

Effect of Hydroxyethyl Starch Solution in Normal Horses and Horses with Colic or Acute Colitis

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With 1 figure and 3 tables

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Summary

Hydroxyethyl starch (HES) solution is an effective colloidal infusion solution in humans for treatment of hypovolaemic shock, but it has not been compared with fluids currently available for use in horses. On the basis of plasma-expanding effect of HES in normal horses, a 10% medium-molecular 200/0.5 solution of HES was subsequently tested in hypovolaemic horses. Six normal horses were given five protocols of a single infusion of HES at varying dosage rates (5, 10, 15 ml HES/kg), as well as isotonic saline (15 ml/kg) and hypertonic saline (4 ml/kg b.w.). Dehydrated horses suffering from acute colitis or those which had been treated surgically for ileus of the small or large intestine were given an i.v. infusion of 10 ml HES/kg in combination with 10 ml saline/kg. Clinical data and blood samples for testing were taken before the infusion, and then 10 min, 1 h, 2, 4, 6, 8, 10, 12 and 24 h after infusion (a.i.). A significant decrease in haematocrit was observed in protocol 1–5 for a period of up to 4, 4, 10 h, 10 min and up to 10 min; in group of colitis, during the entire 24-h testing period, and in groups of ileus of small intestine and of large intestine, up to 4 and 10 h a.i. HES decreases better and longer-lasting haematocrit and total protein than either isotonic or hypertonic saline. Half-life of HES increases due to higher dosage (5.83, 7.63 and 11.48 h) and distribution is exclusively intravascular. In normal horses of protocol 1–3 using HES aPTT, sodium and potassium were within the physiological range. Serum amylase activity is increased in horses using HES. On the basis of this clinical study, the decreasing effect of urea and creatinine in colic patients after surgery and fewer instances of postoperative ileus a dosage of 10 ml HES/kg could be recommended.

Introduction

The enterosystemic circulation of fluids and a water consumption equal to 25–70 ml/kg b.w./day are essential for the physiological preservation of hydration (Argenzio et al., 1974). Moderate to severe dehydration is caused by lack of water consumption or a reduction in/lack of reabsorption of fluid and electrolytes in the gastrointestinal tract which can result from obstruction, strangulation, non-strangulating infarction or enteritis (colitis). Decreased blood circulation in the intestinal tract resulting from this dehydration or from the strangulation of a part of the intestine leads in turn to

damaged mucosa and consequently to the absorption of endotoxins. As a further consequence, the permeability of the capillaries is impaired, which causes a loss of fluids, electrolytes and proteins in the intestine and peritoneal cavity. Subsequent intravascular volume deficit (hypovolaemia) leads to hypovolaemic shock, at times even combined with endotoxaemic shock (White and Moore, 1990). Haematocrit (packed cell volume, PCV) increases and plasma protein decreases significantly due to a loss of protein into the affected gut segment in cases with *Torsio coli totalis* or acute colitis, causing a reduction in oncotic pressure (White and Moore, 1990; Grosche, 2000).

The primary goal of every therapy in colic patients is pain control followed by the preservation of water and electrolyte equilibrium, as well as that of acids and bases. As water, sodium and chloride are always lost in cases of gastrointestinal disease, isotonic saline is usually primary given as an infusion to replace fluid and electrolytes (Grosche and Schusser, 2003). To address the problem of oncotic pressure, blood, plasma and colloidal infusion solutions (e.g. dextrans) are used additionally to stabilize oncotic pressure (Spurlock and Ward, 1990; Meister et al., 1992). A colloidal solution using hydroxyethyl starch (HES) (10%) was applied to treat horses with shock in which haematocrit and total protein (TP) values had dropped significantly (Hermann et al., 1990). The oncotic pressure increased over baseline values in normal ponies and hypoproteinaemic horses using HES (6%) (Jones et al., 1997, 2001). In a retrospective study, HES infusion stabilized systolic pressure of colic horses during general anaesthesia. In this study, the survival rate of surgically treated cases receiving HES (10%) during surgery was significantly higher compared with surgically treated colic horses given isotonic crystalloids (Staehele, 2000). Dose-dependent effects of HES on specific haemostatic variables were observed in clinically normal ponies (Jones et al., 1997). Staehele's study, however, showed no haemostatic abnormalities. HES (10%) metabolic half-life was calculated at 5.59 h in which HES was distributed in the intra vascular space (Meister et al., 1992). In an endotoxin model, hypertonic saline followed by HES infusion resulted in better cardiac output and lower venal lactate concentration when compared to a 60 ml/kg b.w. infusion of polyionic crystalloid solution (Lucas et al., 2006).

The goal of our study was to examine the effect of HES (colloidal infusion solution, a medium molecular HES, 200/

0.5), isotonic and hypertonic saline solution on pharmacokinetic, clinical haematological and chemical variables in normal horses and patients with colic or acute colitis.

