EFFECT OF DILTIAZEM ON PORCINE MALIGNANT HYPERPYREXIA INDUCED BY SUXAMETHONIUM AND HALOTHANE

P. S. FOSTER, K. C. HOPKINSON AND M. A. DENBOROUGH

Porcine MH may be induced by suxamethonium and halothane [1,2]. In the presence of a precipitating agent, the clinical features of the porcine MH syndrome are similar to those described in man. Essentially, MH in swine is characterized by a rapid and sustained increase in core body temperature and muscle rigidity. Biochemical changes such as metabolic acidosis and increased arterial carbon dioxide tensions precede the development of these features [1]. Increases in serum electrolytes and creatine phosphokinase (CPK) concentrations are detected also during and after the syndrome [1,3].

An increased concentration of Ca$^{2+}$ in the myoplasm of skeletal muscle is considered to be the major factor in the development of MH [3,4]. Successful management requires early diagnosis, immediate cessation of anaesthesia, correction of metabolic acidosis by i.v. administration of sodium bicarbonate and prompt i.v. administration of the drug dantrolene sodium [3]. Dantrolene acts to decrease myoplasmic concentrations of Ca$^{2+}$ by suppressing excitation–contraction coupling. One of the rationales for the original use of dantrolene in the treatment of MH was based on the demonstration that the skeletal myoneural blocking drug could prevent and antagonize the contracture responses to the diagnostic agents halothane and caffeine in isolated muscle fibres [5].

The calcium channel antagonist diltiazem also has been shown to inhibit and antagonize the drug-induced hypercontractility of isolated MHS human and porcine muscle preparations [6–8]. It seems that diltiazem acts to inhibit Ca$^{2+}$ fluxes associated with the transverse tubular membrane in skeletal muscle [8,9].

This study has investigated the therapeutic value of diltiazem in inhibiting and antagonizing the porcine MH syndrome.

MATERIALS AND METHODS

Six MH-susceptible (MHS) and four control pigs (body weights 25–47 kg, both sexes) were selected from mixed litters. All were premedicated by i.m. injection of stresnil (4-fluor-4(4-(2-pyridyl)-1-piperazinyl)-butyrophenone) 1.5–2 mg kg$^{-1}$. Tracheal intubation was performed after anaesthesia was induced with thiopentone 3–4 mg kg$^{-1}$. Anaesthesia was maintained with nitrous oxide in oxygen supplemented with thiopentone. Muscle tissue for the diagnosis of susceptibility was removed and anaesthesia maintained subsequently with 66% nitrous oxide in oxygen. Susceptibility to MH was identified by using the established isolated contracture test [10]. Physiological variables were recorded every 1 min for approximately 15 min and venous blood was taken for biochemical analysis. Suxa-
methonium and halothane were administered in the presence (inhibition experiment) or absence (antagonism experiment) of diltiazem.

**Administration of diltiazem**

Diltiazem (Marion Laboratories) was dissolved in sterile water (Abbot Laboratories) to a final concentration of 1 mg ml\(^{-1}\) and administered i.v. through a catheter placed in the ear. The rate of administration varied but did not exceed 1 mg kg\(^{-1}\) min\(^{-1}\). This prevented dramatic and sustained decreases in arterial pressure.

**Inhibition experiments**

Increasing doses of diltiazem were administered i.v. to a total dose of 2, 5 or 10 mg kg\(^{-1}\) (one, one and four pigs, respectively). After physiological variables had stabilized, suxamethonium was administered i.v. (ear vein) in four doses (to reduce fasciculations) to a total dose of 2 mg kg\(^{-1}\). If required, the lungs were ventilated manually at a constant rate until ventilatory function returned.

After the administration of the neuromuscular blocker, nitrous oxide was discontinued and anesthesia with 1\(\%\) halothane in oxygen initiated. Halothane anesthesia was maintained for approximately 90 min. Physiological variables were monitored every 1 min for the duration of the challenge. Blood for biochemical analyses was taken and anesthesia ceased.

**Antagonism experiments**

MHS pigs were allowed to recover for no less than 2 weeks before being challenged again in the absence of diltiazem. After vital signs had stabilized under nitrous oxide-oxygen anesthesia, suxamethonium was administered in four doses to a total concentration of 2 mg kg\(^{-1}\) and 1\(\%\) halothane administered. After the initiation of an MH syndrome, halothane was discontinued and increasing doses of diltiazem were administered i.v. to a total concentration of 7-10 mg kg\(^{-1}\) in five pigs and 2 mg kg\(^{-1}\) in one other. Sodium bicarbonate was also administered i.v. and anesthesia was maintained with 66\(\%\) nitrous oxide in oxygen. Blood samples for biochemical analysis were taken: (1) immediately before the administration of suxamethonium and halothane, (2) when the MH syndrome was established and (3) after administration of diltiazem. Vital signs were monitored every 1 min throughout the challenge.

