DISTRIBUTION OF PCBs IN PROTEIN DERIVATIVES (FACTOR VIII, IMMUNOGLOBULIN AND ALBUMIN) OBTAINED BY HUMAN PLASMA FRACTIONATION

A. Covaci, R. Laub[1], M. Di Giambattista[1], Th. Branckaert[1], V. Hougardy[1], P. Schepens

Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[1]Red Cross, Central Fractionation Department, Joseph Stallaertstraat 5, 1050 Brussels, Belgium

Introduction

Polychlorinated biphenyls (PCBs) and organochlorine pesticides are among the most prevalent environmental contaminants. Because of their lipophilicity and long elimination half-lives, residues of these two groups of persistent organic pollutants (POPs) bioaccumulate in biota and in human tissues[1]. Mainly through the diet, human serum accumulates measurable amounts of these chemicals[2,3]. Plasma derivatives are therapeutic proteins of choice for managing life-threatening coagulation and immune disorders and for shock treatment.

Starting plasma is collected from healthy volunteers with no known specific exposure, qualified by complete batteries of serological, viral, and biochemical tests. Its processing involves extensive purification yielding the proteins of interest. The Cohn process[4], based on cold ethanol fractionation, is commonly used to manufacture many plasma proteins, such as immunoglobulins and albumin, for therapeutic use. Clotting Factor VIII (FVIII) is obtained from cryoprecipitate by a process including a series of precipitations, virucidal treatment with solvent-detergent, and ion-exchange chromatography.

In this work, we measured concentrations of PCBs and major organochlorine pesticides in 3 plasma-pool batches of about 5000 donations from healthy individuals, collected in 2000 after the PCB/dioxin food crisis in Belgium[5]. The distribution and removal of these pollutants were examined in intermediate fractions along the Cohn fractionation process and in the final plasma derivatives. The plasma processing techniques used were found to eliminate the POPs very efficiently from immunoglobulins and FVIII and to reduce POP concentrations in albumin.

Methods

Samples

Three starting plasma-pool batches (1500 l plasma/batch – about 5000 donations) were thawed at 0°C and the cryoprecipitate was centrifuged. Sample size was either 50 ml (plasma and supernatants) or 50 mg (intermediate fractions obtained during Cohn ethanol fractionation – Figure 1). FVIII and immunoglobulin samples were collected after the last production step. Immunoglobulins were purified from Fraction II and treated with the virucide beta-propiolactone. Filtered Fraction V (FV) (about 98% albumin) was obtained before the pasteurisation step. Precipitates were resuspended according to standard production procedures. All fractions were stored at –20°C, except those to be used for POP determinations kept at room temperature and analysed within 48 h of their resuspension.

Analysis

In each process fraction, proteins, lipoproteins, lipids, and selected POPs were measured. The total protein concentration was determined by Biuret's method using seralbumin as standard. Lipoproteins (ApoA and ApoB) were measured by nephelometry (BNA100, Behring) using standardised assay protocols and calibrated standards. Lipids (cholesterol and triglycerides) were measured by an enzymatic assay. The compounds assayed were two organochlorine pesticides,

hexachlorobenzene (HCB) and p,p'-DDE, and nine PCB congeners (IUPAC nos. 28, 52, 101, 118, 138, 153, 156, 170 and 180). The analytical method used has been previously described and validated[6,7]. Briefly, after addition of internal standards (PCBs 46 and 143) and protein denaturation with formic acid, a solid-phase extraction on C18 disk cartridges was carried out with acidified silicagel clean-up. A gas chromatograph coupled with a mass spectrometer equipped with a 50m x 0.22mm x 0.25µm HT-8 capillary column was used for analysis. Two ions of the molecular ion cluster (M^+ and $[M+2]^+$) were monitored for each analyte. Retention times, masses and relative abundances of confirmation ions with respect to quantification ions were used as identification criteria. The method's detection limit ranged between 10 and 30 pg/ml liquid. Results below the detection limit were set at zero. Analyte recovery ranged from 62 to 73%. Regular analysis of calibration curves, reagent blanks, and in-house control serum samples ensured internal quality control. Proficiency was demonstrated by successful participation in inter-laboratory tests.

