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Intravenous and intratracheal administration of trimetoquinol, a fast-acting short-lived bronchodilator in horses with 'heaves'

F. C. CAMARGO*, N. E. ROBINSON†, C. BERNEY†, S. EBERHART†, S. BAKER†, P. DETOLVE†, F. J. DERKSEN†, J. D. HARKINS, A. F. LEHNER‡ and T. TOBIN

Maxwell H. Gluck Equine Research Center and the Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40546; †Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824; and ‡Livestock Disease Diagnostic Center, University of Kentucky, Lexington, Kentucky 40512, USA.

Keywords: horse; Beta-adrenergic agonist; TMQ; pleural pressure; heart rate; sweating

Summary

Reason for performing study: **Trimetoquinol (TMQ) is a potent β-adrenoceptor agonist bronchodilator used in human medicine but has not been evaluated for potential use as a therapeutic agent for horses with 'heaves'.**

Objectives: **To assess the pharmacodynamics of TMQ in horses with 'heaves' to determine potential therapeutic effects.**

Methods: **Increasing doses of TMQ were administered to horses with 'heaves' by i.v. and intratracheal (i.t.) routes. Doses ranged 0.001–0.2 μg/kg bwt i.v. and 0.01–2 μg/kg bwt i.t. Cardiac and airways effects were assessed by measurement of heart rate (HR) and maximal change in pleural pressure (ΔPplmax), respectively. Side effects of sweating, agitation and muscle trembling were scored subjectively. Duration of action to i.v. (0.2 μg/kg bwt) and i.t. (2 μg/kg bwt) TMQ was evaluated over 6 h.**

Results: **Intravenous TMQ was an exceptionally potent cardiac stimulant. Heart rate increased at 0.01 μg/kg bwt, and was still increasing after administration of highest dose, 0.2 μg/kg bwt. Airway bronchodilation, measured as a decrease in ΔPplmax, also commenced at 0.01 μg/kg bwt. By the i.t. route, TMQ was 50–100-fold less potent than by i.v. Side effects included sweating, agitation and muscle trembling. Overall, the onset of HR and bronchodilator effects was rapid, within about 3 min, but effects were over at 2 h.**

Conclusion: **When administered i.v. and i.t., TMQ is a highly potent cardiac stimulant and a modest bronchodilator. It may not be an appropriate pharmacological agent by i.v. and i.t. routes for the alleviation of signs in horses with 'heaves'. Further studies of TMQ by oral and aerosol routes are necessary.**

Potential relevance: **In horses, TMQ is a fast-acting bronchodilator with a short duration of action. It could be used as a rescue agent during an episode of 'heaves'. The i.v. and i.t. administration of TMQ is associated with side effects, similar to those reported for all other β-agonists. However, other routes, such as aerosol and oral, may prove useful and safe for the alleviation of bronchoconstriction typical of 'heaves'.**

Introduction

Trimetoquinol (TMQ) (1-(3',4',5'-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, molecular weight 345) is the prototype tetrahydroisoquinoline pharmaceutical and is marketed in Japan (Inolin)[1] as an oral bronchodilator for the treatment of asthma and other respiratory conditions in man (Iwasawa 1967). It has one centre of chemical asymmetry; the S(-)-isomer is a nonselective (Konkar *et al.* 1999) β-adrenoceptor agonist (Iwasawa 1967). In contrast, the R(+)-isomer possesses thromboxane A_2/prostaglandin H_2 antagonist activity (Shin *et al.* 1993). Inolin is the S(-)-isomer of TMQ, and is therefore primarily a β-adrenoceptor agonist.

'Heaves' is an inflammatory and obstructive airway equine disease and therapeutic agents used for treatment include β_2-adrenoceptor agonists (long and short-acting, e.g. clenbuterol, albuterol), anticholinergics (e.g. ipratropium bromide, glycopyrolate) and corticosteroids (e.g. dexamethasone, beclomethasone). Depending on the agent, these drugs are administered by inhalation, *per os*, intratracheally (i.t.) or i.v. Different agents are effective for different aspects of 'heaves' e.g. albuterol, a short acting bronchodilator (duration of action about 1 h) with rapid onset of action (about 5 min) (Derksen *et al.* 1999), is useful in an acute episode, clenbuterol is longer lasting, improves mucociliary clearance and has mucolytic actions (Turgut and Sasse 1989) and corticosteroids are used to treat inflammation. Treatment should include short-term attenuation of bronchoconstriction, reduction of inflammation and management or prophylaxis to prevent further exacerbations. Bronchodilators should not be used as the sole therapy since they neither suppress airway inflammation nor reduce airway hyper-reactivity. However they are important emergency agents to be administered when the horse is in acute respiratory distress.