Materials and Methods

Normal horses and patients

Six normal warmblood mares formed the control group which was used repeatedly every 2 weeks testing five different protocols. For the six normal mares, the medians of age were 15 years and of body weight 560 kg. A solution of HES (Infukoll[®], HES 10%, 200/0.5, 0.9% saline; Serumwerk-Bernburg, Bernburg, Germany) was given i.v. once every 2 weeks: protocol 1, 5 ml; protocol 2, 10 ml; and protocol 3, 15 ml/kg b.w. In addition, 15 ml of isotonic saline (protocol 4) and 4 ml of 7.5% saline/kg b.w. (protocol 5) were administered, also every 2 weeks. All horses were given a bolus i.v. administered via a venous catheter (G12/8, Braun-Melsungen, Melsungen, Germany) in the 'V. jugularis externa sinister' or in the 'V. thoracica superficialis dexter' or 'sinister' and gravity infusion over a period of at least 20 min. These trials were approved by the Veterinary Trial Commission. The control horses were fed hay *ad libitum* while the infusions were given and blood taken: they were allowed to drink before the trial and then 12 and 24 h after the trial had begun.

There were eight horses in the group with acute colitis (colitis group); seven in the group with ileus of the small intestine (colic group A); and nine in the group with ileus of the large intestine (colic group B): the latter two groups had been treated surgically. In the colitis group, there were seven warmblood horses and one pony (three geldings, two stallions, three mares; 12 years of age; 510 kg b.w.; duration of disease < 6–12 h). Colic group A consisted of one draft horse, three ponies and three warmblood horses (five geldings, one stallion, one mare; 7 years of age; 500 kg b.w.; colic duration 6–48 h; surgical diagnosis: two horses with Foramen omentale herniation, two horses with jejunal impaction, one horse with ileocecal intussusception, one horse with non-strangulating intestinal infarction and one horse with small intestinal adhesion). In colic group B, there were eight warmblood horses and one draft horse (seven mares, two geldings; 7 years of age, 600 kg b.w.; colic duration 6–48 h; surgical diagnosis: four horses with large colon volvulus; five horses with right dorsal displacement).

All patients had haematocrit values of ≥ 0.47 l/l and were included in the trial only if they had survived a minimum of 24 h following the HES infusion. All patients were given 10 ml HES/kg and 10 ml saline/kg b.w. (preoperatively for colic patients with surgery, administration by gravity infusion over a period of at least 20 min.), following which a continuous infusion of saline was administered for 2 days at a rate of 4 ml/kg b.w.: this infusion was then continued depending on haematocrit values. All horses received penicillin and gentamicin and Flunixin meglumine.

Data collected

The following values were recorded before the infusion began and then when blood was drawn: heart and respiratory rate, colour of the mucous membrane, capillary refill time and body temperature.

Blood was drawn from the right 'V. jugularis externa' before administering (b.a.) HES or saline, and then at intervals of 10 min, 1 h, 2, 4, 6, 8, 10, 12 and 24 h after completion of the infusion (a.i.): namely, 2 ml of EDTA blood, 2 ml of citrate blood and 8 ml of whole blood to obtain serum. The serum was extracted immediately and stored at -22°C until processed. EDTA blood was used to determine haematocrit, as well as the number of erythrocytes, leucocytes and thrombocytes. Serum was used to determine the concentration of TP, albumin, amylase, glucose, urea, creatinine, sodium, potassium and HES. Citrate blood was used to establish the activated partial thromboplastin time (aPTT).

Urine was collected from the control mares only before infusion, and then 1 h, 2 and 4 h a.i. The following urine values were measured or calculated: specific gravity, fractional excretion of sodium, potassium and anorganic phosphate as well as the gamma-glutamyl transferase (GGT)/creatinine ratio.

A Hitachi 704 Automatic Analyzer (Roche Diagnostics, Mannheim, Germany) was used to determine TP, albumin, amylase, glucose, urea and creatinine in serum and urine, GGT in urine and serum phosphorus. Haematocrit was determined with the microhaematocrit. The Alvet Cell Counter (Mölab, Karlsruhe, Germany) was employed to analyse erythro-, leuco- and thrombocytes as well as haemoglobin. The aPTT was measured using a Pathromtin test (Dade-Behring, Marburg, Germany). The concentration of sodium and potassium ions in serum was determined with the help of an ion-sensitive electrode (Beckmann Synchron EL-ISE Electrolyte System, Beckmann Coulter GmbH, Krefeld, Germany). Concentrations of sodium, potassium (Ciba-Corning 480 Flammenphotometer, Bayer Diagnostics, Fernwald, Germany) and anorganic phosphate (Beckmann Coulter DU 640 B UV/Vis Spectrophotometer; Beckmann Coulter GmbH) ions in the urine were measured photometrically.

The hexokinase/glucose-6-phosphat-dehydrogenase method was used to determine HES serum concentration in the control mares following the splitting of the polysaccharide using HCl acid (Foerster et al., 1981). The pharmacokinetic evaluation was carried out with the help of the Topfit 2.0 research program (Heinzel et al., 1993).

