The Effects of Mild Hypothermia on Coagulation Tests and Haemodynamic Variables in Anaesthetized Rabbits

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ABSTRACT

Objective: *Hypothermia has been associated with coagulation defects. The purpose of this experimental study was to investigate the effect of mild hypothermia on clinically used coagulation tests and on haemodynamic variables.*

Methods: *Nine New Zealand rabbits were subjected to mild core hypothermia by administration of general anaesthesia and exposure to room temperature of 22°C for 60 minutes. Blood samples were obtained at normothermia and mild hypothermia for measurement of prothrombin time, activated partial thromboplastin time, fibrinogen levels, platelet count and haemoglobin concentration. Hypothermic values were compared to the normothermic values. Additionally, the progressive temperature drop and haemodynamic changes (blood pressure, heart rate) were recorded.*

Results: *Core temperature decreased significantly over time changing from 39.4 ± 0.27 to 36.6 ± 0.28°C (*p = *0.0001). Prothrombin time and activated partial thromboplastin time decreased at hypothermia, but the changes were not statistically significant (*p = *0.203 and* p = *0.109, respectively). Platelet count, fibrinogen levels and haemoglobin concentration decreased significantly (*p = *0.0001,* p = *0.03 and* p = *0.027) but remained within normal limits. Mean arterial pressure and heart rate declined significantly over time (*p = *0.0001 and* p = *0.0001, respectively).*

Conclusion: *The results of this study suggest that short term mild hypothermia may affect the coagulation mechanism to a clinically nonsignificant extent, while haemodynamic responses are significantly suppressed.*

Keywords: Coagulation tests, haemodynamics, mild hypothermia

Efectos de la Hipotermia Leve sobre las Pruebas de Coagulación y las Variables Hemodinámicas en Conejos Anestesiados

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RESUMEN

Objetivo: *La hipotermia ha sido asociada con defectos de coagulación. El propósito de este estudio experimental fue investigar el efecto de la hipotermia leve sobre las pruebas de coagulación de uso clínico, así como sobre las variables hemodinámicas.*

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Métodos: *Nueve conejos de Nueva Zelanda fueron sometidos a hipotermia central leve mediante la administración de anestesia general y exposición a una temperatura ambiente de 22°C durante 60 minutos. Se obtuvieron muestras de sangre en condiciones de normotermia e hipotermia leve para medir el tiempo de protrombina, el tiempo de tromboplastina parcial activada, los niveles de fibrinógeno, el conteo de plaquetas, y la concentración de hemoglobina. Se compararon los valores hipotérmicos con los valores normotérmicos. Además, se registraron la caída progresiva de la temperatura y los cambios hemodinámicos (presión sanguínea, frecuencia cardíaca).*

Resultados: *La temperatura corporal central disminuyó significativamente con el tiempo, cambiando de 39.4 ± 0.27 a 36.6 ± 0.28°C (*p = *0.0001). El tiempo de protrombina y el tiempo de tromboplastina parcial activado disminuyeron en la hipotermia, pero los cambios no fueron estadísticamente significativos (*p = *0.203 y* p = *0.109, respectivamente). El conteo de plaquetas, los niveles de fibrinógeno y la concentración de la hemoglobina disminuyeron significativamente (*p = *0.0001,* p = *0.03 y* p = *0.027) pero permanecieron dentro de los límites normales. La presión arterial promedio y la frecuencia cardíaca disminuyeron significativamente con el tiempo (p = 0.0001 y p = 0.0001, respectivamente).*

Conclusión: *Los resultados de este estudio sugieren que la hipotermia leve a corto plazo puede afectar el mecanismo de la coagulación hasta un punto clínicamente no significativo, mientras que respuestas hemodinámicas se suprimen significativamente.*

Palabras claves: Pruebas de coagulación prueba, hemodinámica, hipotermia leve

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INTRODUCTION

Hypothermia has been associated with coagulation defects that may affect the outcome of trauma victims and surgical patients (1–3). Multiple trauma patients, the elderly and children are more susceptible to hypothermia (4, 5). In trauma victims, morbidity and mortality are significantly increased by hypothermia, acidosis and coagulopathy (2, 6). Also, perioperative hypothermia has been related to increased cardiac morbidity, impaired wound healing and prolonged hospital stay (4, 7).

Anaesthesia *per se* results in mild hypothermia due to vasodilation and core-to-periphery heat redistribution (4). This temperature reduction is further exaggerated intra-operatively by the cold operating room environment, visceral exposure and blood transfusions, especially during prolonged body-cavity procedures.

The reported results on hypothermia induced coagulopathy are contradictory, possibly due to the different study settings, experimental models and methods used for the assessment of the coagulation mechanism (8). Studies on deep hypothermia have shown a significant decrease in fibrinogen levels and platelet count (9–11). Severe hypothermia results in platelet sequestration in the liver and spleen (9, 10) and also in enhanced fibrinolysis due to plasminogen activator release (12).