The same procedure was followed with control pigs, blood being taken for biochemical analysis under nitrous oxide-oxygen anesthesia and at two periods during halothane anesthesia. Halothane anesthesia was maintained for 90 min.

**Criteria for establishing the initiation of an MH episode**

In this study, an MH episode was considered to have occurred when body temperature increased by at least 1 \(^\circ\)C, a clinical score of at least 3 was obtained for extensor rigidity, and tachycardia and a decreasing arterial pressure were observed. Often, abnormal patterns also were observed on the electrocardiogram (ECG). While metabolic changes such as increased \(P_{a\text{O}_2}\) and lactate precede the development of muscular rigidity and hyperpyrexia during an MH crisis, it was not possible to monitor these continuously throughout the duration of the challenge. The syndrome was therefore characterized primarily by muscular rigidity and increased body temperature. The development of these later features of MH was considered sufficient to render any observed response to diltiazem unequivocal.

An increase in rectal temperature of 1 \(^\circ\)C was considered sufficient to indicate an MH reaction, as increased body temperature is not only a late feature of an MH episode, but occurs also against a tendency to hypothermia under halothane anesthesia. Furthermore, death has occurred at rectal temperatures as low as 40 \(^\circ\)C in MH pigs [11].

In two pigs which had been used for inhibition and antagonism experiments, MH was induced again under identical conditions. Halothane anesthesia was discontinued and sodium bicarbonate administered at stages in the reaction which were similar to those of previous antagonism experiments. These experiments were conducted with a view to establishing that withdrawal of the triggering agent would not prevent the development of the hyperpyrexic response.

**Physiological measurements**

Arterial pressure was monitored via a femoral arterial catheter connected to a pressure transducer (Bentley) coupled to a cardiac monitor (Telelectronics HSG). Patency of the catheter was maintained by a constant infusion of (approximately 10 ml h\(^{-1}\)) heparinized saline solution 1000 units litre\(^{-1}\). Electrodes were also placed for the monitoring of heart rate and ECG. Rectal temperature was measured using a mercury-in-
TABLE I. Effect of diltiazem in malignant hyperpyrexia pigs in the presence of suxamethonium and halothane: physiological variables (mean or mean (SEM)) from six MH-susceptible swine. Swine were premedicated with diltiazem before administration of suxamethonium and halothane. After recovery of at least 2 weeks, MH episodes were precipitated by suxamethonium and halothane and the effect of diltiazem observed.

<table>
<thead>
<tr>
<th>Anaesthetic conditions</th>
<th>Temperature (°C)</th>
<th>Extensor rigidity (Clinical score)</th>
<th>Heart rate (beat min⁻¹)</th>
<th>Arterial pressure (SAP/DAP) (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of MH</td>
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<tr>
<td>Nitrous oxide-oxygen</td>
<td>36.0</td>
<td>0</td>
<td>80.3 (2.8)</td>
<td>99/58 (2.5/2.3)</td>
</tr>
<tr>
<td>Diltiazem 2-10 mg kg⁻¹</td>
<td>35.0</td>
<td>0</td>
<td>97 (5.6)</td>
<td>93/48 (2.9/2.6)</td>
</tr>
<tr>
<td>Suxamethonium 2 mg kg⁻¹</td>
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<tr>
<td>and halothane</td>
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<tr>
<td>Antagonism of MH</td>
<td></td>
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</tr>
<tr>
<td>Nitrous oxide-oxygen</td>
<td>37.2</td>
<td>0</td>
<td>77 (1.94)</td>
<td>105/54 (2.6/2.5)</td>
</tr>
<tr>
<td>Suxamethonium 2 mg kg⁻¹</td>
<td>38.3</td>
<td>3–4</td>
<td>163 (21.52)</td>
<td>74/42 (5.9/3.7)</td>
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<tr>
<td>and 1 % halothane</td>
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<td></td>
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<tr>
<td>(precipitation of MH)</td>
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<tr>
<td>After</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Diltiazem 2-10 mg kg⁻¹</td>
<td>37.2</td>
<td>0</td>
<td>114 (11.97)</td>
<td>86/46 (3.4/2.2)</td>
</tr>
<tr>
<td>Halothane off, N₂O/O₂ on, NaHCO₃ i.v.</td>
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Results