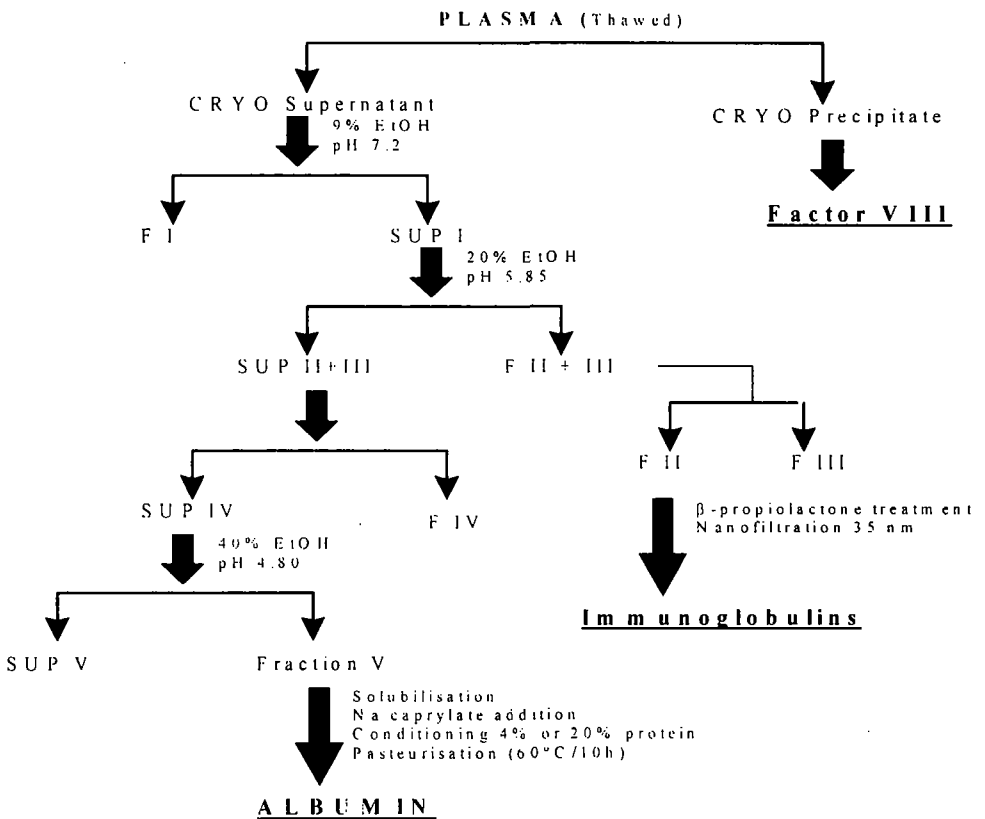


Figure 1. Flow chart of the Cohn fractionation procedure.

Results and discussion

1. Concentrations in plasma pools from healthy donors

Concentrations of PCBs 28, 52, and 101 were below the detection limit (0.02 ng/ml for individual congeners in 10 ml liquid) in the starting plasma pools. The mean concentrations of PCBs (sum of

congeners 118, 138, 153, 156, 170, 180), HCB, and pp-DDE in the plasma pools were respectively 2.38 ± 0.34, 0.20 ± 0.02 and 1.54 ± 0.38 ng/ml. Ratios of detected congeners were similar in the three batches.

2. *Distribution of PCBs, total protein, and total cholesterol and ApoB in Cohn fractions and cryoprecipitate*

Recoveries for the different markers ranged from 71% to 89%. Two fractions (cryoprecipitate and resuspended Fraction I) displayed no PCBs and very low levels of ApoB and cholesterol (Table 1). The other fractions (cryosupernatant, resuspended Fractions II+III, IV, and V) showed variable contents in organochlorine pesticides and PCBs.

PCBs and protein were similarly distributed throughout the fractions. Their distribution differed from that of cholesterol and ApoB. PCBs were found mostly in fractions Fraction II+III and Fraction V. Fraction V was found to contain only low amounts of cholesterol, while Fraction II+III was enriched in cholesterol and lipoproteins (ApoB). The continuous change in pH and polarity of the fractionation medium affected the differential elimination of selected PCB congeners.

In resuspended Fraction V, PCB 180 (the most lipophilic congener) represented 35.3% of the total PCB content. In the starting plasma pool and resuspended Fraction II+III, it represented respectively only 26.6 and 20.4% (Figure 2). This difference was probably due to lowering of the polarity of the medium during fractionation. Levels of PCBs 118 and 156, HCB, and pp-DDE were substantially decreased in Fraction V and increased in Fraction II+III (Figure 2), while the other PCB congeners showed similar levels in these two fractions.

