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Effects of acetylsalicylic acid on coagulation tests and haptoglobin concentrations in rabbits with permanent transvenous pacing

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ABSTRACT: The aim of this study was to evaluate changes in coagulation tests, haptoglobin concentrations and leukocyte counts in rabbits with right-ventricle pacing medicated with acetylsalicylic acid (ASA). Blood was collected from 35 non-anaesthetised males from the jugular vein at baseline, one and two months after pacemaker implantation. Animals were divided into two groups: non-medicated and medicated with ASA. Total leukocyte and platelet counts were measured on an automatic veterinary flow cytometry haematological analyser. Prothrombin time, activated partial thromboplastin time, fibrinogen levels and D-dimers were determined from citrated blood. We found significantly elevated activated partial thromboplastin times and prothrombin times in ASA in comparison to the control group, but not within the ASA group over time. We also observed a decrease in platelet counts in the control group over time, but not in comparison to the ASA group. No significant changes in total leukocyte counts and haptoglobin concentrations were detected. Medication with ASA may alter coagulation profiles in rabbits with permanent transvenous pacing.

Keywords: inflammation; D-dimers; pacemaker; *Oryctolagus*

List of abbreviations

aPTT = activated partial thromboplastin time, **ASA** = acetylsalicylic acid, **PT** = prothrombin time, **PTP** = permanent transvenous pacing, **TT** = thrombin time, **VT** = venous thromboembolism

Implantation of a permanent transvenous pacemaker (PTP) is becoming a routine therapeutic approach in patients with cardiac rhythm abnormalities in both human and veterinary medicine. Implantation of leads is associated with endothelial injury and an association between the number of leads and risk of thrombosis has been documented previously (van Rooden et al. 2004). However, other studies did not confirm these findings and reported that the role of primary disease and patient-related risk factors seems to be more important in the development of thromboembolism (Korkeila et al. 2010; Li et al. 2014).

Fazio et al. (1991) found that the antiplatelet agent ticlopidine decreases the risk of thromboembolism significantly and Ito et al. (1997) suggested anticoagulant therapy to be even more effective. It remains doubtful whether the use of a prophylactic antiplatelet agent or anticoagulant therapy should be recommended routinely after PTP implantation or not, since the pathogenesis of pacemaker-associated thromboembolism is still not fully understood.

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Except for direct endothelial damage, implantation of pacemakers has also been associated with an increase in inflammatory markers (Lelakowski et al. 2012a). Inflammation is known to be associated with the pro-thrombotic state (Saghazadeh et al. 2015) and clinical studies have revealed a higher risk of thrombosis in patients with elevated levels of inflammatory markers (Lelakowski et al. 2012b). Thus, the recommended antiplatelet therapy for electrophysiological device surgery, aspirin, may also be of benefit in this scenario due to its anti-inflammatory action (Amann and Peskar 2002; Zaca et al. 2015).

The utility of preventive anti-aggregative therapy after pacemaker implantation thus remains to be determined in both human and veterinary medicine. The number of transvenous pacemaker implantations in dogs and cats is increasing and post-operative care is not standardised. In the authors' clinical experience, dogs with anti-aggregative therapy after pacemaker placement did not benefit from medication, in contrast to some reports from human medicine (Fazio et al. 1991; Ito et al. 1997). Use of an experimental model with a negative medical history may be more objective for the evaluation of the effect of anti-aggregative therapy after pacemaker implantation. The aim of this study was to evaluate the effect of acetylsalicylic acid on selected laboratory parameters including coagulation tests and inflammatory markers after permanent transvenous pacemaker implantation in clinically healthy rabbits.

MATERIAL AND METHODS

Animals. A group of 35 clinically healthy New Zealand white rabbits (albino) were used in this study. All rabbits were males with an average weight of 3.1 kg (min.–max., 2.7–3.4 kg) and age of 14 weeks. Rabbits were vaccinated against myxomatosis and plague (Pestorin Mormyx Bioveta a.s., Ivanovice na Hane, Czech Republic) two weeks prior to first blood collection. The animals were kept in metal cages (50 × 60 × 70 cm) during the experiment. The cages were situated in a room with a temperature of 19 ± 1 °C and relative air humidity between 55 and 60%. Complete feed mixture (Biostan KV, Biosta Blucina, Czech Republic) and drinking water were administered *ad libitum*. A natural light regimen was maintained and health condition was checked daily.

