Different Heparin Contents in Prothrombin Complex Concentrates May Impair Blood Clotting in Outpatients With Ventricular Assist Devices Receiving Phenprocoumon

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Objectives: **Prothrombin complex concentrates (PCCs) are used to rapidly reverse anticoagulation by oral vitamin K antagonists. They differ in the content of clotting factors, endogenous anticoagulants, and heparin. The authors hypothesized that PCCs' specific heparin content may compromise the hemostatic effect.**

Design: **Prospective ex-vivo investigation.**

Setting: **University hospital.**

Participants: **Venous blood samples were obtained from 8 patients with implanted ventricular assist devices who also were receiving phenprocoumon.**

Interventions: **Four different 4-factor PCCs were added to patient blood to attain a calculated increase in prothrombin time by 20%, 40%, and 60% greater than baseline in paired experiments.**

Measurements and Main Results: **Clotting was measured using thromboelastometry and endogenous thrombin potential. Two heparin-containing PCCs prolonged the clotting times in a concentration-dependent manner compared with baseline ($p < 0.01$) and compared with PCCs containing significantly less or no heparin ($p < 0.01$). The PCCs containing low or no heparin enhanced the area under the curve of thrombin generation and peak thrombin several fold relative to the heparin-containing PCCs ($p < 0.01$). One of the PCCs containing heparin even decreased peak thrombin generation by ~90% compared with baseline ($p < 0.01$). PCC with low or no heparin shortened the lag phase ($p < 0.01$), whereas 1 heparin containing PCC prolonged the lag phase by 66% ($p < 0.01$).**

Conclusions: **Physicians should be aware of the differences in heparin contents. Extrapolation of results from one agent to other PCC preparations may be difficult. Patients with an implanted left ventricular assist device and anticoagulated with vitamin-K antagonists could benefit from the use of PCC with low heparin content when surgery or bleeding requires emergency reversal. Further clinical studies are warranted.**

KEY WORDS: coumarin, prothrombin complex concentrate, thromboelastography, ventricular assist device, heparin

COUMARINS INHIBIT the formation of vitamin K-dependent coagulation factors II, VII, IX, and X, creating a functional deficiency in these proteins. Keeping the target international normalized ratio (INR) of the patient's normal (control) prothrombin time between 2.0 and 3.5 for long-term anticoagulation is recommended, depending on the indication.[1,2] Many outpatients after implantation of a left ventricular assist device (LVAD) are prescribed coumarins to prevent pump thrombosis and embolization.[3,4] However, bleeding is not uncommon in these patients and, similar to cases of emergency surgery, urgent reversal of the coumarin effect with prothrombin complex concentrates (PCCs) containing vitamin K-dependent clotting factors may be required.

For this purpose 4-factor PCCs are recommended and widely used[1,5] because vitamin K supplementation may only suffice for slow elective reversal.[6] Accordingly, administration of PCCs has been shown to be associated with reduced use of blood products.[7,8] An individualized PCC dosing regimen, based on patient weight and initial INR, appears to be more effective in achieving target INR than a standard single dose.[9] However, the active ingredients, including heparin concentration, of available 4-factor PCCs differ significantly.

The authors hypothesized that the specific heparin content of 4 PCCs would adversely affect the clotting time (CT) of whole blood of outpatients with an LVAD who were receiving phenprocoumon after ex-vivo reversal as measured by rotation thromboelastometry and thrombin generation in a dose-dependent fashion.

METHODS

Study Design

This experimental study assessed the effect of heparin as an ingredient in 3 of 4 different 4-factor PCCs on blood clotting, using rotation thromboelastometry and thrombin generation. Table 1 summarizes the active ingredients of the different PCCs available at the authors' hospital.

After institutional review board approval, written consent was obtained from 8 outpatients with an LVAD who were receiving phenprocoumon for anticoagulation. Venous blood was drawn via a peripheral venous cannula. Patients were recruited from January to December 2012. Patients who were enrolled in another trial and/or did not provide consent to participate in this investigation were excluded. There were no restrictions concerning age or sex.

All patients with an LVAD were treated with 100 mg of acetylsalicylic acid (Bayer, Vienna, Austria) once daily and underwent anticoagulation with phenprocoumon (Marcoumar; Meda, Bad Homburg vor der Höhe, Germany), with an INR level of 2.5 to 3.5.