An evaluation of the pharmacodynamics of TMQ in horses with 'heaves' is reported here in order to establish the effective and safe doses of this agent by the i.v. and i.t. routes. Because TMQ is a β-adrenoceptor agonist, we investigated its effects on heart rate, airway function, sweating and temperature regulation, all of which can be modified by β-adrenoceptor activation. This information is fundamental to assess TMQ's potential for use as a therapeutic agent.

*Author to whom correspondence should be addressed.
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Materials and methods

Horses

For dose-response and duration of action experiments, approved by the All University Animal Use and Care Committee of Michigan State University, 6 horses with a history of 'heaves' were used. They included 5 mares and 1 gelding of various breeds, age 17–29 years, weighing 483–570 kg. Horses were maintained on pasture and their diet supplemented with pelleted alfalfa feed as necessary. While on pasture, the animals presented no signs of airway obstruction but, when housed in stalls, bedded on straw and fed hay, they developed characteristic clinical signs of 'heaves'.

For the temperature change experiments, 6 mature Thoroughbred mares weighing 481–525 kg were used from the University of Kentucky herd. They were fed b.i.d. with grass hay and pelleted feed (12%), which was a 50:50 mixture of oats and alfalfa-based protein. During research protocols, horses were provided with water and hay *ad libitum*. They were managed according to the rules and regulations of the Institutional Animal Care Use Committee at the University of Kentucky, which also approved the experimental protocol. A routine clinical examination was performed before each experiment to assure that the animals were healthy.

TMQ acquisition and validation

TMQ was a gift from Tanabe Seiyaku Co., Ltd[1], with a certificate of analysis indicating an estimated purity of 99.3–100.9%, confirmed by in-house GC-MS (gas chromatography-mass spectrometry) evaluation of a BSTFA + 1% TMCS (N,O-bis-(trimethylsilyl) trifluoroacetamide + 1% trimethylchlorosilane)[2] derivatised sample.

Heart rate

Heart rate (HR) was recorded by a heart rate computer (Hewlett Packard M1401A Digital UHF Telemetry System)[3]. The 3 electrodes were placed over the heart at the level of the left elbow, on the withers and on the neck of the horse.

Maximal change in pleural pressure (ΔPplmax)

Maximal change in pleural pressure (ΔPplmax) was estimated by use of an oesophageal balloon catheter inserted into the distal third of the oesophagus so that the balloon was between heart and diaphragm (Robinson *et al*. 2000). The catheter was connected to a pressure transducer (Model DP/45-34)[4] and recording system (Model Dash 18)[5] The position of the balloon was adjusted to obtain maximal change in pleural pressure (ΔPplmax) during tidal breathing. The pressure transducer was calibrated before each study against a water manometer.

Rectal and skin temperatures

Skin and rectal temperatures were measured following the protocol described by Harkins *et al*. (1996): skin temperature with a surface thermistor (Model 409B)[6] attached to the chest wall with a skin adhesive (Vetbond)[7] and rectal temperature with a general purpose thermistor (Model 401)[6] placed 50 cm into the rectum and secured with adhesive tape to the tail after faecal content was removed. The thermistors were attached to a digital thermometer (Model 8402)[8] and temperatures recorded 15 min before, immediately before, and 2, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90 and 120 min after TMQ administration.

Side effects of TMQ

Side effects were scored as follows: 1) *Sweating:* 0 = no sweat and cool flanks; 1 = warm and humid flanks; 2 = warm flanks and wet hands after stroking flanks; 3 = flanks visibly wet; and 4 = sweat dripping from flanks. 2) *Agitation:* 0 = calm horse with no temperament change; 1 = restless; 2 = anxious appearance, or ear pinnae retracted back or eyes wide open; 3 = pawing the ground. 3) *Muscle trembling:* 0 = no trembling; 1 = intermittent trembling of flanks; 2 = constant trembling of flanks; 3 = sustained trembling of flanks and some trembling of other parts of the body.

Experimental design

Cumulative dose-response to TMQ: For the experiments in 'heaves'-affected horses, TMQ was dissolved in ethanol to yield a 1 mg/ml stock solution; this stock was then diluted with saline into the required doses yielding a 2 ml final volume for i.v. jugular and i.t. administration. Intratracheal injections, for all experiments, were administered through a needle inserted between the tracheal rings midway between the larynx and thoracic inlet. The final doses for the i.v. route were: 0.0 (vehicle), 0.001, 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 μg/kg bwt; those for the i.t. route were: 0.0 (vehicle), 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1 and 2 μg/kg bwt.