Statistics

All results were reported as medians, mean values \pm SD and range. All data were analysed by ANOVA for repeated measurements and multiple comparisons were made using the Bonferroni test with $P \leq 0.05$ selected as an indicator of significance.

Results

Normal horses and patients

No side effects or clinical signs were observed following HES infusion in normal horses.

Patients in colic groups A and B experienced a significant reduction in heart rate: in colic group A from 74 to 60 within 10 min a.i. and in colic group B from 64 to 56/min 1 h a.i. In the colitis group, the decrease was not significant. However, 24 h following the end of the HES infusion, heart rates had fallen significantly to 48, 48 and 52/min in the patient groups.

The respiratory rate fell continuously in all patient groups following HES infusion from 22, 20 and 16 to 12, 12 and 14/min respectively. All other clinical signs disappeared or became normal and body temperature fell to within normal limits 24 h a.i. No side effects were detected.

The mean survival rate of 62.5% increased when compared to the survival rate within the horse hospital before HES was used.

Pharmacokinetic results

In normal horses, concentrations of HES in serum were highest in protocols 1 and 3, namely 7.63 and 18.95 mg/ml 10 min a.i. as well as in protocol 2, which registered 12.85 mg/ml 1 h a.i.: these values then fell continuously to reach 2.48, 4.56 and 7.88 mg/ml 24 h a.i. Half-life of HES increases due to the higher dosage given and HES is exclusively distributed in the vascular system. Pharmacokinetic results are listed in Table 1.

Clinical pathological data

Table 2 gives haematocrit levels of control mares using five different protocols and of sick horses following infusions of HES and saline. Although initial haematocrit values b.a. in all five protocols did not vary significantly, these values showed significant variation between normal horses and patients. Normal horses in protocol 3 had significantly lower values than in protocol 5 for up to 2 h and in protocol 4 for up to 4 h a.i. Protocol 3 had significantly lower haematocrit levels 10 min. a.i. than did protocol 1 (Fig. 1). Protocol 2 and 3 did not differ significantly during the entire period. Initial haematocrit values (b.a.) in all patient groups were not significantly different. These values in normal horses in protocol 1 were significantly lower than in the colitis group for up to 10 h a.i. Values in normal horses in protocol 1 never varied significantly from those of colic groups A and B. Normal horses in protocol 2 had significantly lower values than both the colitis group and colic groups A and B for up to 12 or 4 h respectively. Haematocrit values of normal horses in protocol 3 remained significantly lower than values in the colitis group and colic group A for up to 12 h: when compared to colic group B values remained lower for up to 24 h. Only normal horses in protocol 5 had significantly lower values than did the colitis group for up to 1 h a.i., whereby values in protocol 4 were significantly lower for only 10 min. a.i. than in the colitis group (Table 2, Fig. 1). Erythrocyte and haemoglobin levels were similar to those of haematocrit.

Thrombocytes did not change significantly in either normal horses with varying protocols and or in patient groups and always remained within physiological norms. Leucocytes in

normal horses were always within the physiological range. Before infusion leucocyte values in patients in the colitis group were 5.4; in colic group A they were 6.3 and in colic group B they were 4.9 G/l. Twelve hours a.i., they were 9.6 in the colitis group and 6.3 G/l in colic groups A and B. Concentration values for TP in normal and sick horses are shown in Table 3. Albumin levels were similar to those of TP. All protocols had consistently similar TP values except for the fact that protocol 3 had significantly lower levels than in protocol 4 for a period of 2 h a.i. In protocol 2 and 3, TP values were continuously significant lower than the initial value for up to 6 h. Regarding saline protocols only in protocol 5, the TP was significantly decreased 10 min a.i. However, protocol 4 and 5 had significantly higher values than the initial value as of the sixth hour a.i.; these elevated values were not consistent (Table 3). Although initial TP values in all protocols and in all patient groups did not vary significantly, they fell significantly in the colitis group for up to 12 h, in colic group A for up to 2 h and in colic group B for up to 6 h a.i. TP values in all patient groups were significantly lower than in protocols 4 and 5 from 1 to 24 h a.i. (Table 3). Values in protocols 1, 2 and 3 remained significantly higher than in the patient groups for up to 10, 12 and 24 h respectively.

Glucose concentrations rose in protocols 1, 2 and 3 in normal mares and peaked 1 h a.i. (5.0 ± 0.1 to 5.6 ± 0.1 , 4.7 ± 0.4 to 5.8 ± 0.4 and 4.8 ± 0.5 to 5.9 ± 0.4 mmol/l) respectively in protocol 4 12 h a.i. from 5.0 ± 0.5 to 5.7 ± 0.3 , and in protocol 5 8 h a.i. from 4.7 ± 0.1 to 5.7 ± 0.7 mmol/l. They remained significantly higher than initial values for up to 4 h (protocol 1), for up to 12 h (protocol 2 and 3) and for up to 1 h (protocol 4) a.i. Only protocol 5 displayed significantly higher glucose concentrations over a period of 24 h, at which point the level was 5.6 ± 0.6 mmol/l. Among the sick horses, the colitis group displayed a significant increase in glucose concentration from 5.2 ± 1.9 to 7.4 ± 4.9 mmol/l 8 h a.i., after which levels fell continuously. In colic groups A and B, the glucose concentration began to fall significantly from 11.0 ± 3.7 to 7.1 ± 1.4 mmol/l and from 9.3 ± 4.0 to 6.3 ± 1.9 mmol/l 24 h a.i., however, the initial values were significantly higher than in all protocols. Glucose concentrations of all protocols were significantly lower compared with values of colic groups A and B over a period of 1 h a.i.

