COPROPORPHYRINURIA FOUND IN RURAL SOUTH VIETNAMESE EXPOSED TO AGENT ORANGE

Poznyakov, S., Roumak, V., Papkovsky, D., Korotkov, M., An, N., Bykhovsky, V., Bocharov, B., Kountzevitch, A.
Soviet-Vietnam Tropical Center, Ho Chi Minh City, Vietnam
Tropical Department, Institute of Animal Evolutionary Morphology and Ecology, Russian Academy of Sciences, Moscow

It is well known that under appropriate experimental conditions the disturbances in porphyrin metabolism induced by TCDD-like compounds (Ah-locus and other genes involved) may be 10-1000 times more sensitive indicator of exposure than other biological markers are. These effects constitute a rich field of studies on molecular mechanisms of dioxin toxicity and seems to be very useful for biological monitoring and in vitro testing integrated biological activity of isostereochemicals to TCDD in complex mixtures. Also, it is generally accepted that secondary coproporphyrinuria "SCPU" with transition to chronic hepatoporphyria "CHP" may serve as an indicator of human exposure to dioxin. However, still more research is needed to validate diagnostic significance of these effects in different human populations due to its complex ethiology and pathogenesis involving the coincidence of various endogenous (genetic predisposition, certain diseases, state of homeostasis) and exogenous (duration of exposure to dioxin, impact of other cyt P-450 inducers and porphyrinogenic chemicals, bioavailability of Fe(2+) and cofactors of porphyrinogens sythesis, etc.) factors /1,2/. Here, we present initial results of identification coproporphyrinuria "CPU" and CHP among apparantely healthy rural South Vietnamese exposed to Agent Orange (AO).

The comparative clinical laboratory investigations were performed with medico-biologically, socio-demographically, nutritionally and toxicologically homogenous contingent of apparantely healthy Chanh My and Binh My villagers (N=60-216, m, 31-50) selected and divided into 3 risk groups regarding potential contacts with AO as described in other communications in this issue /3,4/.

Samples of a fresh morning (N=216) and a 24-h (N=60) urine were collected at 0°C and stored at -20°C until analysis. Urine aliquots (1 ml) containing appropriate amounts of precipitate were cleared by mixing with HCl (30 mcl, 9 mol/L) for 3 min at 50°C and specimens (50 mcl) were injected into the r.p. HPLC system of Lim et al /5/. Riboflavin, uro- "Uro", hepta- "7C",

hexa- "6C", penta- "5C" carboxylic-porphyrins, coproporphyrin isomers I and III "C1" and "C3" were resolved on the ODS-Hypersil column by a 15-min linear gradient elution from 100% to 10% solvent Afollowed by isocratic elution at 100% solvent B for a further 5 min (1.5 ml/min, FL detection at 404/550 nm, "Bruker" FRG). Porphyrins from the urine samples enriched with nonporphyrin pigments were initially purified by a routine talc adsorbtion procedure. The designed method for detection of porphyrin-Pd(2+) complexes based on the time-resolved luminiscence measurements ("LS-50", Perkin Elmer Cetus, USA) /6/ was employed for large-scale determination of the Uro and total coproporphyrins "TC" in a fresh urine samples. Porphyrin markers were from "Porphyrin Products", Logan, USA.

Selection of the healthy reference group regarding porphyrin metabolism and establishment of the "normal" local ranges for porphyrins excretion within this group were carried out by the described system approach /4/ using the same medico-biological parameters and classification criteria, and generally accepted criteria of normality for porphyrin metabolism /1,2/ taken in the broader ranges.

The established "normal" local porphyrin excretion profiles within the reference group (N=25, a 24-h urine samples) were similar with the obtained for healthy europeoids /1,2/ with a close-to-normal distribution ("Normal Probability Plot") for most parameters studied: * Total porphyrins:

Healthy adult males	Porphyrin excretion profiles, Mean, SD					
	TP* pmol/24h	Uro %	7C %	TC %	TC/Uro ratio	C3/C1 ratio
Europeoids	< 200	< 30	<5	>60	2 << 6	2 << 5
Vietnamese	116.1	19.7	1.46	75.5	4.08	3.14
	21.2	4.2	.83	4.1	.94	.80