Horses were brought from the pasture into the stable, bedded on straw and fed hay. A technician observed horses daily and assigned a clinical score (0–8) to evaluate the severity of airway obstruction (Rush *et al*. 1998; Robinson *et al*. 2000). When the score was 5 or greater, the ΔPplmax was measured. When ΔPplmax exceeded 15 cmH2O, horses qualified for entry into the protocol. Saline and increasing doses of TMQ were administered at 15 min intervals. Maximal change in pleural pressure (ΔPplmax) and heart rate (HR) were recorded continually and peak HR and lowest ΔPplmax during the 15 min period were used to generate a cumulative dose-response curve. Side effects were also recorded. The experiment was terminated and the final response was considered the maximum effective response when one of the following occurred: 2 consecutive doses did not cause any further decrease in ΔPplmax, HR exceeded 220 beats/min or the side effects were considered uncomfortable for the horse.

Duration of action of TMQ: This crossover experiment was conducted 2 weeks after measurement of the dose response. A 1 mg/kg stock solution of TMQ in ethanol was diluted in saline to achieve a concentration of 0.2 μg/kg bwt for i.v. bolus administration and 2 μg/kg bwt for i.t. administration. Final administration volume was 2 ml for either route. In negative control experiments horses received 2 ml saline, i.v. or i.t.

HR and ΔPplmax were recorded at times 0 (before drug administration), and 1, 3, 6, 10, 15, 20, 30 and 45 min, and 1, 2, 4 and 6 h after i.v. administration of 0.2 μg/kg bwt TMQ or saline. For the i.t. administration, measurements were made at times 0 (before drug administration), and 3, 6, 9, 12, 16, 20, 25, 30 and 45 min, and 1, 2, 4 and 6 h after administration.

Rectal and skin temperature

In a crossover experiment conducted at the University of Kentucky, 6 healthy horses were dosed i.v. with saline and TMQ at doses 2 and 8 μg/kg bwt. Skin and rectal temperatures were recorded at times: 10 min prior to administration, immediately before, and 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150 and 180 min after administration. Each horse received only one treatment each day. The minimum time allotted between treatments was 3 days. This experiment was performed before the determination of the dose-response.

Statistical analysis

A repeated measures ANOVA was used to evaluate the effect of TMQ dose and time and their interactions. The confidence interval was set at 95% and results were significantly different when P<0.05. Results are presented as mean and the 95% confidence interval (95% CI).

Results

Cumulative dose-response

Heart rate: Heart rate before administration of i.v. TMQ was 46 beats/min (95% CI: 27.11, 64.89). The HR increased significantly within 1 min of TMQ administration to 71.33 beats/min (95% CI: 52.44, 90.22) at a dose of 0.01 μg/kg bwt (P<0.001). As the TMQ dose was increased, HR increased progressively. At the highest dose of TMQ, administered i.v. (0.2 μg/kg bwt), HR was 162.8 beats/min (95% CI: 143.91, 181.69) and was still increasing; there was a significant increase (P<0.0001) in HR between 0.1 and 0.2 μg/kg bwt (Fig 1A).

Heart rate also increased following i.t. administration of TMQ (Fig 1B). Baseline HR, prior to i.t. TMQ administration, was 40.83 beats/min (95% CI: 32.56, 49.1). The increase first became significant at a dose of 0.5 μg/kg bwt, when the HR was 60.67 beats/min (95% CI: 52.4, 68.94) (P<0.001). At the highest dose administered, HR was 145.7 beats/min (95% CI: 137.43, 153.97) and was significantly higher than at 1 μg/kg (P<0.0001). Whereas the increase in HR occurred within seconds of IV administration of TMQ, the increase was delayed for 6–8 min after i.t. administration.

Bronchodilation: Bronchodilator responses to TMQ, by measurement of ΔPplmax, are shown in Figures 2A and B. Before administration i.v. ΔPplmax mean was 43.94 cmH_2O (95% CI: 27.39, 60.49). Significant decreases in ΔPplmax began at 0.01 μg/kg bwt TMQ (ΔPplmax = 38.19 cmH_2O; 95% CI: 21.64, 54.74) (P<0.05) and continued to decrease to a dose of 0.05 μg/kg bwt (P<0.0001). Doses 0.05 and 0.1 and 0.2 μg/kg bwt caused no further decrease in ΔPplmax (P>0.05) (Fig 2A).

In 3 horses, i.t. administration of 0.01 and 0.02 μg/kg bwt TMQ was followed by an increase in ΔPplmax. Higher doses