In protocols 1, 2 and 3, amylase activity prior to the HES infusion was 14 ± 3 , 13 ± 3.6 , 17 ± 3.1 U/l (range 8–20 U/l) (reference range 14–35 U/l) (Parry, 2003). Initial values for amylase activity did not vary significantly among these protocols. In the control mares, amylase activity peaked as follows: in protocol 1 from 14 ± 3 to 47 ± 9 U/l 8 h a.i.; in protocol 2 from 13 ± 4 to 64 ± 10 and in protocol 3 from 17 ± 3 to 78 ± 14 U/l 12 h a.i. Amylase activity in protocols

Table 1. Pharmacokinetic variables (medians) in six normal adult horses given hydroxyethyl starch (HES) i.v.: 5 ml (protocol 1), 10 ml (protocol 2) or 15 ml/kg b.w. (protocol 3)

	C_{\max} (mg/ml)	$AUC_{t=0 \rightarrow t=24h}$ (mg·h/ml)	$t_{1/2\alpha}$ (h)	$MRT_{0-\infty}$ (h)	Cl (ml/min/kg)	V_z (l/kg)	V_{ss} (l/kg)
Protocol 1	7.63	102.21	5.83	27.9	0.0478	0.0857	0.0812
Protocol 2	12.85	183.60	7.63	29.0	0.0534	0.0959	0.0925
Protocol 3	18.95	301.45	11.48	30.2	0.0444	0.0797	0.0786

C_{\max} , maximal HES concentration after infusion; AUC, area under the concentration time curve; $t_{1/2\alpha}$, distribution half life; $MRT_{0-\infty}$, mean residence time; Cl, clearance; V_z , volume of distribution; V_{ss} , volume of distribution in steady state.

Table 2. Mean values \pm SD of haematocrit (HK) (l/l) in six normal horses using five different protocols before administration (b.a.) and after infusion (a.i.) of 5 ml (protocol 1), 10 ml (protocol 2), 15 ml HES/kg (protocol 3), 15 ml saline/kg (protocol 4) or 4 ml 7.5% NaCl/kg (protocol 5); and in horses with acute colitis (colitis-group, $n = 8$), ileus of small intestine (colic group A, $n = 7$) or with ileus of large intestine (colic group B, $n = 9$) treated with a mixture of 10 ml HES/kg and 10 ml saline/kg b.w.

	Haematocrit at various times a.i.									
	HK b.a.	10 min	1 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
Controls										
Protocol 1	0.36 ^a \pm 0.03	0.27 ^b \pm 0.04	0.29 ^b \pm 0.03	0.30 ^b \pm 0.02	0.30 ^b \pm 0.03	0.31 \pm 0.03	0.33 \pm 0.03	0.34 \pm 0.03	0.36 \pm 0.03	0.32 \pm 0.03
Protocol 2	0.33 ^a \pm 0.03	0.22 ^b \pm 0.03	0.24 ^b \pm 0.03	0.25 ^b \pm 0.04	0.27 ^b \pm 0.03	0.30 \pm 0.02	0.30 \pm 0.02	0.30 \pm 0.02	0.31 \pm 0.02	0.31 \pm 0.02
Protocol 3	0.35 ^a \pm 0.02	0.18 ^b \pm 0.05	0.21 ^b \pm 0.05	0.23 ^b \pm 0.04	0.24 ^b \pm 0.05	0.26 ^b \pm 0.05	0.27 \pm 0.05	0.28 ^b \pm 0.04	0.28 \pm 0.04	0.30 \pm 0.04
Protocol 4	0.34 ^a \pm 0.03	0.29 ^b \pm 0.04	0.31 \pm 0.03	0.34 \pm 0.04	0.35 \pm 0.03	0.34 \pm 0.03	0.35 \pm 0.02	0.35 \pm 0.03	0.35 \pm 0.03	0.33 \pm 0.04
Protocol 5	0.34 ^a \pm 0.04	0.28 ^b \pm 0.03	0.30 \pm 0.03	0.32 \pm 0.03	0.33 \pm 0.04	0.32 \pm 0.04	0.34 \pm 0.04	0.35 \pm 0.04	0.36 \pm 0.04	0.35 \pm 0.05
Patients										
Colitis-group	0.55 ^a \pm 0.09	0.38 ^b \pm 0.06	0.38 ^b \pm 0.07	0.38 ^b \pm 0.07	0.41 ^b \pm 0.08	0.42 ^b \pm 0.08	0.42 ^b \pm 0.08	0.44 ^b \pm 0.09	0.43 ^b \pm 0.10	0.39 ^b \pm 0.05
Colic-group A	0.49 ^a \pm 0.03	0.32 ^b \pm 0.03	0.33 ^b \pm 0.03	0.35 ^b \pm 0.04	0.38 ^b \pm 0.05	0.39 \pm 0.06	0.40 \pm 0.07	0.40 \pm 0.06	0.40 \pm 0.08	0.39 \pm 0.08
Colic-group B	0.50 ^a \pm 0.03	0.33 ^b \pm 0.04	0.34 ^b \pm 0.04	0.35 ^b \pm 0.05	0.38 ^b \pm 0.05	0.38 ^b \pm 0.04	0.40 ^b \pm 0.05	0.41 ^b \pm 0.06	0.41 \pm 0.06	0.41 \pm 0.07

