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Biochemical characterization of prothrombin complex concentrates in China

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ABSTRACT

Despite increasing use of prothrombin complex concentrates (PCCs), there is little knowledge about the biochemical characterization of Chinese PCCs. Six Chinese PCCs were investigated and compared with PCCs (Octaplex®) from Europe. The levels of coagulation factors and inhibitors were detected. The presence of activated coagulation factors was assessed. Furthermore, their thrombin inhibitory capacities, specific activity and purity were assayed. All above parameters of biochemical properties were statistically analyzed.

Chinese PCCs contained FII, VII, IX and X, protein C, S and Z, heparin and extremely low level antithrombin, as well as Octaplex®. The measured FIX activities were similar to those declared, however the measured potency of FII, VII and X greatly exceeded the labeled. Though all preparations were negative for activated coagulation factors in non-activated partial thromboplastin time test, the activated coagulation factor VII (FVIIa) remained in all PCCs and its content differed greatly. Overall, FVIIa content of Chinese PCCs was higher than that of Octaplex®. Further, Chinese PCCs were inferior to Octaplex® in the thrombin inhibitory capacities, specific activity and purity. In summary, compared with Octaplex®, Chinese PCCs' errors about the labeled activity of coagulation factors and probably high risks of thrombosis should be considered.

1. Introduction

Prothrombin complex concentrates (PCCs), traditional plasma-derived product, are the heterogeneous combination of coagulation factors and counterbalancing inhibitor components [1]. These preparations contain the vitamin K-dependent coagulation factor (F) II, IX and X, with variable amounts of FVII [2]. Besides, they may contain coagulation inhibitors, such as protein C (PC), protein S (PS), protein Z (PZ), and antithrombin (AT) [3]. Although originally developed as a source of FIX for the treatment of patients with haemophilia B, PCCs have been used for treating other inherited and acquired deficiencies of coagulation factor (FII, VII or X) and coagulation inhibitor (PC, PS), liver disease, trauma-induced severe bleeding and other acquired coagulopathy as an alternative to fresh frozen plasma [4–9]. At present, the use of PCCs has been further broadened to encompass emergency reversal of oral vitamin K antagonists therapy [10–14] and even novel oral anticoagulants such as rivaroxaban therapy [15,16].

In China, since the high-purity single coagulation factor and inhibitor are hardly available, PCCs play more important role in treating the patients with genetic and acquired deficiencies of single vitamin K-dependent coagulation factor or inhibitor. For PCCs in clinical use, the thromboembolic events are always of great concern. Though the present product has been improved and the thrombotic risk after current PCCs administration appears to be fairly low [17], the thrombotic complications associated with PCCs sometimes occur [11,18]. These adverse events could be attributed to underlying patient-specific factors or clinical circumstances [19].

Abbreviations: PCCs, prothrombin complex concentrates; FII, factor II; FVII, factor VII; FIX, factor IX; FX, factor X; PC, protein C; PS, protein S; PZ, protein Z; AT, antithrombin; APTT, activated partial thromboplastin time; PT, prothrombin time; CV, coefficient of variation; NAPTT, non-activated partial thromboplastin time; FVIIa, activated coagulation factor VII; OD, optical density; ELISA, enzyme-linked immunoassay; DIC, disseminated intravascular coagulation.

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Moreover, PCCs composition is also a very important factor for associated thromboembolism [20,21].

Until now, the haemovigilance system in China has not yet been established and there is no knowledge about the transfusion-associated adverse events including thromboembolic side effects after PCCs administration. In order to strengthen the monitoring for PCCs in clinical use, biochemical characterization of PCCs from different manufacturers in China was evaluated. Only one Western brand of PCCs was available for the study and served as the contrast.

2. Materials and methods

2.1. PCCs preparations

PCCs preparations A–F were obtained from 6 different manufacturers of China and the preparation A, B, C, D, E and F was obtained from Shanghai pharmaceutical Co., Ltd., North China biological engineering Co., Ltd., Southwest China biological product Co., Ltd., East China biological product Co., Ltd., Central China biological product Co., Ltd. and Sichuan pharmaceutical Co., Ltd., respectively. The preparation G was Octaplex® (Octapharma company). All preparations except for preparation F included 3 batches. The lyophilized preparations were dissolved according to manufacturer instructions. The vials were then carefully mixed to avoid foaming and stored at room temperature for 20 min [22]. Aliquots were stored at −60 °C for further assay. The samples were thawed at 37 °C for 10 min before use.