There are limited data about the possible coagulation defects induced by mild hypothermia *in vivo* (13–15), even though increased blood loss and need for transfusions have been reported in mildly hypothermic surgical patients (16, 17). Mild hypothermia may impair the coagulation-fibrinolysis balance by reducing the activity of natural anticoagulant and antifibrinolytic factors, while increasing the activity of plasminogen (15).

The aim of the present study was to investigate the possible impact of mild hypothermia on the integrity of the coagulation mechanism in rabbits without haemorrhage or surgical intervention. We assessed prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen levels and also platelet (PLT) count, since these are the standard coagulation tests and markers commonly used in clinical practice. Haemodynamic responses to progressive hypothermia were also recorded.

METHODS

The study was approved by the Committee on the Care of Experimental Animals and the animals were housed and cared for in conformance with the Animal Care Committee guidelines (National law 160/91–12).

Nine male New Zealand white rabbits were premedicated with ketamine HCl (Ketaset, Ceva) 25 mg/kg and Xylazine HCl (Rompun, Bayer) 5 mg/kg intramuscularly, shaved and positioned on the surgical table in an air-conditioned operating theatre at 22°C. Electrocardiograph (Graseby-Rigel 429), pulse oximetry (Kontron Instruments Pulse Oximeter 7840), blood pressure (Dinamap-Criticon Vital Signs Monitor 1840), and distal oesophageal temperature monitoring (thermistor connected to Graseby-Rigel 429 monitor) were applied to all animals.

Baseline blood samples were obtained at normothermia and 0.9% NaCl was started at 8 ml/kg/h *via* a marginal ear vein catheter for volume replacement. Anaesthesia was induced and maintained with intravenous (IV) propofol

(Propofol, Fresenius 1%), 10 mg/kg bolus and 30 mg/kg/h infusion, respectively. After the insertion of a tracheal tube (ID: 3-3.5 mm, uncuffed), mechanical ventilation was applied. An oxygen/air mixture was administered (fractional inspired oxygen, FiO_2: 0.45) and proper adjustments were made to correct possible hypercarbia, according to arterial blood gas values. Sixty minutes after induction of anaesthesia, at mildly hypothermic conditions, the second blood samples were obtained from the auricular artery. They were used for assessment of PT, APTT, PLT count, fibrinogen levels and haemoglobin (Hb) concentration. For coagulation tests, blood (4.5 ml) was collected in a 0.105 M sodium citrate tube and centrifuged immediately for 15 minutes at 2500 g. Commercially available reagents were used to measure PT (kit STA-Stachrom® Neoplastine), APTT (kit STA-Stachrom® PTTA 5) and fibrinogen (kit STA-Stachrom® Fibrinogen). For all determinations, the manufacturers' instructions were followed. For measurements of Hb concentration and PLT count, blood (2 ml) was collected in K_3EDTA tubes. Temperature and haemodynamic measurements (blood pressure and heart rate) were recorded at 10-minute intervals.

Statistics

Statistical analysis was conducted using the SPSS version 16.0 (SPSS Inc, Chicago IL). Haemoglobin, PLT and fibrinogen values followed normal distributions and were analysed with student's paired test. Prothrombin time and APTT values did not follow normality and were analysed with Wilcoxon Signed Rank test. Body temperature, mean arterial blood pressure (MAP) and heart rate (HR) values, measured every 10 minutes during the protocol, were analysed with ANOVA Repeated Measures. Values are expressed as Mean ± SD. A statistical level of 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Nine rabbits with mean body weight 3516.66 ± 158.11 g were anaesthetized and exposed to 22°C environmental temperature for 60 minutes. During the experiment, the animals' mean core temperature significantly decreased from 39.4 ± 0.27 to 36.6 ± 0.28 ($p = 0.0001$), as shown in Fig. 1. Coagulation tests at mild hypothermia, as expressed by PT and APTT values, did not differ significantly from baseline measurements at normothermia (Table). Platelet count, fibrinogen levels and Hb concentration at hypothermia were significantly lower compared to the normothermic values ($p = 0.0001$, $p = 0.03$ and $p = 0.027$ respectively), as shown in the Table. Haemodynamic variables, thus MAP and HR significantly declined over time ($p = 0.0001$ and $p = 0.0001$ respectively) as shown in Fig. 2. Vasoactive or anticholinergic drugs were not administered to any of the animals.

