ORGANOCHLORINE DETERMINATION IN HUMAN PLACENTAS FROM DIFFERENT REGIONS

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Introduction

Organochlorine compounds are common contaminants of human tissue as a result of environmental exposure to man-made chemicals[1]. Organochlorine pesticides accumulate in the food chain, are metabolized with difficulty and, because of their lipophility, are deposited in adipose tissue. Organochlorine substances can be released to the blood from fat deposits in very different situations, such as in the metabolism of fat during pregnancy and breastfeeding or during slimming regimes. Maternal-infant exposure is of special importance because it can lead to severe and irreversible effects at critical moments of development. Many studies have been conducted on the levels of these compounds in adipose tissue, serum, and maternal milk. However, there has been much less direct measurement of foetal exposure[2-5]. The present work studies the intrauterine exposure of the foetus to some organochlorine compounds, selected for their oestrogen-mimicking character, through the analysis of placenta samples from women in hospitals in Granada, Almeria, Turku, and Copenhagen.

Material and methods

Placenta tissue was analysed in: 39 samples from Turku (Finland), 25 from Copenhagen (Denmark), 24 from Almeria (Spain), and 65 from Granada (Spain). The placentas were collected at the time of delivery in the hospitals of each city in the study. They were immediately frozen at -70 ºC and stored until their analysis. Biocumulative compounds were extracted from placenta by a previously described method [1]. An aliquot of 400 mg placenta was dissolved in hexane and eluted in a glass column filled with Alumina Merck 90 (70-230 mesh, nº 1097) that had been dried at 600 ºC for 4 h followed by the addition of 5 % water. The eluate obtained was concentrated at reduced pressure and then under a stream of nitrogen to a volume of 400 mL and then injected twice (200 µL) into the preparative HPLC. One µL of fractions of HPLC chromatography were dried, dissolved in n-hexane, spiked with an internal standard (p,p´-dichlorobenzofenone) and then injected into a gas chromatographer with an electron capture detector (ECD). Standard solutions of organochlorine pesticides were previously analysed by gas chromatography in order to determine the retention times and calibration curves of these chemicals. The calibration linearity of seventeen pesticides in pure and processed standards was greater than 0.98.

Results and discussion

The mean values of the organochlorine pesticides content measured in the extracted placenta samples from each city are shown in Tables 1 and 2. GC/MS was needed to confirm the presence of the

organochlorine pesticides in the samples. The percentage of lipids in each sample could account for the variability of the results, because the organochlorine compounds analyzed are lipophilic and the amount of fat per gram of tissue is very different in some cases. For this reason were also corrected by lipid content (data not shown). All samples were positive for at least one of the organochlorine compounds. DDE and derivates predominated in samples from Almeria, whereas lindane predominated in the Danish samples. Mirex was virtually undetectable in the samples from Turku and Copenhagen. This distribution of compounds in the human placentas of different geographic origin may reflect the use of these compounds in each area. Another study described lindane, aldrin, and DDE as the most frequently detected compounds in placentas and cord serum samples[2-3], which are similar to our findings in that lindane and DDE were among the compounds detected in the greatest number of samples from each country, and in some cases were more frequent than endosulphan metabolites. In addition to these data on human placenta appreciable levels of organochlorine pesticides have been reported in umbilical cord serum, indicating that these compounds cross the placental barrier and represent a further form of maternal-infant exposure [4,5].

Table 1. Mean values of organochlorine pesticides measured in placentas from Turku (Finland) and Copenhagen (Denmark).

	Samples from Finland (N=39)			Simples from Denmark (N=25)		
	Mean	**SD**	**%Frequency**	**Mean**	**SD**	**%Frequency**
o,p´DDT	4.26	8.60	29	5.96	12.3	76
p,p´DDT	7.82	5.86	5	2.37	0.03	8
o,p‘DDD	49.3	37.2	22	133.9	6.5	4
p,p‘DDE	4.59	8.39	56	6.80	15.4	52
Metoxychlor	5.04	13.3	85	3.60	7.14	28
Lindane	0.26	0.16	71	0.44	0.41	92
HCB	15.5	39.2	66	4.7	4.52	8
Aldrin	1.11	1.15	19	0.94	0.42	24
Endrin	8.34	17.7	51	7.43	7.36	68
Dieldrin	4.25	3.95	34	4.86	6.87	20
E-I	9.22	15.6	46	14.5	22.1	60
E-II	12.5	16.6	17	3.54	4.73	24
E-eter	0.25	0.27	83	0.90	1.31	20
E-lactona	38.1	35.1	63	97.8	165.9	88
E-diol	13.4	33.1	90	12.30	11.9	88
E-sulfato	2.17	1.27	19	2.73	1.95	60

Values expressed en ng/g placenta

Table 2. Mean values of organochlorine pesticides measured in placentas from Granada and Almeria (Spain)

	Samples from Granada (N=65)			Samples from Almeria (N=24)		
	Mean	**SD**	**%Frequency**	**Mean**	**SD**	**%Frequency**
O,p´DDT	7.67	10.5	55	5.94	11.9	46

p,p´DDT	7.76	8.10	17	66.45	85.6	8
O,p‘DDD	51.8	36.2	29	112.4	249.4	83
p,p‘DDE	3.32	5.62	65	3.90	3.40	96
Metoxychlor	1.85	5.23	65	2.55	5.35	46
Lindane	0.32	0.42	85	0.22	0.13	58
HCB	14.5	14.9	60	6.85	8.25	96
Aldrin	1.10	0.83	34	1.07	0.28	12
Endrin	6.99	11.9	66	4.10	2.72	46
Dieldrin	5.83	13.5	57	12.6	14.8	12
E-I	6.97	10.4	71	5.45	5.14	54
E-II	12.8	20.6	29	1.98	1.91	46
E-ether	0.24	0.17	88	0.19	0.16	96
E-lactone	33.8	19.9	61	43.2	29.2	33
E-diol	9.45	8.57	71	9.80	10.3	67
E-sulphate	3.80	4.81	58	9.91	10.8	71

Values expressed in ng/g of placenta.

References

1. Rivas A, Fernández M.F, Cerrillo I, Ibarluzea J, Olea-Serrano MF, Pedraza V, Olea N. Human exposure to endocrine disrupters: Standardisation of a marker of estrogenic exposure in adipose tissue. APMIS 109:1-13 (2001).
2. Saxena MC, Seth TD, Mahajan PL. Organochlorine pesticides in human placenta and accompanying fluid. Int. J. Environ Anal Chem 7(3): 245-51 (1980).
3. Cooper SP, Burau K, Sweeney A, Robbinson T, Smith MA, Symanski E, Colt JS, Laseter J, Zahm SH. Prenatal exposure to pesticides: A feasibility study among migrant and seasonal farmworkers. Am J Ind Med 40 (5): 578-85 (2001).
4. Sala M, Ribas-Fito N, Cardo E, De Muga ME, Marco E, Mazon C, Verdu A, Grimallt JO, Sunyer J Levels of HCB and other organochlorines compounds in cord blood: exposure across placenta. Chemosphere, 43 (4-7): 895-901 (2001).
5. Waliszewski SM, Aguirre AA, Infanzon RM, Siliceo J. Carry-over of persistent organochlorine pesticides through placenta to fetus. Salud Pública México 42(5): 384-90 (2000)