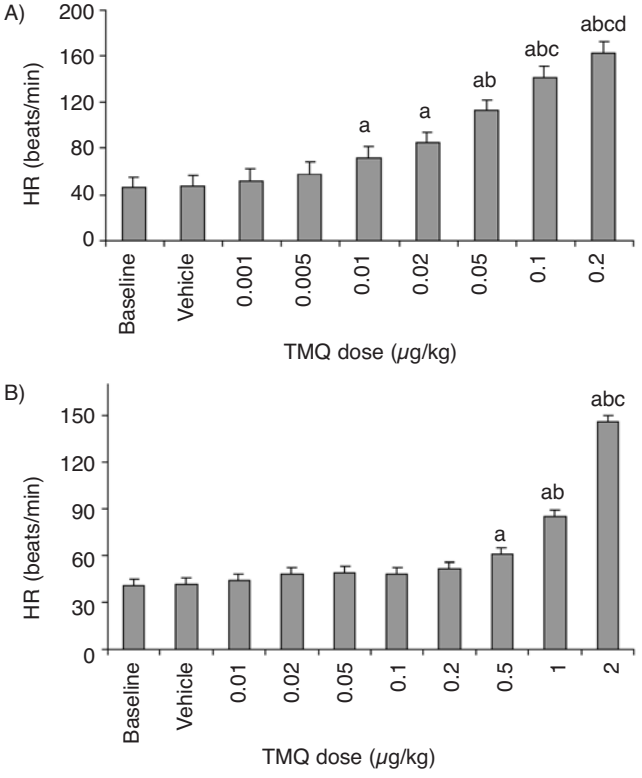

Fig 1: Effect of trimetoquinol (TMQ) on heart rate (HR) in 6 horses affected with 'heaves'. Upper panel (A) = i.v. administration; lower panel (B) = i.t. administration. Data are shown as mean ± s.e. Panel A: a = significantly different from baseline (P<0.05), b = significantly different from 0.01 and 0.02 μg/kg (P<0.001), c = significantly different from 0.05 μg/kg (P<0.0001), d = significantly different from 0.1 μg/kg (P<0.01). Panel B: a = significantly different from baseline (P<0.05), b = significantly different from 0.5 μg/kg (P<0.0001), c = significantly different from 1 μg/kg (P<0.0001).

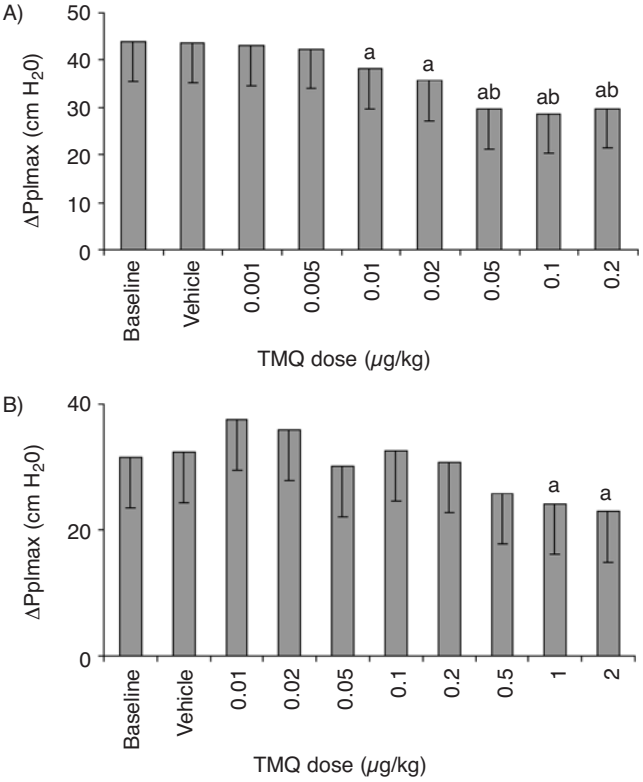

Fig 2: Effect of trimetoquinol (TMQ) on ΔPplmax in 6 horses affected with 'heaves'. Upper panel (A) = i.v. administration; lower panel (B) = i.t. administration. Data are shown as mean ± s.e. Panel A: a = significantly different from baseline (P<0.05), b = significantly different from doses 0.01 and 0.02 μg/kg (P<0.01). Panel B: a = significantly different from baseline (P<0.05).

caused a decrease in ΔPplmax back to baseline. In the other 3 horses, no increase in ΔPplmax occurred and i.t. TMQ decreased ΔPplmax dose dependently. When data from all horses were evaluated, the increase in ΔPplmax from those 3 horses did not result in a significant overall increase in ΔPplmax (P>0.05). A significant reduction in ΔPplmax below baseline values (31.59 cmH_2O; 95% CI: 15.83, 47.35) occurred at an i.t. TMQ dose of 1 μg/kg bwt (ΔPplmax = 24.17 cmH_2O; 95% CI: 8.41, 39.93) (P<0.05), a 100-fold higher dose compared to i.v. administration. The effect of 1 and 2 μg/kg bwt TMQ were not significantly different from each other (Fig 2B) (P>0.05). Figure 3 compares the dose responses to i.v. and i.t. TMQ and shows clearly the greater potency and efficacy of the i.v. route. There was a significant difference of effect between i.v. and i.t. routes of administration, for all doses administered by both routes, 0.01, 0.02, 0.05, 0.1 and 0.2 μg/kg bwt (P<0.01).

