Distribution of PCBs, DDE, Hexachlorobenzene and Methylsulfonyl Metabolites of PCB and DDE among Lipoprotein and Protein Fractions of Human Blood Plasma

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Introduction

Several studies have been performed in order to explain the transport of persistent organochlorine compounds in the body. In vitro studies of 2',4,4',5,5'-hexachlorobiphenyl (CB-153) indicate that this compound is associated with lipoproteins in plasma (1-3) as well as albumin and a steroid binding globulin (3). For certain organochlorine compounds differences in the distribution have been found between man and rat (4,5). Furthermore, the distribution of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) among plasma compartments was found to be dependent of the number of chlorine in the molecule (6). As the number of chlorine atoms in the molecule increased higher amounts were found in the protein fraction.
In the present investigation (7) the concentrations of polychlorinated (PCBs), DDE, hexachlorobenzene and methylsulfonyl metabolites of PCBs and DDE were determined in human plasma samples and in the fractions obtained by ultracentrifugation of plasma into very low density (VLDL), low density (LDL), high density (HDL) lipoprotein and lipoprotein depleted (LPDP) fractions. The concentrations of triacylglycerols, cholesterol, phospholipids and apolipoprotein B (apoB) were also determined.

Materials and Methods

Subjects. Fasting plasma was obtained from five subjects (aged 22-45).
Isolation of lipoprotein fractions Approximately 200 ml of plasma was fractionated by ultracentrifugation with sequential flotation. The fractions VLDL (d<1.006 kg/l), LDL (1.006<d<1.063 kg/l), HDL (1.063<d<1.21 kg/l) and LPDP (d>1.21 kg/l) were isolated (8).

Determination of lipids and apolipoproteins. Cholesterol, triacylglycerols and phospholipids were determined by combination of preparative ultracentrifugation and precipitation of apo B-containing lipoproteins (9). Free and esterified cholesterol, triacylglycerols and phospholipids were determined in triplicate in the isolated lipoprotein subfractions using enzymatic methods. ApoB was quantified in LDL and VLDL as the total protein (10) content minus the isopropanol-precipitated supernatants of the isolated fractions (11).

Determination of organochlorine compounds. A sample of 50 ml was analyzed for PCBs, DDE and their methylsufonyl metabolites according to Weistrand et al. (12,13). Another technique was used for LPDP fraction. An aliquot of LPDP (40ml) was extracted with chloroform-methanol (2:1 v/v, 125 ml). The methylsulfone metabolites were separated from CBs on aluminium oxide. The fraction containing CBs and DDE was purified by chromatography on silica gel and the

concentrations of CBs and DDE were determined by GC-ECD. Further clean-up of the methylsulfone metabolites was performed by gel permeation chromatography on Bio-Beads S-X3 (Bio-Rad Laboratories, Richmond, CA). GC/MS selected ion recording was used for identification and determination of the concentrations.

Results and discussion

Distribution of lipids and apolipoprotein B

The sum of the concentrations of triacylglycerols, cholesterol and phospholipids, ranged 6.39-7.16 g/l. The total lipids in plasma, as determined gravimetrically by the extraction method used for organochlorine contaminants (12), were on an average 96% (range 84-109%) of the sum of triacylglycerols, cholesterol and phospholipids. Most of the triacylglycerols (60%) were recovered in VLDL, the cholesterol in LDL (63%) and the phospholipids in HDL (47%). The concentration of apoB in LDL was about ten times higher than in VLDL.

Organochlorine compounds in plasma

The sum of CBs in the plasma samples was 2.5-4.3 μg/l plasma (corresponding to 0.35-0.64 μg/g lipids) and the distribution of individual congeners was similar in all five samples. The congeners CB-153, CB-180, CB-138 and CB-170 were the most abundant. The sum of $MeSO_2$-CBs was about two orders of magnitude lower than the sum of CBs. The methyl sulfonyl metabolites of CB-149, CB-87 and CB-101 occurred at highest concentrations and the profiles of the compounds were similar in all plasma samples.

The concentrations of p,p'-DDE in the plasma samples differed more, 0.75-6.1 μg /l (corresponding to 0.11-0.88 μg/g lipids), than those of CBs. The levels of 3-$MeSO_2$-DDE were two to three orders of magnitude lower than those of DDE. Hexachlorobenzene (HCB) was found at levels of 0.12-0.39 μg /l plasma (corresponding to 16-56 ng/g lipids).

Distribution of organochlorine compounds among plasma lipoprotein fractions

The organochlorine compounds were found in all plasma fractions, Fig. 1-3. The recoveries of total CBs and $MeSO_2$-CBs from the plasma fractions (sum from VLDL, LDL, HDL and LPDP) were 71-100% and 76-95%, respectively, of the concentrations in plasma. The sum of DDE, $MeSO_2$-DDE and HCB in plasma fractions were 73-108, 89-116 and 81-110 %, respectively of the concentrations in plasma.