After a period of adaptation (four weeks), rabbits underwent anaesthesia and a right-ventricle pacemaker was placed in all animals. Induction of anaesthesia was performed using diazepam (2 mg/kg body weight, *i.m.*, Apaurin inj., Krka d.d. Novo mesto, Slovenia), ketamine (35 mg/kg body weight, *i.m.*, Narketan 100 mg/ml inj., Vetoquinol Ltd. Nymburk, Czech Republic) and xylazine (5 mg/kg body weight, *i.m.*, Xylapan 20 mg/ml inj., Vetoquinol Ltd. Nymburk, Czech Republic). After surgery, rabbits received marbofloxacin (4 mg/kg, *s.c.*/48 h, Marbocyl 10% A.U.V. inj. Vetoquinol Ltd. Nymburk, Czech Republic) and tolfenamic acid (4 mg/kg body weight, *s.c.*/48 h, Tolfedine 4% inj. Vetoquinol, Lure, France) for seven days and metroprolol tartarate (100 mg/l of drinking water, Vasocardin SR 200, Takeda GmbH Konstanz, Germany) until the end of the experiment.

After that, rabbits were randomly divided into two groups: the control group (n = 23) did not receive additional medication, the ASA group (n = 12) received acetylsalicylic acid (125 mg/l of drinking water, Acylpyrin 500 mg tablets – Herbacos Recordati Ltd., Pardubice, Czech Republic) until the end of the experiment.

The experiment was performed in compliance with Act. No. 246/1992 on the Protection of Animals from Maltreatment, as later amended. The experimental protocol was approved by the expert committee for ensuring the welfare of experimental animals and Ministry of Education, Youth and Sport under the number 53/2013.

Blood collection and laboratory analyses. Blood was collected one day before (baseline), one month and two months after pacemaker placement from the *vena jugularis externa* into citrate- and EDTA-containing (Dispolab Ltd., Brno, Czech Republic) test tubes. Haematological examination (measurement of leukocyte and platelet counts) was performed from EDTA blood on an automated haematological analyser (Sysmex XT 2000iV, Sysmex Corporation, Kobe, Japan) within 10 min. Citrated blood was centrifuged (1000 *g*, 10 min) and used for coagulation analysis. Measurement of prothrombin time (PT; Tromboplastin-S, Dialab, Ltd., Prague, Czech Republic), activated partial thromboplastin time (aPTT; APTT-S, Dialab, Ltd., Prague, Czech Republic; 0.025M $CaCl_2$, Dr. Kulich Pharma, Ltd., Hradec Kralove, Czech Republic), thrombin time and fibrinogen (Bovinni trombin 100 NIH-U/ml, Dialab, Ltd., Prague, Czech Republic) was performed on a two-channel analyser (Coatron M2,

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Teco, Hilden, Germany). Coagulation analysis including D-dimers (NycoCard D-dimers, Axis-Shield PoC, Oslo, Norway) was performed within 1 h after blood collection.

Haptoglobin was measured using an ELISA kit according to the manufacturer's instructions (Haptoglobin rabbit ELISA kit, Abcam plc, Cambridge, United Kingdom). Samples were measured in doublets, accepted CV 10%, observed mean CV 5.7%.

Statistical analysis. Data underwent statistical analysis (MedCalc bvba, Ostend, Belgium). Parameters in each measurement (baseline, one month, two months) were compared between control and ASA groups using the Mann-Whitney *U*-test. Blood samplings within each group were compared using the Wilcoxon paired test. Values are shown as median (min.–max.). The level of significance was set at $P < 0.05$.

RESULTS

Results of the coagulation test are shown in Table 1. A significant difference between the control and ASA groups was found in aPTT one month ($P = 0.046$) and in PT two months ($P = 0.017$) after pacemaker placement. Both groups exhibited a significant decrease of platelets after one month in comparison to baseline values ($P = 0.02$ and $P = 0.001$, Mann-Whitney *U*-test) with values not significantly different between the groups. Using a pair-test, a significant decrease in platelet counts was observed only in the control group in both measurements (baseline vs one month, one month vs two months, $P = 0.023$ and $P = 0.0043$, respectively).

Total leukocyte counts and haptoglobin levels were not significantly different between groups or samplings (Table 1).

