CLINICAL CONCENTRATIONS OF VERAPAMIL AFFECT THE IN VITRO DIAGNOSIS OF SUSCEPTIBILITY TO MALIGNANT HYPERPYREXIA


SUMMARY

We examined the effects of verapamil on the in vitro caffeine and halothane tests for malignant hyperpyrexia (MH) susceptibility. Ten consecutive MH-susceptible patients were investigated according to the protocol of the European MH group. Additional tests were carried out in the presence of verapamil $10^{-6}$ mol litre$^{-1}$. In four of the 10 patients, the halothane contracture response following pretreatment with verapamil was classified as positive to halothane. In contrast, in nine of the 10 patients, contracture tests of muscle in the presence of verapamil were classified as negative to caffeine. It is advised that verapamil should be discontinued before performing a contracture test.

KEY WORDS


The diagnosis of susceptibility to malignant hyperpyrexia (MHS) is commonly based upon abnormal contracture of susceptible muscle in response to halothane and caffeine [1-3]. Various drugs such as procaine [4], dantrolene [5] and propranolol [6] have been described as interfering with the in vitro halothane and caffeine contracture tests. However, the studies reported with verapamil on MHS human muscle have been poorly examined. To our knowledge, only one study reported that verapamil $8 \times 10^{-6}$ mol litre$^{-1}$ was effective in blocking halothane-induced contracture of MHS human muscle [7], but this concentration is much higher than that achieved clinically [8-11]. Verapamil is used widely in patients with cardiovascular disease [12] and some of these may undergo elective muscle biopsy for diagnosis of MH susceptibility.

In the present study, we have examined the effects of verapamil $10^{-6}$ mol litre$^{-1}$—a concentration which is close to the clinical range—on both caffeine- and halothane-induced contractures of susceptible human muscle.

PATIENTS AND METHODS

Biopsies were obtained from the vastus medialis muscle under femoral nerve block anaesthesia with lignocaine and dissected carefully in muscle strips (approximately 15 mm long and 2 mm diameter). One end was pinned to the silicone bottom of the experimental bath which was perfused continuously (4-5 ml min$^{-1}$) with Krebs-Ringer solution of the following composition (mmol litre$^{-1}$): NaCl 118.1; KCl 3.4; CaCl$_2$ 2.5; MgSO$_4$ 0.8; KH$_2$PO$_4$ 1.2; NaHCO$_3$ 25; glucose 11.1; pH 7.35 ± 0.05 and bubbled with carbogen (5% carbon dioxide in oxygen). The other end of the strip was attached by a thin silk thread to a force transducer (Bioscience dynamometer UF1 and biological amplifier 120). The preparations were stimulated directly via silver electrodes with rectangular current pulses of 2 ms duration and at twice the threshold intensity, delivered at a frequency of 0.2 Hz by a stimulator CEA-DAM model GP1-GE219. The preparation was stretched until the amplitude of muscle twitches could not be increased further (2 g
tension approximately) and allowed to stabilize during 15 min of isometric relaxation. Baseline and twitch tensions were recorded continuously at low speed on a Kompensograph C1013 (Siemens).

Halothane was mixed with carbogen by means of a calibrated vaporizer (Fluotec Mark III) in concentrations of 0.5, 1, 1.5, 2 and 3 vol % as confirmed by gas chromatography. Caffeine was added to the Krebs-Ringer solution in increasing concentrations of 0.5, 1, 1.5, 2, 3, 4, 8, 16 and 32 mmol litre⁻¹. Each concentration of either halothane or caffeine was maintained for 3 min.

The study involved 10 patients investigated according to the European MH protocol [3]. The criteria for MH susceptibility (MHS) are an increase in resting muscle tension of at least 0.2 g induced by 2 vol % of halothane or less and with caffeine 2 mmol litre⁻¹ or less. Halothane and caffeine effects were always tested on separate strips. In additional muscle strips obtained from the same biopsies, verapamil 10⁻⁶ mol litre⁻¹ was added to the Krebs-Ringer solution 15 min before testing with halothane or caffeine according to the procedure described above.

The increase in resting tension and the amplitude of twitch tension were determined. We analysed differences between the means of the groups for maximum contracture response and maximum increase in twitch tension to each concentration of halothane and caffeine separately using Student’s t test for independent samples. A value of P < 0.05 was regarded as significant.