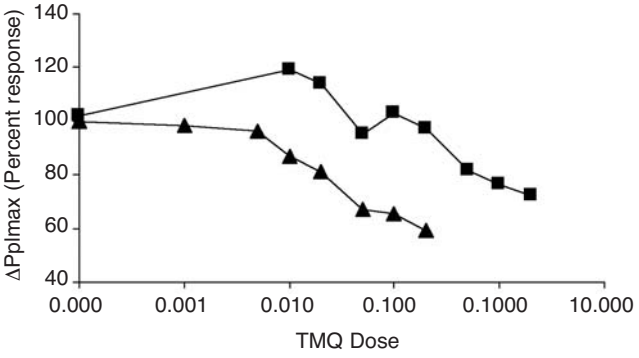

Fig 3: Comparison of i.v. and i.t. dose response to TMQ in 6 horses affected with 'heaves'. The percent decrease in mean ΔPplmax is shown for i.v. (▲) and i.t. (■) routes. Note the right shift in the i.t. dose-response curve. The overall decrease in ΔPplmax was 41% for i.v. route and 28% for i.t. route.

TABLE 1: Side effects observed after i.v. and i.t. TMQ administration. The side effects following i.t. were similar to those following i.v. administration, but the onset was earlier, and at lower doses for i.v. when compared to i.t.

Dose	Sweating Median range	Agitation Median range	Muscle trembling Median range
Intraveneous (i.v.)			
0	0 (0–0)	0 (0–0)	0 (0–0)
0.001	0 (0–0)	0 (0–0)	0 (0–0)
0.005	0 (0–0)	0 (0–0)	0 (0–0)
0.01	0 (0–3)	0 (0–1)	0 (0–1)
0.02	0 (0–3)	0 (0–1)	0 (0–1)
0.05	1 (1–4)	0.5 (0–2)	1 (0–2)
0.1	4 (4–4)	1.5 (0–3)	2.5 (1–3)
0.2	4 (4–4)	3 (3–3)	3 (3–3)
Intratracheal (i.t.)			
0	0 (0–0)	0 (0–0)	0 (0–0)
0.01	0 (0–0)	0 (0–0)	0 (0–0)
0.02	0 (0–0)	0 (0–0)	0 (0–0)
0.05	0 (0–0)	0 (0–0)	0 (0–0)
0.1	0 (0–0)	0 (0–0)	0 (0–0)
0.2	0 (0–0)	0 (0–0)	0 (0–0)
0.5	0 (0–1)	0 (0–0)	0 (0–0)
1	2 (0–4)	1 (0–2)	1 (0–2)
2	4 (4–4)	3 (0–3)	3 (2–3)

Side effects

Most horses started to show side effects of i.v. TMQ after a dose of 0.02 μg/kg bwt (Table 1). Sweating was the most observable response with score reaching 4 in all horses at a TMQ dose of 0.1 μg/kg bwt. The scores for muscle trembling and agitation were considerably less than for sweating, but both increased with TMQ dose. Side effects were no longer evident 1–2 h after the last dose of TMQ. With i.t. administration, sweating appeared at a TMQ dose of 0.5 μg/kg bwt, trembling and agitation at 1 μg/kg bwt (Table 1).

Duration of action

Heart rate: Following administration of a single dose of 0.2 μg/kg bwt TMQ i.v., HR peaked at 1 min (baseline = 45.67 beats/min, 95% CI: 25.07, 66.27; 1 min = 159 beats/min, 95% CI: 138.4, 179.6) (P<0.0001). Heart rate was not significantly different from baseline starting at 1 h (65 beats/min, 95% CI: 44.4, 85.6) and not significantly different from saline starting 2 h (45.83, 95% CI: 25.23, 66.43) after TMQ administration (Fig 4A) (P>0.05).

After i.t. administration (2 μg/kg bwt), HR peaked at 9 min (baseline = 50.5 beats/min, 95% CI: 32.74, 68.26; 9 min = 141 beats/min, 95% CI: 123.24, 158.76). Nevertheless, at time 3 min, HR was increased above baseline (79.33 beats/min, 95%