Inhibition experiments

Pretreatment of MHS pigs with diltiazem modified the induction of the MH syndrome by suxamethonium 2 mg kg⁻¹ and 1 % halothane (tables I, II) over a 90-min period. In the absence of diltiazem, all reactor pigs developed the syndrome within 15–30 min (see antagonism experiments). In the presence of diltiazem, extensor muscles remained flaccid and body temperature decreased by approximately 1 °C. Arterial pressure decreased following administration of diltiazem and recovered within 5–10 min (results not shown). Heart rate did not exceed 100 beat min⁻¹. Blood pH and base excess values decreased slightly, but not to the same degree as observed during an MH episode (table II). Similar observations were made during anaesthesia in control swine (table III). Furthermore, in MHS pigs treated with diltiazem, plasma lactate concentration did not increase, and blood-gas analysis indicated that PaO₂ and PaCO₂ did not change markedly. Plasma concentrations of calcium, potassium and inorganic phosphate also remained essentially unchanged. CPK concentration did increase in pretreated animals during anaesthesia, by 638 u. litre⁻¹ (table II). However, this increase was substantially less than that in untreated pigs (2112 u. litre⁻¹) (table II) and was probably a result of muscular fasciculations induced by suxamethonium.

Antagonism experiments

Treatment with diltiazem was partially effective against a mild (or early) MH response. Exposure of MHS pigs to suxamethonium 2 mg kg⁻¹ and 1 % halothane in the absence of diltiazem resulted in the development of the MH syndrome (tables I and II). Pigs developed pronounced muscular rigidity (clinical score of 3–4), followed by an increase in rectal temperature. Heart rate doubled.

Biochemical measurements

Arterial blood samples were taken and analysed for pH, PaO₂, PaCO₂, potassium, inorganic phosphate, calcium, lactate and CPK. Blood-gas analysis was conducted within 30 min of sampling. All biochemical measurements were conducted by the Department of Clinical Biochemistry at Royal Canberra Hospital.
TABLE II. Effect of diltiazem in malignant hyperpyrexia pigs in the presence of suxamethonium and halothane: biochemical variables (mean (SEM)) from six MH-susceptible swine. Swine were premedicated with diltiazem before administration of suxamethonium and halothane. After recovery of at least 2 weeks, MH episodes were precipitated by suxamethonium and halothane and the effect of diltiazem observed.

<table>
<thead>
<tr>
<th>Anaesthetic conditions</th>
<th>CPK (u. litre⁻¹)</th>
<th>pH</th>
<th>$P_{a,\text{CO}_2}$ (kPa)</th>
<th>$P_{a,\text{O}_2}$ (kPa)</th>
<th>Base excess (mmol litre⁻¹)</th>
<th>Lactate (mmol litre⁻¹)</th>
<th>K (mmol litre⁻¹)</th>
<th>PO₄ (mmol litre⁻¹)</th>
<th>Ca (mmol litre⁻¹)</th>
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<tbody>
<tr>
<td>Inhibition of MH</td>
<td></td>
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<tr>
<td>Nitrous oxide-oxygen</td>
<td>1410 (250)</td>
<td>7.41 (0.01)</td>
<td>5.8 (0.3)</td>
<td>40.7 (2.9)</td>
<td>3.12 (0.34)</td>
<td>2.8 (0.51)</td>
<td>3.1 (0.21)</td>
<td>2.3 (0.21)</td>
<td>2.3 (0.25)</td>
</tr>
<tr>
<td>Diltiazem 2-10 mg kg⁻¹</td>
<td>2048 (287)</td>
<td>7.36 (0.03)</td>
<td>6.0 (0.3)</td>
<td>33.5 (4.0)</td>
<td>1.9 (0.44)</td>
<td>1.9 (0.63)</td>
<td>3.7 (0.31)</td>
<td>2.4 (0.21)</td>
<td>2.4 (0.32)</td>
</tr>
</tbody>
</table>
| and blood-gas analysis showed an increase in $P_{a,\text{CO}_2}$ tensions of 2.31 kPa and a decrease in $P_{a,\text{O}_2}$. Plasma lactate concentration increased from 3.8 to 6.9 mmol litre⁻¹ while blood pH and base excess decreased by 0.16 units and 4.4 mmol litre⁻¹, respectively, consistent with metabolic acidosis (table II). Plasma potassium and inorganic phosphate concentrations were increased during and after the challenge, while calcium concentration remained virtually unchanged. CPK concentration also increased, approximately two-fold (table II). In three of the

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six challenged pigs, blotchy cyanosis of the skin was also observed. When the clinical features of MH had been identified, with muscular rigidity and an increase in body temperature of at least 1 °C, diltiazem 2–10 mg kg⁻¹ was administered i.v. Diltiazem caused a rapid decrease in both systolic and diastolic arterial pressures (results not shown). However, administration of small doses of diltiazem with careful monitoring of arterial pressure prevented sustained hypotension. Arterial pressure recovered to pre-administration values within 5–10 min (allowing further administration if required). The administration of diltiazem caused a rapid loss of extensor rigidity, followed by a progressive decrease in rectal temperature. Heart rate decreased, while arterial pressure remained low in the presence of diltiazem. Blood pH, base excess and \( P_{a\text{CO}_2} \) returned to values approaching those before the challenge. \( P_{a\text{CO}_2} \) also increased (table II). A total concentration of diltiazem 7–10 mg kg⁻¹ was required to antagonize the MH syndrome in five pigs and 2 mg kg⁻¹ was needed in another. The only ancillary treatment pigs received was the administration of sodium bicarbonate; active cooling of one pig was required after body temperature had increased above 40 °C. All pigs survived.