The organochlorine compounds were associated with all fractions, but predominantly with the LPDP fraction. On an average 44 % of PCB, 61% $MeSO_2$ -PCB, 73% DDE, 77% $MeSO_2$ -DDE and 45% of HCB were distributed in the LPDP fraction. A tendency to greater association of 3-methylsulfonyl substituted (3- $MeSO_2$ -CBs) than of corresponding 4-methylsulfonyl substituted chlorobiphenyls (4- $MeSO_2$ -CBs) to the LPDP fraction was noticed. Among the lipoprotein fractions, LDL was the main carrier of HCB, DDE and PCB, although a tendency of the more highly chlorinated CBs to associate with HDL was observed. $MeSO_2$ -DDE was predominantly found in HDL and $MeSO_2$ -PCB was distributed equally among the LDL and HDL fractions. Calculating the concentrations of organochlorine compounds in relation to the content of apoB, the levels were about ten times higher in VLDL than in LDL.

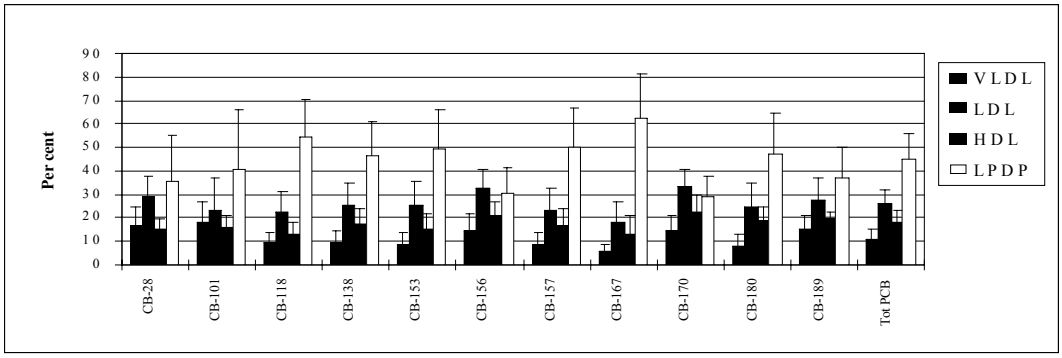

Fig. 1. Distribution of PCB congeners among plasma fractions.

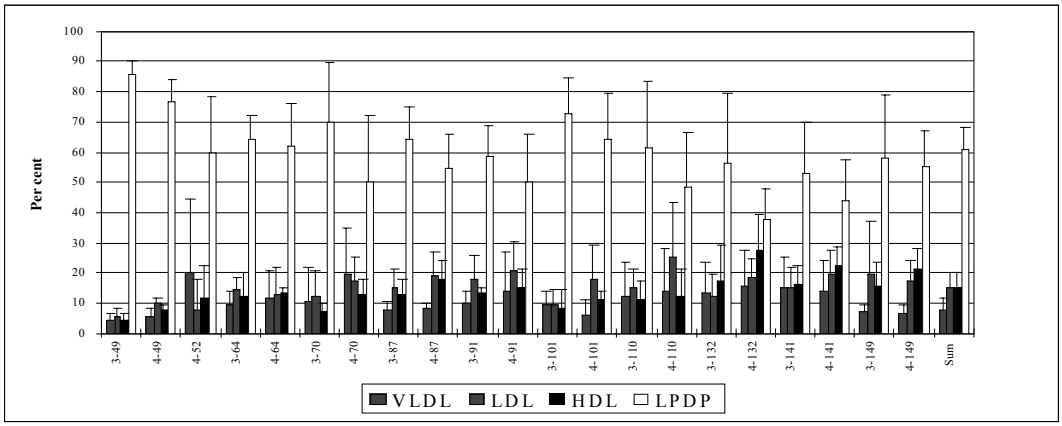

Fig. 2. Distribution of 3-$MeSO_2$-PCBs and 4-$MeSO_2$-PCBs among plasma fractions

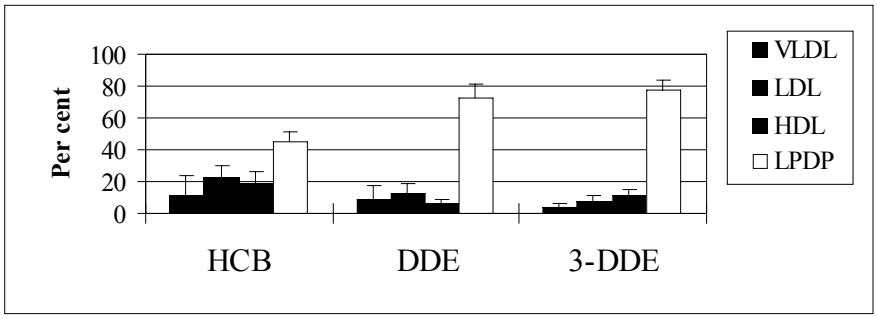

Fig. 3. Distribution of HCB, DDE and $MeSO_2$-DDE among plasma fractions.

The samples were obtained after 12 hours of fasting and it cannot be excluded that the distribution may differ with food intake. Further studies are needed to clarify possible influence of lipid intake.

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