect in a more sensitive manner the concentration changes seen in MH. Peak heights are used to calculate the R value of the control sample (R) and of the sample exposed to halothane (R_H) by the following formula:

$$R = \frac{ATP + ADP}{AMP + HYPX}$$

The % reduction in the R value is calculated as:

$$\% \text{ Reduction in } R = \left[1 - \frac{R_H}{R} \right] \times 100$$

Results. The 8 muscle biopsy positive patients had a mean % reduction in R of 76.1 + 4.7 SEM and the 15 clinically diagnosed MH patients had a mean % reduction of 74.1 + 3.4 SEM. There was no statistically significant difference (p < .0001) between these two groups which were combined to give a mean % reduction of 74.9 + 2.7 SEM. The mean % reduction in R was 23.3 + 3.0 for the 25 normal patients and 15 + 7.8 SEM for the 10 muscle and metabolic disease patients. The latter two groups were not statistically differ-

ent (p < .0001). However, when normals were compared to clinically diagnosed MH patients, the difference was highly statistically significant (p < .0001).

Discussion. Platelets were considered for study because they have a calcium-activated ATPase actinomyosin contractile system and a membrane storage organelle for intracellular calcium analogous to skeletal muscle (2). The platelets were used for the study of MH because their observed decrease in R value in response to halothane in MH patients was highly significant compared to controls. This assay has a high degree of reproducibility and specificity which recommends it for study in elucidating the etiology of the MH syndrome and in monitoring the intracellular effect of drugs administered for the control of MH episodes and it is currently in clinical use at our institution.

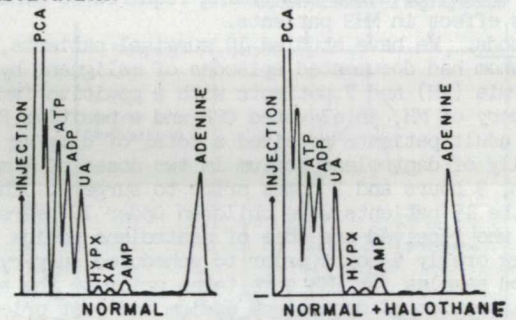


Figure 1

Figure 2

HPLC graph of platelet nucleotides from a normal control before (Fig.1) and after the addition of halothane (Fig.2) The % reduction of R = 31.2%

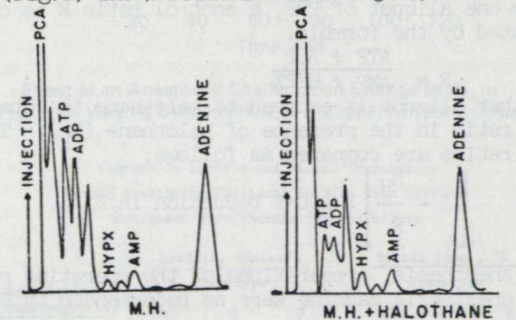


Figure 3

Figure 4

HPLC graph of platelet nucleotides from an MH patient before (Fig. 3) and after the addition of halothane (Fig. 4). The % reduction of R = 73.7%

References.

- 1) Atkinson, D.E. *Cellular Energy Metabolism and It's Regulation*. New York: Academic Press, 1977, pp. 88 - 91.
- 2) Lüscher, E.F., Probst, E., Bettex-Galland, M. Thrombosthenin: Structure and function. *Ann. N.Y. Acad. Scis.* 1972, pp. 122-129.