Table 1. Distribution (in %) of PCBs, proteins, and cholesterol among the different fractions

Fractions	Protein	Sum PCBs	Cholesterol	Apo B
Cryoprecipitate	2.3	0	0.2	0
Fraction I	4.9	0	2.1	3.3
Fraction II+III	32.5	35.9	67.2	89.2
Fraction IV	12.3	16.0	9.7	7.5
Fraction V	47.1	48.1	1.2	0
Supernatant V	0.8	0	19.6	0
Process recovery (%)	89	71	73	80

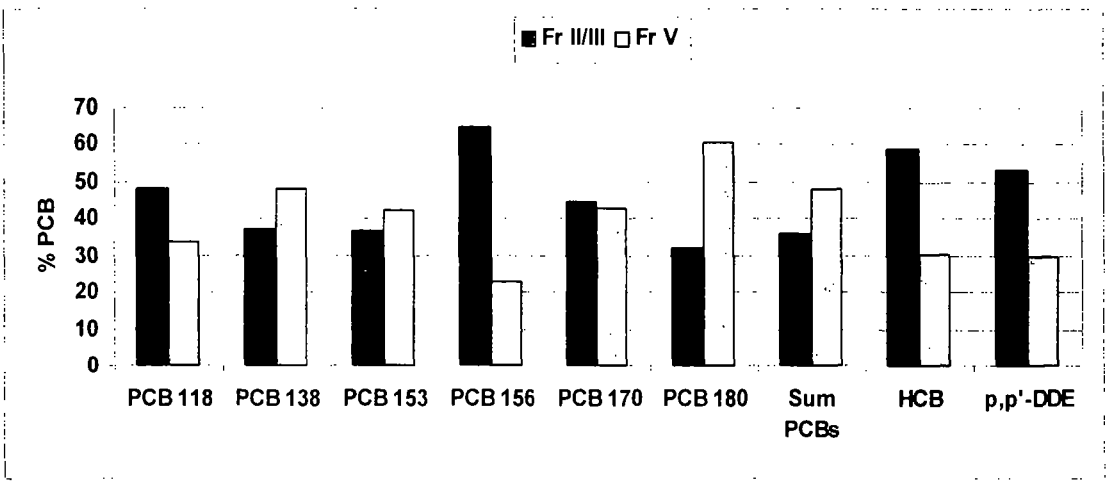
3. Concentrations of PCBs and major organochlorine pesticides in therapeutic protein derivatives

Due to selective elimination, pollutant levels were below the detection limit in therapeutic proteins prepared from cryoprecipitate (Factor VIII, von Willebrand Factor) and in Fraction I (fibrinogen). PCB congeners found in Fraction II+III (rich in IgG and IgM) were completely eliminated in the subsequent steps of the manufacturing process for Immunoglobulins (IgG). However, Fraction V (with 98% albumin) was found to contain organochlorine contaminants. As a result of fractionation and filtration, the PCB concentration (sum of 6 congeners) decreased from 36 ng/g protein (2.37 ng/ml plasma) in the starting material to 26 ng/g protein in the filtered Fraction V.

It has been reported previously that lipophilic contaminants are transported in the blood by lipoproproteins or proteins such as albumin[8]. The authors of these reports, however, used a single

ultracentrifugation step to purify albumin, and this purification method does not exclude the presence of another possible carrier.
Albumin is infused into severely burned and sepsis patients when their life is threatened. The increase in POPs body burden (0.023%) for patients administered with a treatment dose of this therapeutic concentrate is negligible. compared to the concentration found in plasma.

Figure 2. Distribution of organochlorine pollutants in Cohn Fraction II+III and Fraction V



Conclusion

Processing of plasma pools leads to complete elimination of PCBs and pesticides from cryoprecipitate, i.e. the starting fraction for producing important clotting factors such as FVIII, von Willebrand Factor, and fibrinogen. Processing also yields, from Cohn Fraction FII+III, a PCB- and pesticide-free immunoglobulin concentrate. Our results show residual POP concentrations in the albumin fraction.

References

1. De Voogt P., Wells D.E., Reutergardth L. and Brinkman U.A.Th. (1990) Intern J Environ Anal Chem 40, 1-46.
2. Covaci A., Koppen G., Van Cleuvenbergen R., Schepens P., Winneke G., van Larebeke N., Nelen V., Vlietinck R. and Schoeters G. (2001) submitted to Chemosphere.
3. Pauwels A., Covaci A., Weyler J., Delbeke L., Dhont M., De Sutter P., D'Hooghe T. and Schepens P. (2000) Arch Environ Contam Toxicol 39, 265-270.
4. Cohn E.J. (1946) J Am Soc Chem 68, 459-466.
5. Schepens P., Covaci A., Jorens P., Hens L., Scharpe S. and Van Larebeke L. (2001) Env Health Persp, 109, 101-103.
6. Covaci A., Pauwels A. and Schepens P. (2000) Intern J Environ Anal Chem 76, 167-178.
7. Covaci A. and Schepens P. (2001) Chemosphere 43, 49-57.
8. Gomez-Catalan J. and To-Figueras J. (1991) Arch Environ Contam Toxicol 20, 61-66.