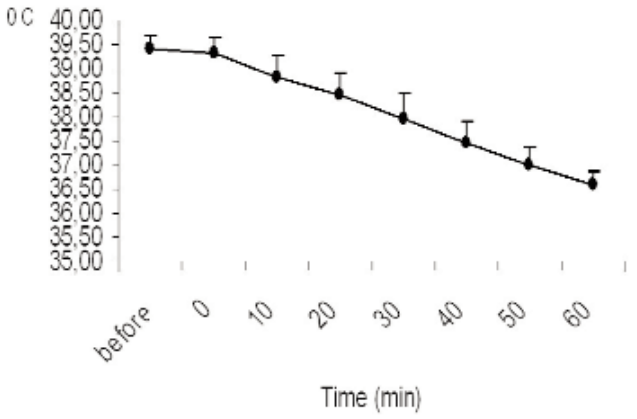

Fig. 1: Progressive decrease of core temperature in rabbits over 60 minutes of anaesthesia and exposure to 22°C room temperature.

Table: Prothrombin time (PT), activated partial thromboplastin time (APTT), platelet count (PLT), fibrinogen levels and haemoglobin concentration (Hb) at normothermia and mild hypothermia in anaesthetized rabbits exposed to 22°C for 60 minutes. Values are expressed as mean ± SD.

		Mean ± SD	T	Z	*p*
PT	Normothermia	7.61 ± 0.78		-1.27	0.203
	Mild hypothermia	8.22 ± 1.27			
APTT	Normothermia	52.42 ± 13.92		-1.601	0.109
	Mild hypothermia	57.86 ± 15.18			
PLT	Normothermia	343.33 ± 76.79†	4.81		0.001
	Mild hypothermia	264.11 ± 78.59†			
Fibrinogen	Normothermia	408.33 ± 71.94††	4.24		0.03
	Mild hypothermia	350.11 ± 82.28††			
Hb	Normothermia	11.76 ± 1.26*	2.69		0.027
	Mild hypothermia	10.31 ± 1.48*			

p value < 0.05 was considered statistically significant, †PLT at normothermia *versus* mild hypothermia, ††Fibrinogen at normothermia *versus* mild hypothermia, *Hb at normothermia *versus* mild hypothermia.

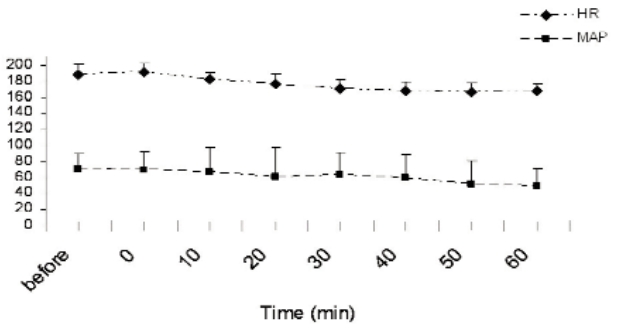

Fig. 2: Decline of mean arterial pressure (MAP) and heart rate (HR) in rabbits after 60 minutes of anaesthesia and exposure to 22°C room temperature.

DISCUSSION

According to the results, PT and APTT were not significantly affected by mild hypothermia, while fibrinogen levels and PLT count were significantly reduced. Haemodynamic variables, thus MAP and HR declined significantly over time. Rabbits were used as study animals because they are considered to be a good model for studies on the human coagulation mechanism (18, 19).

In the present experiment, mild hypothermia was induced by anaesthetizing the rabbits, by exposing them to an environment of 22°C, and by administering intravenous fluids at room temperature. Core temperature monitoring was based on deep oesophageal temperature, since it reliably reflects cardiac and blood temperature (3, 5, 20).

After one hour, the animals' core temperature decreased by 2.8°C. It has been shown that the body temperature of anaesthetized patients is greatly affected by the ambient temperature (7, 21). The suggested mechanisms are that anaesthetic drugs reduce by 20% the metabolic heat production and they also inhibit shivering and peripheral vasoconstriction (7, 21). General anaesthesia also produces peripheral vasodilation and core-to-periphery heat distribution with rapid decrease of core temperature (7, 21) and expansion of the core compartment (22). Twenty minutes after the induction of anaesthesia, the size of the core compartment is increased from 66% to 71.2% of body mass (22). Anaesthetic drugs impair thermoregulation to a different extent; it has been found that propofol, which was used in the present experiment, produces more core hypothermia than sevoflurane (23). Nevertheless, propofol has not been related to coagulation abnormalities when administered for long term sedation in the Intensive Care Unit (24).

The majority of clinical studies have shown that mild perioperative hypothermia increases blood loss (16, 17, 25). According to a recent meta-analysis, mild perioperative hypothermia may increase blood loss by 16% and risk for transfusion by 22% (17). Our results show a tendency towards prolongation of PT and APTT , but the changes were not statistically significant. Staab *et al* also reported no significant changes of PT and APTT values using both *in vitro* and *in vivo* cooling (from 40°C to 37°C) in minipigs (14). In humans with trauma, PT and APTT were not significantly affected at core temperatures of 34°C or more (26). It should be noted though, that multiple trauma may induce hypercoagulability due to massive release of tissue thromboplastin (26).