Rotation Thromboelastometry

Rotation thromboelastometry (ROTEM; Tem Innovations GmbH, Basel, Switzerland) is a whole blood assay used to detect properties during clot formation.[10] It measures the

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Table 1. Comparison of the Active Ingredients in 4 Different Prothrombin Complex Concentrates

	Beriplex P/N 500 IU CSL	Cofact 500 IU	Prothromplex 600 IU	Octaplex 500 IU
Active ingredients (IU per vial)				
Human coagulation factor II	400-960	280-700	600	600
Human coagulation factor VII	200-500	140-400	500	180-480
Human coagulation factor IX	400-620	500	600	500
Human coagulation factor X	440-1200	280-700	600	360-600
Other active ingredients (IU)				
Protein C	300-900	222-780	150-450	140-620
Protein S	240-760	20-160	140-260	140-640
Heparin	4-20	0	(max, 0.5 IU/IU factor IX max 300)	0.2-0.5 IU/IU factor IX 80-310
Anti-thrombin III	5-15	0	Anti-thrombin III (0.75-1.5 IU/mL)	0

Abbreviation: IU, international units.

correction of coagulation factors after administration of PCC.[11] Bedside testing may help tailor the treatment of perioperative coagulation disorders by selective substitution management.[12] Rotation thromboelastometry[13] is related to but differs from classical thromboelastography and is used to assess blood coagulation disorders[14] or the effect of anticoagulants.[15] A ball bearing guides the firmness sensor in ROTEM, making the analysis less susceptible to mechanical stress, movement, and vibration.

ROTEM measurements were performed within 4 hours of blood sampling. Citrated blood samples (3.8%) were mixed separately with 4 different PCCs at 3 different concentrations and incubated for 30 minutes. The coagulation parameters of the blood samples were tested using ROTEM at baseline and after the addition of aliquots of the 4 PCCs at 3 different concentrations as described in the following.

Citrated blood samples were recalcified with 20 μL of $CaCl_2$ 0.2 mol/L before the assay was run. Subsequently, CT, clot formation time (CFT), and maximum clot firmness (MCF) were assessed using INTEM and HEPTEM assays (Tem Innovations). INTEM is sensitive to heparin, low-molecular-weight heparin, and fondaparinux.[16,17] HEPTEM eliminates heparin's effects with heparinase. Thus, the difference in CT (INTEM–HEPTEM) is related directly to the effects of heparin. In each ROTEM analysis, 300 μL of blood were mixed with the desired reagents for INTEM or HEPTEM (Table 2).

CT is defined as the time from the start of the measurement to the start of clot formation (reference range, 137-246 seconds in INTEM). CFT is the time from the beginning of clot formation until a clot amplitude of 20 mm is attained (CFT reference range, 40-100 seconds in INTEM). MCF is a measure of clot stability and firmness (reference range, 52-72 mm in INTEM).[18]

The quantity of PCC is expressed in international units (IU) and follows the current World Health Organization standard for coagulation factor IX content.[19] The concentration of the added PCC was calculated and was dependent on the weight and the intended increase in Quick value.

Baseline

1 × 700 μL (INTEM and HEPTEM and 100 μL waste)

4 (4 PCC) × 700 μL blood × 3 concentrations = 8400 μL

1 IU/kg body weight of PCC is required for a 1% increase in Quick value

Required units (IU) = body weight (kg) × desired increase in Quick value (%) × 1 (IU = kg × % × 1])

Table 2. ROTEM Results at Different Quick Values

Parameter	Cofact 500 IU	Beriplex P/N 500 IU	Prothromplex 600 IU	Octaplex 500 IU
Reference range CT (s) 137-246	INTEM HEPTEM	INTEM HEPTEM	INTEM HEPTEM	INTEM HEPTEM
CT 0	167 185	167 185	167 185	167 185
CT + 20%	174 171	180 183	202 175	206 172
CT + 40%	188 181	187 179	261 190	280 189
CT + 60%	199 192	200 189	298 195	346 198
Reference range CFT (s) 40-100	INTEM HEPTEM	INTEM HEPTEM	INTEM HEPTEM	INTEM HEPTEM
CFT 0	71 79	71 79	71 79	71 79
CFT + 20%	73 56	74 87	77 87	93 87
CFT + 40%	73 56	74 99	92 86	97 84
CFT + 60%	76 81	78 87	97 92	101 84
Reference range MCF (mm) 52-72	INTEM HEPTEM	INTEM HEPTEM	INTEM HEPTEM	INTEM HEPTEM
MCF 0	65 63	65 63	65 63	65 63
MCF + 20%	62 61	63 61	62 61	59 60
MCF + 40%	63 61	62 59	61 60	59 60
MCF + 60%	61 60	61 60	57 59	57 59

NOTE: ROTEM measurements of clotting time (CT), clot formation time (CFT), and maximum clot firmness (MCF) for Cofact 500 IU, Beriplex P/N 500 IU, Prothromplex 600 IU, and Octaplex 500 IU when a 20%, 40%, and 60% increase in Quick value was achieved.
Parameter reference range.[18]