Diabetes, Cardiovascular Disorders and 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Body Burden in Czech Patients 50 Years After the Intoxication

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Abstract: The correlation between 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) intoxication and the parameters of metabolic impairment was examined in the last eight male survivors of 80 workers exposed to TCDD during the production of herbicides in a chemical factory in 1965–1967. Their median TCDD blood level was 112 (46–390) pg/g lipids, and the median TCDD body deposit was 3.9 (0.8–11.7) μg. This puts these patients into the most severely intoxicated group of subjects, according to back-calculated levels of TCDD. The median TCDD blood level in eight controls was 12 pg/g (<0.10 to 22.2 pg/g). Markers of metabolic impairment – diabetes, dyslipidaemia, arterial hypertension, carotid artery plaque, skin microvascular reactivity, eye fundus hypertensive angiopathy and history of coronary heart disease – were assessed and compared to a general male population of comparable age. Measured parameters compared with a population of comparable age were as follows: prevalence of diabetes (62.5% versus 17.6%), arterial hypertension (87.5% versus 71.8%), dyslipidaemia (87.5% versus 88.8%), history of coronary heart disease (62.5% versus 26.0%) and eye fundus hypertensive angiopathy (50% versus 14%). All eight patients (100%) developed plaques in carotid arteries, six had stenosis >50% and two had a carotid intervention (stening or endarterectomy).

Total cholesterol levels decreased compared to the earlier study this patient group in 2008, most likely due to a more intensive use of lipid-lowering drugs. Several metabolic parameters were higher (diabetes as much as 3.5-fold) in the group of severely TCDD-intoxicated subjects than in a general population of comparable age. This suggests that TCDD plays a role in the development of metabolic impairment and vascular changes.

Cardiovascular and metabolic diseases attributable to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are associated with aryl hydrocarbon receptor (AhR) activation and subsequent induction of metabolic changes, and inflammation in blood vessels which is combined with premature cell senescence mediated by produced reactive oxygen species [1,2]. The association of environmental exposure to TCDD with increasing incidence of diabetes mellitus is a topic of interest; however, it remains unanswered [3], as do similar questions on coronary heart disease, hypertension and on dyslipidaemia [4–6].

From this point of view, the follow-up study of the severely intoxicated Czech chemical workers brings new data to bear on these questions. We examined the last eight survivors of 80 workers, who became seriously intoxicated during the production of herbicide 2,4,5-trichlorophenoxyacetic acid from the unintentional by-production of TCDD between the years 1965 and 1968.

The first TCDD blood analysis was possible in 1996 [7] when a median of 305 pg/g fat (74–760 pg/g fat) was found in these workers. The back-calculated TCDD plasma level at the time of exposure (time zero) using the physiologically based model [8] might have reached about 35,000 and 350,000 pg/g fat in patient No. 8 and No. 1, respectively [9]. This made them one of the most severely intoxicated groups of subjects in the world among herbicide producers and users in several countries, including veterans of the war spraying TCDD-contaminated Agent Orange in Vietnam and the associated affected Vietnamese population [8,10]. The half-life of TCDD during the first months after exposure is about 3 months. However, the half-life prolongs 50 years after exposure to more than 10 years, in agreement with the physiologically based model [8].

Due to the long elimination half-life, TCDD stays in the human body, bound on the lipids, for decades, and there is no antidote that would increase the elimination of this toxic agent or mitigate its toxic effect. At the time of exposure, the mean age of the group of the male workers was 35 (20–58) years. All subjects developed chloracne during this occupation, 36% of the subjects had skin hyperpigmentation and/or hypertrichosis, and 50% had dyslipidaemia. During the first 2 years after exposure, 15% of the workers were diagnosed with type 2 diabetes [10]. The levels of cholesterol, total plasma lipids and triglycerides (TG) in the past correlated well with the TCDD levels [11,12]. Vascular dysfunction was observed in these subjects in 2004 [13]. In addition, this group of patients showed severe neurological
and neuropsychological impairment throughout the following years [9,14,15].

Our objective was to evaluate the long-term metabolic and vascular changes in the last survivors of the TCDD intoxication and TCDD elimination half-life, as, to the best of our knowledge, no such data exist for humans 50 years after such exposure.

Results

The median TCDD level was 112 (46–390) pg/g of blood lipids. For comparison, a median of 12 pg/g (<0.10 to 22.2 pg/g) TCDD was found in eight controls. The median plasma half-time prolonged from 8.5 (5.9–9.0) years 30–35 years after exposure [20], and 50 years after exposure reached 10.2 (5.5–24.1) years, which is in agreement with the physiologically based model.

The median body fat content was 31.6 (27.8–44.6) %, or 25.6 (17.8–69.7) kg, and the median TCDD body deposit was 3.9 (0.8–11.7) μg, (average 4.95 ± 3.65 μg). Individual levels and characteristics of the subjects are shown in table 1. Mean metabolic parameters are as follows: total cholesterol 4.38 ± 0.68 mmol/l, TG 1.56 ± 0.54 mmol/l, fasting glucose 6.76 ± 2.3 mmol/l and HbA1c 47 ± 13 mmol/mol.