2.2. Coagulation factors potency measurement

The potency of FII, VII, IX and X was measured with one-stage clotting assays following Chinese Pharmacopoeia 2010. Before the assay for FIX potency, heparin neutralization was performed with protamin sulfate (Sigma, Missouri, USA). The standard plasma (Siemens Healthcare, Marburg, Germany) was used to create calibration curve to detect the potency of FIX, II, VII and X and the normal plasma (Siemens Healthcare, Marburg, Germany) was applied to control measure. The potency of FIX, II, VII and X in Siemens standard plasma was calibrated against WHO-standard. FIX was determined by APTT reagent (Siemens Healthcare, Marburg, Germany) and FIX-deficient plasma (Siemens Healthcare, Marburg, Germany). FII, VII and X were measured by PT reagent (Siemens Healthcare, Marburg, Germany) and respective corresponding factor-deficient plasma (Siemens Healthcare, Marburg, Germany) [22]. These were performed by CA-1500 coagulometer (Sysmex, Kobe, Japan). All tests were carried out in triplicate and the coefficients of variation (CV) value in these tests were $\leq$9.6%.

2.3. Coagulation inhibitor component assay

Before assay of the coagulation inhibitor, PCCs preparations were further diluted to 1 IU FIX per milliliter according to the labeled amount. The activity of PC and AT was measured by chromogenic assay, and the functionally active PS was assayed by coagulation method. All procedures were completely based upon the previous description [23]. These assays of PC, AT and PS activity were standardized with Siemens standard plasma and controlled by the normal and abnormal plasma (Siemens Healthcare, Marburg, Germany). The PC, AT and PS activity of Siemens standard plasma was calibrated by WHO-standard. PZ antigen was analyzed by one step immuno-assay.

PC activity was assayed with PC activator (protac®) (Hyphen BioMed, Andrésy, France) and activated PC substrate (S2166) (Hyphen BioMed, Andrésy, France). The prediluted preparation and protac® were mixed and incubated, and then S2166 was added and mixed. The color reaction was stopped by acetic acid, and then the adsorption was immediately measured at optical density (OD) 405 nm using SpectraMax M2^e microplate readers (Molecular Devices, California, USA) [23]. The CV value in this PC activity assay was 6.7%.

The measurement for AT activity was performed using AT kit (ADI, Stamford, USA) and the measurement process included two stages. First, the preparation diluted by dilution buffer was added into micro plate and incubated, and then Thrombin reagent (thrombin) was introduced and continuously incubated. Second, Spectrozyme TH (thrombin substrate) was added, mixed and incubated. At last, the reaction was quenched by acetic acid. The rate of change of absorbance at OD 405 nm was detected [23]. The CV was 8.6% in this measurement.

PS activity was measured by PS Ac kit (Siemens Healthcare, Marburg, Germany) on the CA-1500 coagulometer. Firstly, the prediluted preparation was mixed with PS-deficient plasma. Secondly, the above mixture was added into PS-deficient plasma and then the whole system was incubated. Thirdly, APC Reagent (activated protein C) was added and incubated. Lastly, the coagulation time which was directly proportional to PS activity was determined after addition of Starting Reagent (activator) [23]. The CV was 5.7% in the measurement for diluted PCCs preparation.

The protein Z kit (Hyphen BioMed, Neuville-Sur-Oise, France) was used to analyze PZ antigen. The specific polyclonal antibody coupled with horse radish peroxidase was carried out to capture PZ. The content of PZ was detected according to the amount of color developed by hydrogen peroxide and peroxidase substrate. The assay step was in accordance with the manufacturer instructions [23] and the CV in this assay was 6.1%.

Heparin activity was measured according to European Pharmacopoeia 7.0 and the CV in this measurement was 7.5%.

2.4. Activated coagulation factors detection

Non-activated partial thromboplastin time (NAPTT) was detected according to European Pharmacopoeia 7.0. Thrombin was examined in accordance with Chinese Pharmacopoeia 2010. Prior to these assays, protamin sulfate neutralized heparin in the ratio of 10 μg to 1 IU. The activated FVII (FVIIa) content was analyzed through an enzyme-linked immunoassay (ELISA) and performed with the FVIIa ELISA kit (Sekisui Diagnostics, Connecticut, USA). This ELISA employed a biotinylated enzyme inhibitor of FVIIa and anti-FVII/FVIIa monoclonal antibody as the capture antibody. Firstly, diluted PCCs were incubated with the biotinylated inhibitor, which covalently attached to the FVIIa but not to FVII and then the samples were added to microwells precoated with the FVIIa capture antibody. Secondly, FVIIa/biotinylated inhibitor complex was detected by binding of the streptavidin conjugated horseradish peroxidase. The TMB substrate was added, and then sulfuric acid solution was introduced to stop the reaction. The absorbance at 450 nm which was directly proportional to the content of FVIIa was detected and FVIIa levels were determined by the standard curve developed with FVIIa standard.

The pooled plasma from 10 healthy donors, obtained from Guanghan plasma apheresis station, served as the contrast. In order to avoid further activation of FVII in this plasma, the citrate anticoagulant was successively added in the process of collecting this plasma, the 10 ml apheresis plasma from each healthy donor was almost simultaneously obtained and gently mixed. Then the pooled plasma was immediately stored in ice-cold water bath no more than 2 h before use. The CV was 7.9% in this detection.

2.5. Thrombin inhibitory capacities assay

The thrombin inhibitory capacities were determined using modification of the method as previously reported [22]. First, all

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