HES, hydroxyethyl starch.

Decreasing values in the same row with different superscripts differ significantly ($P \leq 0.05$).

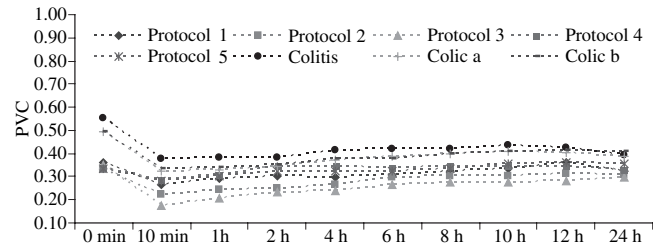


Fig. 1. Mean haematocrit before (0 min) and described minutes and hours after infusion of 5, 10 and 15 ml hydroxyethyl starch (HES)/kg (protocol 1, 2 and 3). Ten millilitre isotonic (protocol 4) or 4 ml hypertonic saline (protocol 5) solution/kg of normal mares and of patients with acute colitis, small intestine (colic A) or large colon (colic B) ileus which were treated with 10 ml HES and 10 ml isotonic saline solution/kg b.w.

1–3 was significantly higher than initial values during the first 24 h of the observation period, following which the amylase activity in protocol 3 (69 ± 15.5 U/l) was significantly higher than in protocol 1 (32 ± 8.7 U/l) and 2 (47 ± 10 U/l). Protocols 4 and 5 displayed no significant change in amylase activity during the 24-h observation period (range: 11–30 U/l b.a.; 10–23 U/l 24 h a.i.). In the colitis group, two of eight had amylase activity of 54 and 105 U/l prior to the HES infusion. In colic group A, one of seven had a value of 1501 U/l and in colic group B, five of nine had a value of 305–1019 U/l. Among the sick horses, amylase activity peaked significantly as follows: in the colitis group from 31 ± 3 b.a. to 57 ± 48 U/l 4 h a.i.; in colic group A from 230 ± 560 b.a. to 248 ± 583 U/l 1 h a.i. and in colic group B from 292 ± 370 b.a. to 366 ± 414 U/l 4 h a.i. Values fell to 16 ± 8 , 19 ± 4 and 26 ± 17 U/l respectively 24 h a.i. which are significantly lower than values of the protocols 2 and 3.

The aPTT was prolonged significantly in the control mares only in protocol 3, namely from 42 ± 3 b.a. to 44 ± 3 s 1 h a.i.. Sick horses also experienced a prolongation, but only in colic group B was it significant, i.e. from 67 ± 13 b.a. to 89 ± 20 s 1 h a.i.. Only in colic group A, the aPTT (75.7 ± 26.6 s) was significantly prolonged b.a. compared with values of all protocols.

Urea and creatinine concentrations in serum in all protocols of normal mares were always within the physiological range during the entire 24-h period. Urea concentrations in patients increased 4 h a.i. ($P > 0.05$): in the colitis group from 6.5 ± 2.3 b.a. to 6.6 ± 2.6 a.i.; in colic group A from 8.4 ± 2.3 b.a. to 8.7 ± 1.8 a.i. and in colic group B from 7.1 ± 1.9 b.a. to 7.1 ± 2.1 mmol/l a.i., and then fell continuously to 4.9 ± 1.6 , 6.7 ± 2.4 and 6.3 ± 1.8 mmol/l respectively 24 h a.i. Creatinine concentration fell significantly among patients in the colitis group from 168 ± 54 b.a. to 134 ± 46 μ mol/l 24 h a.i. In colic group A, the concentration rose ($P > 0.05$) from 170 ± 41 b.a. to 190 ± 50 μ mol/l 1 h a.i. and then fell ($P > 0.05$) to 143 ± 40 μ mol/l 24 h a.i. In colic group B, it fell significantly from 214 ± 72 b.a. to 159 ± 67 μ mol/l 24 h a.i.