In everyday clinical practice, PT and APTT are standard screening tests used for the assessment of clotting factors' adequacy. Prothrombin time assesses the extrinsic pathway of coagulation (factors II, V, VII, X) while APTT assesses the intrinsic pathway (factors VIII, IX, XI, XII). Plasmatic coagulation tests are temperature sensitive and are routinely performed at 37°C. It has been suggested that hypothermia induces reversible coagulation defects, mainly by affecting the kinetics of clotting factors, rather than their concentrations (27). In hypothermic patients, if coagulation tests are performed at 37°C they may give "falsely" normal results (28, 29). In the present study, PT and APTT were measured at 37°C which also was the animal core and blood temperature, so we consider that our results are reliable.

In this study, a statistically significant reduction of PLT count and fibrinogen levels was found. These findings could be attributed, at least in part, to haemodilution by the fluids administered for volume replacement, since the levels of Hb were also reduced. All values remained within normal limits, suggesting that the reductions may be considered as clinically not significant. There are limited published data on the effects of mild hypothermia on PLT and fibrinogen (13, 14). In mildly hypothermic minipigs, a decrease in PLT count has been reported, which was not statistically significant (14). The authors also report fibrinogen levels within normal limits, but without comparing pre- and post-hypothermic values to assess any possible change (14). In humans, no significant decrease of PLT number has been reported at 34°C, while a statistically significant 5% PLT decrease was found at 32°C (13). In trauma patients with core temperatures below 35°C, only a tendency towards decreased PLT count has been found (26).

Even though it is likely that PLT count and fibrinogen levels were affected by haemodilution, the decrease of their mean values was quite significant, probably indicating a subclinical abnormality in the haemostatic-fibrinolytic mechanism. Such a condition, developing quite early during the course of hypothermia, could be expected to worsen over time or with further temperature reduction, leading to a clinically evident coagulopathy. The possibility of an early developed subclinical haemostatic-fibrinolytic abnormality is supported by our previously published data that α_2-antiplasmin activity is significantly reduced and plasminogen activity is significantly increased at mild and moderate hypothermia (15). These changes indicate a tendency toward increased fibrinolysis, probably not severe enough to be evident early in the routine coagulation tests. Also, an acute

decrease of fibrinogen levels may be related to its increased consumption, as in abnormal fibrinolysis or in disseminated intravascular coagulation (DIC). Accidental hypothermic victims have been reported to develop a DIC-like syndrome (30).

Another finding of the present study was that MAP and HR were significantly reduced over time. The results are in accordance with that of Tanaka *et al* who found that in hypothermic (33.9°C) anaesthetized humans, the systolic blood pressure decreases and the arterial baroreflex control of HR is significantly depressed (31). The authors consider that hypothermia may significantly impair the baroreflex function (31). Also, Krause *et al* in a porcine model of haemorrhagic shock found that the hypothermic animals had significantly reduced cardiac output and were unable to maintain the tachycardic response to haemorrhage (3). On the other hand, Sessler *et al* report minor haemodynamic effects of perioperative hypothermia during isoflurane anaesthesia, with similar heart rates between normothermic and hypothermic volunteers [1.5°C temperature drop] (32). The different haemodynamic profile of the subjects and anaesthetic agents, propofol *versus* isoflurane, as well as the lower degree of hypothermia in volunteers could probably play a role in these findings. Most studies have investigated the haemodynamic responses to hypothermia postoperatively in awake patients with conflicting results (32–34). Decreased heart rates, but increased arterial pressure measured *via* the radial artery have been reported post-operatively in hypothermic elderly patients, possibly due to increased norepinephrine concentration (33). It has been suggested that vasoconstriction at the site of blood pressure measurement could play a role in the increased blood pressure measurements *via* radial artery catheterization (32). Heart rate and blood pressure measured oscillometrically were comparable in normothermic and hypothermic infants and children after general anaesthesia (34). The different findings of these studies may be attributed to the different measurement methods and also to the different clinical settings and age groups, since thermoregulatory mechanisms and haemodynamic responses are significantly impaired in anaesthetized and elderly patients.

We chose to study mild hypothermia, since this is the type of hypothermia most commonly seen perioperatively, especially in elderly and paediatric patients, as well as during prolonged major abdominal surgery.

We conclude that even short duration mild hypothermia may impair the haemostatic mechanism to some extent, especially platelets and fibrinogen, but the effects are clinically not significant. On the other hand, we found that haemodynamic responses are significantly suppressed during mild hypothermia. We consider that the possible coagulation defects induced by mild hypothermia and their clinical significance in humans should be further investigated.

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