This data permitted to estimate the incidence of certain porphyrinurias and intrahepatic cholestasis in risk groups using western diagnostic criteria /1,2/: TP excretion 200 pmol/24h: SCPU - Uro < 20%, 7C < 3%, TC > 80%, TC/Uro > 6, CHP type A - Uro < 40%, 7C > 5%, TC > 40%, TC/Uro < 6, CHP type B - Uro > 40% 7C < 20%, TC < 40%, TC/Uro < 1, CHP type C - Uro > 50%, 7C > 20%, TC > 10%, TC/Uro < 1, cholestasis - C3/C1 < 1: (a 24-h urine):

Porphyrinuria, cholestasis	Percentages of risk groups, N, %, rounded		
	I (N=21)	II (N=16)	III (N=23)
CPU	1/21=4.8%	3/16=18.9%	19/23=82.6%
CHP type A	0	0	1/23=4.4%
CHP type B	0	0	0
CHP type C	0	0	0
Cholestasis	6/21=28.6%	2/16=12.5%	4/23=17.4%

Elevated excretion of TP, 7C, and TC in AO-exposed groups with a concomittant increase in TC/Uro ratios resembling a dose-response relationship was further confirmed using the greater number of a fresh urine samples and Z-value based probability approach /4/:

Parameter	Percentages of risk groups with excessive (+) or inadequate (-) values of the parameters as compared to the "normal" ranges established for a fresh urine samples within selected reference group		
	I (N=109)	II (N=74)	III (N=33)
TP,pmol/ml	15.3% -	2.5% +	33.1% +
Uro, %	24.7% -	44.8% -	56.7% -
7C, %	2.7% +	31.3% +	32.8% +
TC, %	17.8% -	9.2% +	36.3% +
TC/Uro	28.7% +	42.6% +	59.5% +
C3/C1	53.2% -	58.3% -	50.5% -

The similar results were obtained by a time-resolved luminiscence method with Pd(2+) acting as porphyrin-specific porphyrinogen-oxidizing agent /6/(data not shown).

These results indicated that: 1 - the "normal" rates of urinary porphyrins excretion in healthy adult rural South Vietnamese are close to the observed in corresponding groups of europeoids, 2 - high prevalence of low Uro and elevated C1 excretions is the characteristic feature of the study subpopulation, 3 - high incidence of CPU in the selected apparantely healthy medico-biologically, socio-demographically, nutritionally, and toxicologically homogenous contingent is associated with history and degree of potential exposure to AO. Taking into consideration the close-to-normal levels of serum enzymes, bilirubin, and lipids in these AO-exposed persons /4/, we may suppose that TCDD-depressed activity of coproporphyrinogen oxidase is the main cause of this acquired CPU development. Performance of the porphyrinogens synthesis "normalizing" (supplementation with pyridoxal phosphate, zinc,etc.) and porphyrinuria "provocating" (glycine and iron loadings) tests, estimation of the urinary uroporphyrinogen precursors (ALA, PBG) and heme synthesizing enzymes activities in isolated blood cells as well as the deeper examinations of liver and renal functions in these patients are need to be done to investigate mechanisms of the dioxin-induced coproporphyrinuria development, to increase specificity and sensitivity of this indicator.

1 Strik J. The occurence of chronic hepatic porphyria in man caused by halogenated hydrocarbons. In:Strik J, Koeman J, eds. Chemical porphyria in man. N. Holland: Elsevier, 1979:3-10.
2 Doss M. Porphyrinurias and occupational disease. Ann NY Acad Sci 1987; 514:204-18.
3 Roumak V, Poznyakov S, An N, Kim Chi H, Thu T, Sofronov G, Sokolov V, Kountzevitch A. Epidemiological and clinical labora-

tory studies on health consequences of Agent Orange in the South Vietnam. Dioxin'92;this issue.
4Poznyakov S, Roumak V, An N, Bykhovsky V, Kountzevitch A, System study of alterations in vit A status among rural South Vietnamese exposed to Agent Orange. Dioxin'92;this issue.
5 Lim C, Rideout J, Wright D. Separation of porphyrin isomers by HPLC. Biochem J 1983;311:435-8.
6 Papkovsky D, Savitsky A, Ponomarjov G, Poznyakov S, Roumak V A method for selective determination of biogenic porphyrins. Pat for Application N 4869584/25. Moscow, 1991.