The specific gravity of urine in protocol 1 and 2 increased significantly from 1039 ± 0.01 and 1038 ± 0.01 b.a. to 1056 ± 0.01 and 1057 ± 0.01 respectively 1 h a.i. and then reverted to the physiological range 24 h a.i. No change could be determined in protocol 3 over a period of 24 h. In protocol 4 and 5, values decreased significantly from 1039 ± 0.01 and

Table 3. Mean values \pm SD of total protein (TP) concentrations in the serum (g/l) of six normal horses using five different protocols before administration (b.a.) and after infusion (a.i.) with 5 ml (protocol 1), 10 ml (protocol 2), 15 ml HES/kg (protocol 3), 15 ml saline/kg (protocol 4) or 4 ml 7.5% NaCl/kg (protocol 5); and of horses with acute colitis (colitis group, $n = 8$), ileus of small intestine (colic group A, $n = 7$) or with ileus of large intestine (colic group B, $n = 9$) treated with a mixture of 10 ml HES/kg and 10 ml saline/kg b.w.

	Serum concentrations of TP at various times a.i.										
	TP b.a.	10 min	1 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h	
Controls											
Protocol 1	63.2 ^a \pm 6.6	55.0 \pm 8.8	60.4 \pm 11.2	57.5 ^b \pm 5.9	56.3 ^b \pm 5.9	57.4 \pm 6.9	61.0 \pm 6.4	61.4 \pm 6.7	63.3 \pm 6.5	57.8 \pm 7.0	
Protocol 2	64.7 ^a \pm 5.5	53.4 ^b \pm 5.3	54.0 ^b \pm 4.4	53.7 ^b \pm 3.2	56.0 ^b \pm 4.2	60.5 ^b \pm 5.9	60.3 \pm 6.1	60.1 \pm 4.7	63.2 \pm 4.7	61.6 \pm 5.7	
Protocol 3	62.3 ^a \pm 5.3	45.8 ^b \pm 3.6	50.0 ^b \pm 3.8	51.3 ^b \pm 4.5	52.1 ^b \pm 4.2	54.5 ^b \pm 4.7	57.1 \pm 2.5	56.3 ^b \pm 4.1	57.4 ^b \pm 4.0	58.3 \pm 5.8	
Protocol 4	62.2 ^a \pm 5.8	58.2 \pm 5.3	62.0 \pm 6.8	63.1 \pm 4.7	63.7 \pm 4.1	64.5 \pm 5.7	66.1 ^x \pm 4.7	65.6 \pm 5.4	66.1 ^x \pm 5.2	63.8 \pm 5.6	
Protocol 5	60.3 ^a \pm 5.9	53.1 ^b \pm 6.6	59.0 \pm 6.1	62.8 \pm 6.5	61.2 \pm 6.4	62.4 ^x \pm 6.3	64.2 \pm 4.7	64.6 ^x \pm 6.1	66.2 ^x \pm 6.2	67.5 ^x \pm 5.3	
Patients											
Colitis-group	57.3 ^a \pm 6.6	43.7 ^b \pm 5.2	44.8 ^b \pm 5.7	45.2 ^b \pm 7.1	47.4 ^b \pm 7.3	46.4 ^b \pm 6.7	47.8 ^b \pm 6.5	48.9 ^b \pm 6.0	47.8 ^b \pm 6.3	50.6 \pm 7.7	
Colic-group A	61.7 ^a \pm 13.1	45.9 ^b \pm 8.3	44.9 ^b \pm 7.8	45.4 ^b \pm 8.3	47.1 \pm 8.3	48.5 \pm 8.0	50.1 \pm 9.0	50.2 \pm 8.1	51.0 \pm 8.0	52.5 \pm 9.9	
Colic-group B	58.9 ^a \pm 7.7	46.9 \pm 12.9	43.8 ^b \pm 6.8	45.4 ^b \pm 6.1	49.6 ^b \pm 5.9	49.4 ^b \pm 6.8	50.8 \pm 7.8	51.3 \pm 5.4	53.2 \pm 5.5	53.1 \pm 5.7	

HES, hydroxyethyl starch.

Decreasing^b respectively increasing^x values in the same row with different superscripts differ significantly ($P \leq 0.05$).

1032 \pm 0.01 b.a. to 1022 \pm 0.01 and 1010 \pm 0.01 respectively 1 h a.i., and then rose to normal 24 h a.i.

Gamma-glutamyl transferase/creatinine ratio in the urine did not change in protocol 1 through 4; only in protocol 5, there was a non-significant rise from 18 \pm 8.9 to 23 \pm 12.6 U/g 1 h a.i.

Sodium concentrations in the serum were elevated during the entire trial period in the protocols in normal mares, but only in protocols 3, 4 and 5, there was a significant increase, namely from 137 \pm 2, 139 \pm 3 and 137 \pm 2 mmol/l b.a. to 140 \pm 2, 142 \pm 3 and 146 \pm 3 mmol/l respectively 4 h a.i.

Fractional excretion of sodium in protocols 1 through 4 was under 1% over a period of 24 h a.i. In protocol 5, there was a significant rise from 0.50 \pm 0.75 to 6.6 \pm 3.3% 1 h a.i.; this value then sank to 3.2 \pm 1.6% 4 h a.i.