DISCUSSION

It is well described that patients with permanent transvenous pacing often suffer from venous thromboembolism (VT). Partial or total obstruction has usually been found in examined patients to varying degrees – 14% in the study of Korkeila et al. (2007), 50% in the study of Costa et al. (2009) and 79% in

Table 1. Selected laboratory parameters in rabbits with permanent transvenous pacing over time

Parameter (UI)		After one month		After two months	
		control group	ASA	control group	ASA
D-dimer (mg/l)	median	0.2	0.3	0.1	0.3
	range	DL–0.5	0.2–0.5	DL–0.4	0.1–0.4
aPTT (s)	median	22.55	28.8*	27.2	34.95
	range	16.0–35.8	20.1–35.3	16.9–48.4	20.6–78.5
Prothrombin time (s)	median	10.2	10.1	9.75	10.9*
	range	8.6–13.7	7.9–12.7	8.4–13.5	9.6–12.9
Thrombin time (s)	median	16	15.1	15.55	14.4
	range	12.7–18.5	13.3–16.3	8.5–19.9	12.6–17.1
Fibrinogen (g/l)	median	2.1	2.53	2.45	2.42
	range	1.23–4.82	1.37–4.74	1.66–5.16	0.68–4.52
Platelet count ($\times 10^9$/l)	median	370.5†	292	333.5	285.5
	range	214–577	122–544	69–460	84–403
Haptoglobin (ng/ml)	median	20.4	29	18.9	10.2
	range	DL–51.7	DL–47.9	DL–53.6	DL–51.3
Leukocyte count ($\times 10^9$/l)	median	7.38	6.8	8.3	8.3
	range	5.3–11.9	4.8–14.0	3.1–12.1	6.1–13.3

aPTT = activated partial thromboplastin time, ASA = group medicated with acetylsalicylic acid, DL = detection limit

*value significantly different from control group (Mann-Withney *U*-test)

†value significantly different from previous sampling (Wilcoxon paired test)

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the study of Stoney et al. (1976). Venous thromboembolism is, however, usually asymptomatic due to the development of collateral circulation (Ito et al. 1997). The pathogenesis of thromboembolism in patients with permanent pacing is not known yet and some authors consider that pacemaker electrodes or the absence of anticoagulant treatment contribute to the occurrence of thrombosis (van Rooden et al. 2004; Khairy et al. 2006). However, in recent studies on patients suffering from venous or pulmonary thromboembolism after pacemaker implantation, whether patients were on antithrombotic therapy or not had no significant difference on the incidence of the condition (Khairy et al. 2006; Korkeila et al. 2010; Noheria et al. 2016). Since prophylactic antithrombotic medication is routinely used, we wondered if there are any changes in laboratory parameters associated with acetylsalicylic acid medication. As a model animal we used clinically healthy rabbits in order to eliminate patient-related risk factors for the development of VT. Moreover, electrodes are relatively large when implanted in rabbits in comparison with humans. We collected blood before, and one and two months after, pacemaker implantation.

Our results revealed almost no significant changes between the control group and the group medicated with ASA. Only aPTT after one month, and PT after two months, were significantly higher in rabbits medicated with ASA in comparison to the control group. The reason for the elevated aPTT is not clear. Even though the median haptoglobin concentration was high in this group, which could explain the increased activity of the coagulation system, this did not correlate with aPTT (data not shown). A significant difference in prothrombin time was caused by a slight decrease in PT in the control group. However, neither aPTT nor PT was significantly different among samplings within each group.

The decrease in platelets was observed in all groups over time but was significant only in the control group. Platelet consumption during microthrombi formation could be a logical explanation; however, values of D-dimers which could indicate increased fibrinolytic activity were not significantly different among samplings.

Regarding the inflammatory markers, neither haptoglobin concentration nor leukocyte counts were significantly different over time and between groups. Haptoglobin is a major acute phase protein in rabbits (Cray et al. 2009); its concentration is increased at 8 h after stimulus and remains elevated for three days (Jethanandani et al. 1995). Although surgery almost always results in increased concentrations of acute phase proteins, these remain high for more than six days only in infected patients (Panda et al. 1987). The presence of a pacemaker was not associated with elevation of haptoglobin levels in this study. Similar results were reported from human medicine, where C-reactive protein levels measured at implantation of the pacemaker and one month later do not fluctuate significantly (Balli et al. 2014).

We conclude that the mere presence of a pacemaker is not associated with significant changes in the coagulation profile and inflammatory markers in the rabbit.

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