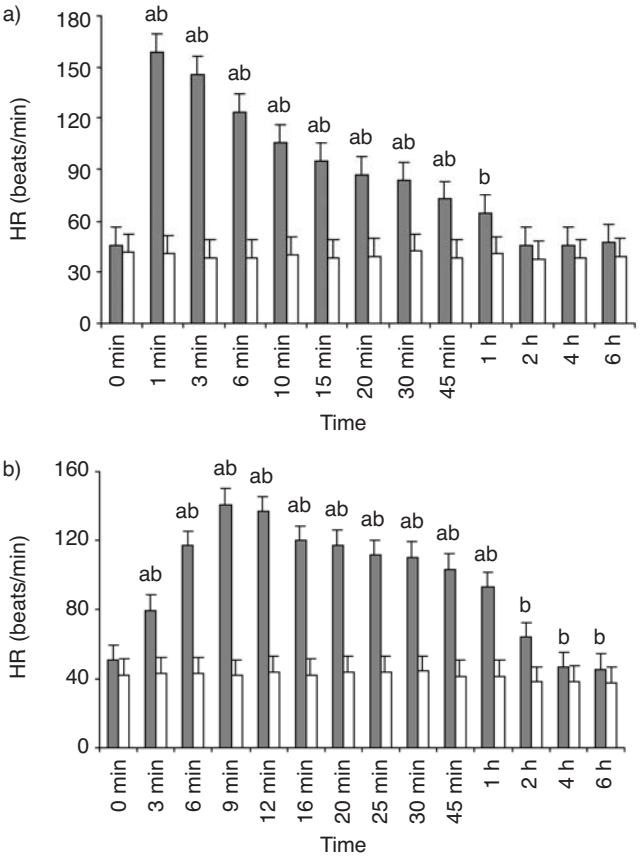

Fig 4: Duration of heart rate (HR) response to TMQ administered i.v. (0.2 μg/kg bwt, A, upper panel) and i.t. (2 μg/kg bwt, B, lower panel) to 6 horses affected with 'heaves'. Grey bars = TMQ, white bars = saline. Data are shown as mean ± s.e. a = significant difference from baseline (0 min) (P<0.05); b = significant difference between saline and TMQ (P<0.05).

CI: 61.57, 97.09) (P<0.01). HR was not different from baseline at 2 h (63.5 beats/min, 95% CI: 45.74, 81.26) (P>0.05). However, HR remained significantly elevated above the saline value throughout the entire time starting at 3 min (P<0.05) (Fig 4B). Saline administration (i.v. and i.t.) had no effect on HR (P>0.05).

Bronchodilation: Before i.v. administration, ΔPplmax averaged 31.8 cmH_2O (95% CI: 16.33, 47.27). Following i.v. administration, ΔPplmax decreased significantly at 1 min (22.76 cmH_2O, 95% CI: 7.29, 38.23) (P<0.05), reached its nadir at 3 min (19.03 cmH_2O, 95% CI: 3.56, 34.5) (P<0.01), and returned to baseline value at 15 min (28.46 cmH_2O, 95% CI: 12.99, 43.93) (P>0.05). There was a secondary significant decrease in ΔPplmax at 2 and 4 h after TMQ administration (P<0.05) (Fig 5A). Saline i.v. had no effect on ΔPplmax (P>0.05).

ΔPplmax averaged 38.65 cmH_2O (95% CI: 24.86, 52.44) before i.t. administration, following which it decreased at 6 min (25.22 cmH_2O, 95% CI: 11.43, 39.01) (P<0.01), reached a nadir at 9 min (23.82 cmH_2O, 95% CI: 10.03, 37.61) (P<0.01), and was no longer different from the saline value at 45 min (39.36 cmH_2O, 95% CI: 25.57, 53.15) (P>0.05) (Fig 5B). There was a secondary decrease in ΔPplmax 4 and 6 h after TMQ administration (P<0.05). Saline i.t. had no effect on ΔPplmax (P>0.05).

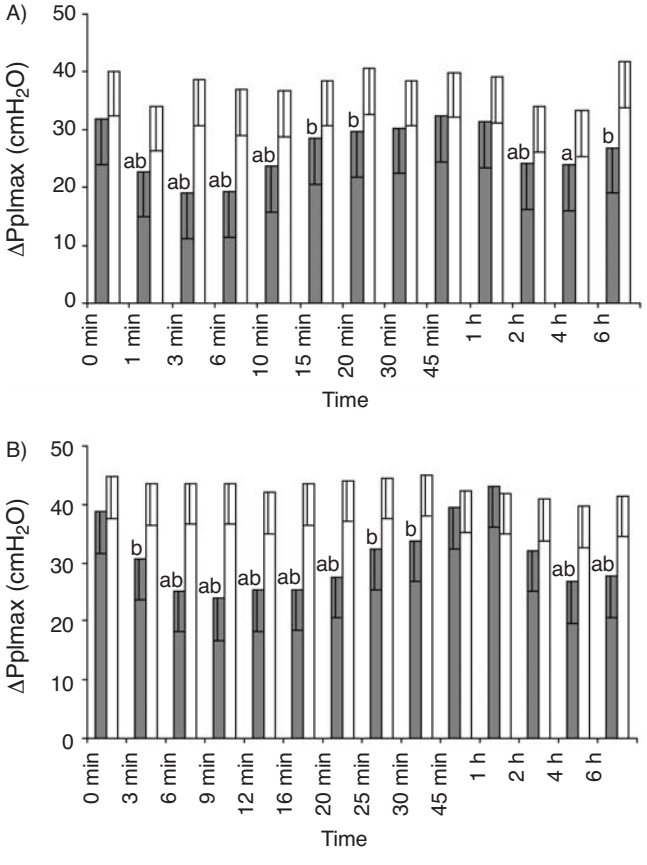

Fig 5: Duration of ΔPplmax response to TMQ administered IV (0.2 µg/kg bwt, A, upper panel) and i.t. (2 µg/kg bwt, B, lower panel) to 6 horses affected with 'heaves'. Grey bars = TMQ, white bars = saline. Data is shown as mean and standard error of mean. a = significant difference from baseline (0 min) (P<0.05); b = significant difference between saline and TMQ (P<0.05).