Potassium concentrations in serum fell significantly in the normal mares: in protocol 1 and 5 from 4.2 \pm 0.4 and 4.0 \pm 0.2 mmol/l b.a. to 3.8 \pm 0.4 and 3.3 \pm 0.4 mmol/l 1 h a.i. Protocols 2, 3 and 4 displayed no significant changes.

Fractional excretion of potassium rose in normal mares in protocols 1 through 5 from values of 71 \pm 24%, 80 \pm 32%, 69 \pm 26%, 90 \pm 32% and 88 \pm 24% b.a. respectively to 110 \pm 27% ($P < 0.05$), 133 \pm 30% ($P < 0.05$), 109 \pm 29% ($P > 0.05$), 99 \pm 39% ($P > 0.05$) and 134 \pm 30% ($P < 0.05$) 4 h a.i.

Concentrations of anorganic phosphate in serum fell significantly in all protocols of normal mares from 1.1 \pm 0.1, 1.2 \pm 0.2, 1.1 \pm 0.1, 0.9 \pm 0.3 and 1.2 \pm 0.4 mmol/l b.a. respectively to 0.9 \pm 0.2, 0.9 \pm 0.2, 0.8 \pm 0.1, 0.8 \pm 0.2 and 0.9 \pm 0.2 mmol/l respectively 4 h a.i.

Fractional excretion of anorganic phosphate was always under 1% in all protocols, both b.a. and 4 h a.i.

Discussion

Hydroxyethyl starch concentration in serum depends on the dosage administered. HES metabolic half-life in horses of 5.59 h (Meister et al., 1992) is comparable with the 5.83 h which we calculated at a dosage rate of 5 ml/kg. In humans, HES 200/0.5 metabolic half-life (2.5–3.5 h) is shorter (Asskali and Foerster, 1999). However, based on our results, half-life increases due to the higher dosage given. Amylase activity is within reference values for isotonic (protocol 4) and hypertonic (protocol 5) saline 24 h a.i.: following HES infusion (protocols 1–3) it is significantly raised, although it remains below the value of 84 U/l. Serum amylase is responsible for the intravascular metabolism of HES, the speed of which is determined by the degree of HES substitution (Foerster, 1989; Weidhase et al., 1998). The sharpest increase in amylase activity in protocol 3 (highest HES dosage) occurred 12 h a.i. In patients with large intestinal ileus (colic group B), however, the greatest amylase activity was recorded 4 h a.i. Initial values for amylase activity did not differ significantly in control mares in protocols 1 through 3 versus horses with acute colitis (colitis group) or small intestinal ileus (colic group A); however, five out of nine horses with large intestinal ileus (colic group B) had extremely elevated values. It is interesting to note that the highest values for amylase activity were 4 and 1 h a.i. following HES infusion in horses suffering from acute colitis or small intestinal ileus and showed no significant difference when compared to those of control mares in protocols 1 through 3. Horses with large intestinal ileus (colic

group B) displayed higher activity for a period of 12 h a.i. as compared with control mares in protocols 1 through 3: the reason could be injury to the pancreas resulting from large colon displacement. Possible pancreatitis in this group could be excluded because amylase activity fell back to 26 ± 17 U/l 24 h a.i. Although initial glucose values were significantly higher among the horses in colic group A and B as compared with control mares. Despite the highest values for amylase activity, the concentration of glucose in horses with ileus of the large intestine sank continually: consequently higher glucose values could not be caused by intravascular HES metabolism.

An anticipated rise in intravascular glucose resulting from hydrolysis of HES following infusion at various dosage rates could not be established in the control mares, the reason being that isotonic or hypertonic saline in and of itself caused an increase in blood glucose in these horses. Indeed, following infusion of hypertonic saline, horses in protocol 5 displayed significantly higher glucose concentrations for a period of 24 h a.i. No significant difference in glucose concentrations could be established among the three groups: normal horses, those which were given HES and those which received isotonic or hypertonic saline.

Although haematocrit, haemoglobin, TP and albumin are all considered important parameters for haemodilution, haematocrit is essential in determining the fluidity of blood (Brueckner, 1991; Asskali and Foerster, 1999). In control mares given an infusion of isotonic or hypertonic saline, a reduction in haematocrit levels by 14.7% and 17.6% was measured 10 min a.i. In control mares given three different dosages of HES, the drop in haematocrit was 25.0%, 33.3% and 48.6% respectively. The drop of TP in serum, however, was much lower and of shorter duration than that of haematocrit in all protocols, regardless of the product administered: it was 6.4% and 11.9% respectively in protocols 4 and 5 following isotonic or hypertonic saline infusions. Following HES infusion, on the other hand, TP fell to 13.0%, 20.8% and 26.5%, depending on the dosage. Although initial TP values varied only modestly, a highly significant difference in these values could be established when comparing the controls with the sick horses. In the three groups of sick horses, haematocrit values sank following HES administration by 30.9%, 34.7% and 34.0%. This study showed that an infusion of 15 ml/kg of isotonic saline (protocol 4) was less effective than a dosage of 5 ml/kg infusion of HES. One would have to administer four times the volume of electrolyte solution as compared with HES to achieve the same intravascular volume effect (Dietrich, 2001). Based on the literature, we believe that volume expansion with HES causes an increase in colloid osmotic pressure in the intravascular space, resulting in better blood fluidity and a corresponding increase in the supply of oxygen to the tissues (Jones et al., 1997, 2001; Asskali and Foerster, 1999; Dietrich, 2001; Lucas et al., 2006). A single infusion of isotonic or hypertonic saline elevated the TP concentration 8 resp. 6 h a.i. and simultaneously lowered the specific gravity of urine: the HES infusion had the opposite effect.