All patients had residues of chloracne. All eight patients had atherosclerotic plaques on carotid arteries as can be seen in table 1. Six patients (No. 1, 4, 5, 6, 7 and 8) had stenosis >50% and among them, subjects No. 1 and No. 4 had a history of surgery or stenting due to significant carotid stenosis. Eye fundus examination showed stable hypertensive angiopathy in four of the subjects and physiological age-relevant vascular findings with a mild improvement since 2004 in the remaining four subjects. The patient with the highest TCDD burden (No. 1) had developed all observed characteristics (diabetes mellitus, dyslipidaemia, hypertension, coronary heart disease, carotid artery stenting) during his life.

For microvascular reactivity, a mean basal perfusion showed increase in comparison with the results from 2004 on the forearm (10.8 ± 4.9 versus 7.1 ± 2.4 perfusion units (PU), p < 0.05), while a decrease in basal perfusion was observed on the finger (132 ± 45 versus 257 ± 110 PU, p < 0.05). Improvement in microvascular reactivity on the forearm was observed both in PORH and in TH (PORHmax: 42 ± 24 versus 26 ± 7 PU, p < 0.02; PORH time-to-max 6 ± 4 versus 13 ± 4 sec., p < 0.02; THmax 80 ± 32 versus 59 ± 18 PU, p < 0.02, TH time-to-max 76 ± 24 versus 89 ± 20 sec., NS). Microvascular reactivity did not improve on the finger. There was no improvement in parameters of microvascular reactivity when the results were compared with the findings in 2001.

Discussion

Recent TCDD level classifies this group in those with the highest exposure – patient No. 1 is comparable with individuals with the highest ever documented levels of TCDD as measured a few months after exposure [21,22] and shows multiple signs of metabolic impairment, more severe than patients with lower TCDD levels.

The prevalence of diabetes in these chronically TCDD-exposed patients is more than 3.5-fold higher compared to the male population of a comparable age [15]. Also, the prevalence of hypertension and hyperlipidaemia exceeds general male population values, as shown in table 1. Atherosclerosis was highly prevalent, based on carotid ultrasonography and on a substantial history of cardiovascular disease.

In 2004, four patients were diagnosed with diabetes and two of them were treated with glucose-lowering drugs. By 2016, already five patients used glucose-lowering drugs. The higher proportion of diabetes as compared with the population is also in accordance with experimental studies [23–25]. Activators of peroxisome proliferator-activated receptor alpha (PPAR-α), a key regulator of systemic insulin sensitivity, delay the onset of type 2 diabetes by lowering plasma TG. The inhibition of PPAR-α through the Ah receptor could explain TCDD-mediated diabetes [26]. Dioxin has been recognized as an environmental endocrine disruptor. A study in Taiwan that evaluated the association between exposure to dioxin and diabetes in TCDD-contaminated areas found exposure to TCDD is a risk factor for diabetes, independent of age and body mass index (BMI) [27]. Also, cross-sectional studies of populations with low-level TCDD exposures (serum
concentrations <10 pg/g lipid) found positive dose–response. Heterogeneous results were seen in studies of subjects with high TCDD body burdens [3]; however, our study of very high exposure supports the association.

Oxidative stress is the crucial pathogenic mechanism inducing impairment of vascular function, which can be accelerated by dyslipidaemia and both oxidative stress and dyslipidaemia. Elevated markers of oxidative stress have been found in these TCDD-exposed patients [12].

We found a rather unexpected improvement in the microvascular reactivity as compared with the findings in 2004. This may be explained by a more efficient hypolipidaemic treatment in these patients and their lower cholesterol level. This lowering can be observed in the whole group already from 1991. At that time, the mean total cholesterol and TG levels reached 7.7 and 3.7 mmol/l, respectively [28], which follows the total trend in the general population [16]. Another positive finding is that the last clinically relevant coronary heart disease, acute myocardial infarction and/or ischaemic stroke were diagnosed in 2011. These positive results may also be associated with about 50% lowering of TCDD levels.

Obviously, a limitation of this study is the low number of highly exposed survivors of TCDD intoxication, which complicates the generalization of the findings and the comparison with the general population. The advantage, on the other side, is the long-term follow-up of highly exposed subjects using the same methods.

**Conclusion**

The results in the last eight surviving men who have been severely intoxicated with TCDD for 50 years suggest that TCDD may promote metabolic impairment. The proportion of diabetes, hypertension, carotid plaques, eye fundus angiopathy and coronary heart disease in this group of patients is higher than in the general population. These data support the associations between this persistent organic pollutant and diabetes [6].

On the other hand, cholesterol and TG levels further decreased due to more efficient pharmacological treatment. Dyslipidaemia treatment may therefore have a beneficial effect both on the plasma lipid profile and on the vascular activity, even if there is no causal treatment and increased elimination of TCDD from the human body.

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**Conflict of Interests**

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the manuscript.

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