Rectal and skin temperature

We assessed skin and rectal temperature before and after i.v. administration of saline, and 2 and 8 µg/kg bwt TMQ. Rectal temperature was not significantly affected by TMQ administration (overall mean ±s.d.: 40.12ºC ± 0.423) (P>0.05). Skin temperature decreased significantly at 60 and 90 min (Table 2) (P<0.05). Saline administration had no effect on skin temperature.

Discussion

The scope of this study was to evaluate the efficacy of TMQ, a β-agonist, as a bronchodilator in horses and also to assess the possibility of side effects. We therefore evaluated the bronchodilator and cardiac responses to TMQ following its i.v. and i.t. administration in horses with 'heaves'. Because bronchospasm is a defining feature of this disease, these horses were used to determine if TMQ is a bronchodilator. The results presented here showed that TMQ produced bronchodilation in horses with 'heaves'. In general the effects were fast-acting and short-lived. By the i.v. route, it was up to 100 fold more potent than by i.t. Side effects included tachycardia, sweating, agitation and muscle tremors.

The results of the study confirm that TMQ administered i.v. or i.t. is a nonspecific β-adrenoceptor agonist. While bronchodilation and sweating are initiated via β_2-adrenoceptor activation, tachycardia is initiated by activation of β_1-adrenoceptors. Inspection of Figures 1 and 2 indicates that first significant effects of i.v. TMQ on HR and ΔPplmax occurred at the same dose (0.01 µg/kg bwt). Also the i.v. dose of TMQ producing half maximal effects was similar (0.02–0.05 µg/kg bwt) for HR and bronchodilation (ΔPplmax). There are differing reports about TMQ β-adrenoceptor selectivity (Farmer *et al*. 1970; Feller *et al*. 1975; Fedyna *et al*. 1987; Konkar *et al*. 1999), some report a selective β_2-adrenoceptor activation, and some a nonselective β-adrenoceptor activation, our results favour nonselectivity.

To be optimally active by the i.t. route, medication should have potent respiratory effects and relatively fewer systemic effects. However, when administered by the i.t. route at doses equivalent to those administered i.v., TMQ produced no measurable pulmonary responses and no apparent cardiac responses. Increasing the dose showed that TMQ, by the i.t. route, was up to 100 x less potent in producing pharmacological

TABLE 2: Skin temperature (ºC) in 6 horses following i.v. administration of saline and TMQ (2 and 8 µg/kg bwt). Results are mean ± s.d.

Time (min)	Saline	2 : g/kg bwt	8 : g/kg bwt
-10	32.58 ± 2.36	33.17 ± 2.05	32.13 ± 2.18
0	32.88 ± 2.31	33.13 ± 2.19	32.57 ± 1.89
2	32.68 ± 1.78	34.17 ± 0.93	33.97 ± 1.25
5	33.26 ± 2.07	34.02 ± 1.39	34.20 ± 1.15
10	32.78 ± 1.96	34.40 ± 1.09	34.25 ± 0.99
15	33.26 ± 1.97	34.52 ± 0.86	34.63 ± 1.17
20	33.04 ± 1.55	33.92 ± 1.77	34.73 ± 1.37
30	33.54 ± 1.80	32.73 ± 1.87	34.33 ± 1.92
45	33.80 ± 1.66	31.10 ± 2.19	32.10 ± 2.67
60	33.64 ± 1.66	30.32[a] ± 1.90	30.83[a] ± 2.66
90	33.64 ± 1.69	30.72[a] ± 1.11	31.17[a] ± 1.94
120	33.68 ± 1.69	31.85 ± 2.17	31.90 ± 2.11
150	33.14 ± 1.58	31.83 ± 2.40	31.60 ± 2.29
180	33.80 ± 1.66	32.85 ± 1.73	32.15 ± 2.34

a = significantly different from saline treatment (P<0.05).

responses than by the i.v. route. Furthermore, even at the highest doses administered, TMQ was less efficacious i.t. than i.v. in causing bronchodilation. The percent reduction in ΔPplmax for i.v. route was 41%, whereas for the i.t. route it was only 28% (Fig 3). One possible explanation is that TMQ, administered i.t. in a small volume (2 ml), deposits on the tracheal mucosa, is absorbed through it, and then passes through the peripheral circulation before it causes an effect in the airways. This is probably the reason why i.t. TMQ elicited an effect only after 6–8 min, compared to 1–2 min by the i.v. route.