Animal experiments with ischaemic reperfusion models have established that HES infusions result in both improved microcirculation as well as a significant reduction in reperfusion injuries when compared to electrolyte solutions, dextran or serum albumin (Wisselink et al., 1998). Hypovolaemic shock and endotoxaemia cause a malfunction in the microcirculation in horses with colic or colitis, which in turn

increases the possibility of formation of intestinal oedema. Increasing microcirculatory malfunction leads to greater protein concentrations in the oedema liquid, consequently increasing the probability of an inflammation. HES could potentially eliminate swelling and consequently reduce inflammation. Potential capillary leakage can be avoided by administering a 10% solution of HES 200/0.5 (Friedmann et al., 2003).

Although aPTT was prolonged due to haemodilution by 2 s following infusion of 15 ml HES/kg, values of normal mares were still within the physiological range of 37–54 s. The fact that aPTT was lengthened significantly in the sick horses can be attributed to existing hypovolaemia and endotoxaemia and resulting disseminated intravascular coagulopathy (Jones et al., 1997; Monreal et al., 2000). Staehli (2000), however, was able to show in a retrospective study that there was no difference in the aPTT of horses with or without HES treatment. HES 200/0.5 had the least effect on blood coagulation when compared with other colloidal plasma-substitution products, such as dextran and high molecular or highly substituted HES (Petroinau et al., 2000).

Hydroxyethyl starch metabolic products (threshold 40 000 Da) are excreted primarily through the kidneys (Foerster et al., 2001). HES improves kidney perfusion, as well as increases urine volumes and creatinine clearance, which means that a single infusion of HES can be given to geriatric patients with mild to severe renal failure (Jungheinrich et al., 2001). In controls, the specific gravity of urine increased following 5 and 10 ml/kg infusions of HES, although the concentration of urea and creatinine remained the same during the entire observation period. No change in the specific gravity of urine was observed following a 15 ml/kg infusion. Most likely the rise in colloid osmotic pressure in intravascular space led to increased cardiac output, resulting in turn in higher hydrostatic pressure: consequently the temporary rise in colloid osmotic pressure appeared to be compensated for and the glomerular filtration rate returned to normal. The influence of HES on the specific gravity of urine is unknown, but urine viscosity increased 2.4-fold. In comparison, urine viscosity can increase 5-fold following an infusion of dextran-40 (Glaser, 1997).

Serum concentrations of urea and creatinine were significantly higher in sick horses than in the controls. The reason for these increases is haemodynamic kidney failure, whereby the proximal tubule is primarily affected in patients with strangulation obstruction (Halbmayer, 1999; Schulze et al., 2004). Additional benefits of HES infusion could include a reduction in damage to the epithelia of the proximal tubule, as neither the GGT/creatinine relationship nor the fractional excretion of sodium and anorganic phosphate was elevated in normal horses. Renal excretion of HES metabolic products depends on glomerular water filtration and consequently on the concentration of the glomerular filtrate. This in turn means that administration of a mixture of HES and a sufficient electrolyte solution not only increases HES renal excretion: it also contributes to hydration (Ragaller et al., 2001).

To summarize: a mixture of 10 ml HES/kg and 10 ml isotonic saline/kg optimizes the speed of infusion and consequently also kidney perfusion and decreases the specific gravity of urine. The efficacy of a bolus of HES (10 ml/kg) followed by isotonic saline (10 ml/kg) infusion in patients was determined due to the decrease of haematocrit in the patient groups. High serum concentrations of urea and creatinine revert to almost normal values in patients within 24 h a.i. Based on this, HES

infusion did not induce renal failure in horses with acute colitis or small or large intestine ileus. In horses with strangulation obstruction, a single infusion of isotonic saline has the same effect only 8 days after surgery (Halbmayr, 1999), therefore, HES should be administered before as a single dose. No side effects of HES could be observed in horses with acute colitis or colic. HES infusion stabilized circulation and hydration: in addition, 24 h a.i. heart rate had fallen significantly, increasing oxygen transportation to the tissues and limiting haemodilution. Clinical experience showed fewer instances of postoperative ileus. All of these factors could reduce postoperative costs in the treatment of horses with colic.

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