In two MHS swine which had previously been included in inhibition and antagonism experiments, MH was induced under identical conditions. Halothane anaesthesia was discontinued and sodium bicarbonate administered at stages in the reaction which were similar to previous antagonism experiments. Both pigs developed fulminant MH and died. This suggests that metabolism had been stimulated sufficiently and withdrawal of the triggering agent did not prevent the development of the hyperpyrexic response.

Suxamethonium and halothane anaesthesia in control pigs

Temperature decreased by approximately 1 °C and extensors remained flaccid during suxamethonium 2 mg kg⁻¹ and 1% halothane-induced anaesthesia in control swine (table III). Physiological and biochemical features remained essentially constant.

DISCUSSION

The calcium channel antagonist diltiazem modified and partially antagonized a mild (or early) MH response. In the absence of diltiazem, MH-susceptible pigs developed the characteristic clinical features of MH. These included hyperpyrexia, muscular rigidity, metabolic acidosis, increased plasma potassium, inorganic phosphate and CPK concentrations and increased \( P_{a\text{CO}_2} \). Tachycardia, arrhythmias (results not shown) and hypotension were also observed.

Blood-gas analysis in unpretreated swine was consistent with the early expression of the MH syndrome. In this study, all pigs underwent continuous or intermittent ventilation at a constant rate. Alterations in blood-gas values occurred in the absence of any obvious respiratory cause (eg. underventilation) and reflected metabolic acidosis and increased carbon dioxide production. A rapid decrease in base excess corresponding to the increase in \( P_{a\text{CO}_2} \) was also observed. Furthermore, despite relatively high arterial \( P_{a\text{CO}_2} \) (> 27 kPa) at all times, lactate production reflecting anaerobic glycolysis was always observed during halothane anaesthesia in the absence of diltiazem in MH pigs. By comparison, \( P_{a\text{CO}_2}, P_{a\text{O}_2} \), pH and base excess measurements remained essentially constant during halothane anaesthesia in control and diltiazem-pre-treated MH pigs.

When MHS pigs were pretreated with diltiazem, suxamethonium and halothane did not trigger the MH syndrome. Skeletal muscle metabolism was not accelerated, and plasma lactate and arterial pH and blood-gas values remained essentially unchanged. Subsequently, extensors remained flaccid and cardiac dysfunction and increased plasma concentrations of inorganic phosphate and potassium were not observed. Also, rectal temperature decreased over the duration of the challenge. Plasma CPK concentrations increased, but not to the same degree as observed in unpretreated MHS pigs. This increase probably reflected increased muscular activity, observed as fasciculation after the administration of suxamethonium, as CPK concentrations increased also in control swine after the administration of suxamethonium. The clinical features of halothane anaesthesia in pretreated MHS pigs were essentially the same as those observed in control swine.

Furthermore, two MHS pigs in which MH was initiated by halothane (but which did not receive diltiazem), developed fulminant MH and died. This suggests that metabolism had been stimulated sufficiently and withdrawal of halothane...
did not prevent the development of the MH response. Similar observations have been made by other investigators who have shown that, when metabolism has been stimulated sufficiently, the MH syndrome can continue unabated with further increases in temperature in the absence of the triggering agent [1, 12, 13].

Pharmacological experiments indicate that diltiazem may modify Ca\textsuperscript{2+} fluxes at the level of the T-tubule membrane [8]. Ca\textsuperscript{2+} entering the muscle via T-tubules may therefore be important in the aetiology of the MH syndrome. Diltiazem not only acts on skeletal muscle, but also has well known cardiovascular effects [14]. Diltiazem prevents arrhythmias and protects the heart from ischaemia, effects which would be beneficial during an MH episode. Although diltiazem causes hypotension, this was not an adverse effect in this study during MH. The release of catecholamines from the adrenal medulla is a Ca\textsuperscript{2+}-dependent process [15]. The increased concentration of circulating catecholamines observed during an MH episode [12] may also be mediated by Ca\textsuperscript{2+} [3]. Diltiazem may also inhibit this Ca\textsuperscript{2+}-dependent process and attenuate cardiovascular changes [16].

In common with dantrolene, diltiazem modifies the onset of MH and the further expression of an initiated MH syndrome. However, diltiazem is not as effective as dantrolene, and should not displace this drug in the treatment of MH.

REFERENCES
2. Gronert GA, Milde JH, Theye RA. Porcine malignant hyperthermia induced by halothane and succinylcholine.