We also noticed that by the i.t. route, low doses of TMQ produced an increase in ΔPplmax (more bronchospasm) in 3 horses (mean increase 48%, or 12 cmH_2O in these 3 horses). However, this increase was not statistically significant. By the i.t. route, only at the 2 higher doses did TMQ produce significant decreases in ΔPplmax. One possibility is that the drug is an irritant that provokes bronchospasm, and the lower concentrations of TMQ cannot alleviate it. This may be due to the nonspecific hyperresponsiveness characteristic of 'heaves', in which TMQ, an alkaloid, saline and residues of ethanol may have acted as a stimulus to further contract the airway smooth muscles. With higher doses, the drug was able to overcome the irritant effect and caused bronchodilation, seen by decreased ΔPplmax.

When administered in a single bolus dose by the i.v. route (0.2 μg/kg bwt), the onset of action of TMQ was rapid both in increasing the HR, causing bronchodilation and initiating sweating. Peak responses occurred in less than 3 min. The peak response occurred slightly later (9 min) when TMQ was administered i.t. (2 μg/kg bwt). The duration of effect of TMQ was short by these routes, with HR and ΔPplmax returning to basal levels within 2 h, comparable to albuterol (Derksen *et al*. 1999). With both i.v. and i.t. routes of administration, there was an intriguing secondary decrease in ΔPplmax without an accompanying increase in HR 2–6 h after TMQ administration. This may be explained if serological analyses show that a β2-adrenoceptor active metabolite is being re-released into circulation, perhaps from fat tissues. It is unlikely that this decrease is due to a circadian change in the severity of airway obstruction because there was no significant decrease when horses were treated with saline.

Trimetoquinol was highly active on the cardiovascular system, and it also produced agitation, sweating and muscle trembling in horses, which was not surprising since these are the same responses elicited by epinephrine and other sympathomimetic agents (Hoffman 2001) and all β2-agonists. The possibility of developed tolerance to these side effects following multiple administrations, as happens with clenbuterol (Erichsen *et al*. 1994), was not investigated.

In a pilot experiment, it was noticed that sweating was one of the responses observed following TMQ administration. We, therefore, followed the changes in rectal and cutaneous temperatures after i.v. TMQ. Rectal temperature was used because measuring blood temperature in the pulmonary artery is an invasive technique, despite the fact that it may be the best estimate of core temperature (Tomasic 1999). Tomasic and Nann (1999) showed that mean rectal temperature and mean core temperature were not significantly different from each other in anaesthetised horses. While there are limitations to measuring rectal temperature as a reflection of core temperature, we have described previously how the sweat response induced by isoxsuprine administration produces a substantial decline in rectal temperature (Harkins *et al*. 1996) and, by extrapolation, core temperature. Since the technique utilised previously for isoxsuprine and now for TMQ was the same, had a drop in core temperature occurred following TMQ administration, it would probably have been detectable rectally. However, TMQ-induced sweating was associated with decreased skin temperature but no significant reduction in rectal temperature. It may be that TMQ-induced agitation and muscle trembling increased metabolic heat production and prevented the decrease in rectal temperature in the face of sweating. Trimetoquinol also has been reported to activate β_3-adrenoceptors that promote lipolysis and thereby increases heat production (Konkar *et al*. 1999).

In conclusion, although TMQ is a highly potent agent, it is not a very efficacious bronchodilator when compared to atropine (Robinson *et al*. 1993). Furthermore, the presence of side effects of TMQ precludes its clinical use by the i.v. and i.t. routes. Nevertheless, when administered by nebulisation in man (Yamamura *et al*. 1968) TMQ produced no side effects and yielded a clinically useful bronchodilation. Even though by the i.t. route TMQ elicited an inferior response when compared to the i.v. route, a small dose administered by aerosol may be very effective with symptomatic treatment of 'heaves'. For these reasons, we plan to investigate the efficacy of TMQ as an aerosol in horses for symptomatic treatment of 'heaves'.

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Manufacturers' addresses

[1]Tanabe Seiyaku Co., Ltd, Chuo-ku, Osaka, Japan.
[2]Pierce, Rockford, Illinois, USA.
[3]Phillips Medical, Andover, Massachusetts, USA.
[4]Validyne, Northridge, California, USA.
[5]Astro-Med Inc., West Warwick, Rhode Island, USA.
[6]YSI Incorporated, Yellow Springs, Ohio, USA.
[7]3M Animal Care Products, St. Paul, Minnesota, USA.
[8]Cole-Parmer Instruments Co., Niles, Illinois, USA.

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