RESULTS

The distribution of patients is shown on the European MH diagram (fig. 1). A 10-min pretreatment with verapamil 10⁻⁶ mol litre⁻¹ reduced significantly the halothane-induced contracture (P < 0.05). However, in four of the 10 patients, the contracture responses following pretreatment with verapamil still exceeded 0.2 g and therefore could be classified as positive to halothane (fig. 1). Verapamil lowered the initial part of the caffeine dose–response curve from 0.5 to 2 mmol litre⁻¹ (P < 0.05) and, in nine of 10 MHS patients, contracture tests of muscle in the presence of verapamil could be classified as negative to caffeine (fig. 1). According to the European MH protocol, the 10 MHS patients tested in the presence of verapamil can thus be classified as four MHE(h), one MHE(c) and five MHN patients.

Fig. 1. Threshold concentration for each patient (I–X) to halothane and caffeine in the absence (●) and presence (○) verapamil 10⁻⁶ mol litre⁻¹. MHS = malignant hyperpyrexia susceptible; MHN = MH negative; MHE = MH equivocal.

The positive inotropic effect of both 0.5–2% halothane and caffeine 0.5–2 mmol litre⁻¹ was enhanced in the presence of verapamil. However, there were no significant differences in the percentage of maximum twitch increases between the pretreated and untreated muscles (data not shown).

DISCUSSION

The present results demonstrate that verapamil may affect the diagnosis of Malignant Hyperpyrexia susceptibility based on in vitro muscle contractures in response to halothane and caffeine. In four of 10 patients, verapamil 10⁻⁶ mol litre⁻¹ decreased the halothane-induced contracture to such an extent that the test became negative. Furthermore, in nine of 10 patients, the contracture induced by caffeine 2 mmol litre⁻¹ (the highest concentration admitted for MHS diagnosis) was antagonized completely by verapamil 10⁻⁶ mol litre⁻¹.

There are few studies of the effect of calcium channel blockers on halothane–caffeine contracture tests in human MHS muscle. However, our results are consistent with the report of Gruener and Blanck [7], who found that verapamil 8 × 10⁻⁶ mol litre⁻¹ was effective in blocking halothane-induced contractures. It is of interest that the concentration of verapamil used in the present work is more comparable to the clinical range than that reported by these authors. However, the use
of verapamil $10^{-6}$ mol litre$^{-1}$ corresponds to the highest therapeutic concentration. Indeed, some studies suggest a therapeutic range of 150–500 mg ml$^{-1}$ [8–11]. The greatest concentrations are close to $10^{-6}$ mol litre$^{-1}$, but there is obviously an order of magnitude difference in the lower concentration. However, higher plasma concentrations are easily attained as verapamil is known to have a large first-pass metabolism in the liver [13]; this would result in a considerable inter-individual variation in plasma concentration. Hence the use of a concentration of verapamil less than $10^{-6}$ mol litre$^{-1}$ for in vitro testing may have no clinical relevance for patients with high plasma concentrations of the drug.

Furthermore, we found that verapamil interfered more with caffeine than with halothane contractures. To our knowledge, these observations have not been described previously in human MHS skeletal muscle. However, verapamil effects on halothane- or caffeine-induced contracture have been investigated in skeletal muscle obtained from various animal species, with conflicting results. Thus very high concentrations ($500 \times 10^{-6}$ mol litre$^{-1}$) of verapamil have been reported to potentiate the halothane-induced contracture in pig MHS muscle [14]. In cat soleus muscle, halothane-induced contractures were abolished by verapamil $10^{-6}$ mol litre$^{-1}$, whereas $7.5 \times 10^{-6}$ mol litre$^{-1}$ of the same drug was ineffective; verapamil $10^{-6}$ mol litre$^{-1}$ reduced the caffeine-induced contracture [15]. Similar reduction or suppression of halothane- and caffeine-induced contractures has been observed when extracellular Ca$^{2+}$ was removed [15, 16], thus providing evidence that halothane- and caffeine-induced contracture depends on an extracellular source of Ca$^{2+}$.

We have shown that clinical concentrations of verapamil affect the in vitro discrimination of MH susceptibility by producing false negative results for some MHS patients. If these results may be extrapolated to in vivo conditions (i.e. patients under verapamil treatment), we conclude that verapamil should be discontinued before performing a contracture test.

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REFERENCES


