SEVERE 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN INTOXICATION: A FOLLOW-UP OF THE PATIENTS FROM VIENNA

Alexandra Geusau[1], Klaus Abraham[2]

[1]Medical University Of Vienna
[2]Federal Institute for Risk Assessment , Berlin

Introduction

In recent decades much effort has been focused on examination of the health of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), and in particular of the most toxic congener in this group of compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[1]. A variety of health effects have been attributed to TCDD, but little information is available on the course of a verified high level TCDD intoxication in humans. Chloracne is considered the characteristic symptom of severe poisoning [2,3,4]. TCDD exposure leads to a change in the phenotype and function of epithelial, and possibly also other cell types [1,3,5].

Here we present a seven year follow up study addressing the clinical, biochemical and immunologic parameters of two patients with chloracne, resulting from severe TCDD poisoning. We also investigated the elimination pathways of TCDD in these patients, the effects of various methods to increase its elimination, and methods for chloracne treatment.

Subjects and clinical findings

In October 1997, a 30-year old woman (patient 1) started to develop acneiform skin symptoms accompanied by nausea, vomiting, fatigue, exhaustion and epigastric pain shortly after she had moved into a new office space at a textile research institute in Vienna. In spring 1998 she presented at the Department of Dermatology, Medical University of Vienna, with severely inflamed, atypically distributed acne lesions. Her colleague, a 27-year old woman (patient 2) working in the same room, consulted us at about the same time. During the past few months she, too, had developed multiple cysts and comedones, restricted to the malar crescent and the pre- and retroauricular area of her face, accompanied by gastrointestinal symptoms. Neither women was directly occupied with chemical laboratory work. Chloracne was clinically suspected and confirmed soon thereafter by the measurement of highly elevated levels of TCDD in both patients.

In the first blood samples taken, patient 1 had a TCDD level of 144,000 pg/g blood fat (April 1998), the highest levels ever reported in an individual, and patient 2 had 26,000 pg/g blood fat (June 1998). These and the following measurements were performed by the ERGO Forschungsgesellschaft (Hamburg/Germany) using HRGC/HRMS. The total body burden at the time the first blood sample was collected was calculated to be 1.5 mg in the first and 0.34 mg in the second patient, corresponding to an approximate dose of 25 and 6 µg/kg body weight, respectively (initial dosages in autumn 1997 can be assumed to be even higher). For the investigation of the first blood samples, all 2,3,7,8-substituted PCDDs and PCDFs were determined. The only congener besides TCDD above the background range was 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin. The source of the TCDD and the route of exposure have never been identified in the two patients. Because of the high blood levels, oral ingestion appears to be the most likely mode of intoxication. Elevated TCDD blood levels were also detected in three out of 30 additional employees working at the same institute (149, 93, 865 pg/g blood fat); however, they did not develop clinical symptoms [6].

In patient 1, a continuous progression of chloracne was observed within the first year of observation. Almost every single follicle was involved and the entire skin surface was covered with inflamed, painful cysts, draining smelly content [6]. In addition, she had brownish-grey hyperpigmentation of the face, hypertrichosis, acral granuloma annulare-like lesions, distal onycholysis and flattening of the nails. At a later time point, she developed palmoplantar keratoderma, a condition associated with circumscribed hyperkeratosis, which has never been reported before in an individual with TCDD poisoning [7]. The patient received numerous known treatments for acne-like skin diseases including topical and systemic retinoids, but these could not prevent progression of the disease. In the following years, incisions of the cysts were and are still required on a regular (initially weekly) basis, as well as mechanical extraction of the open and closed comedones (so called *acne toilet)*. Because of the heavy (sterile) inflammation, systemic corticosteroids were administered initially for almost two years, and the painful skin condition required analgesic drugs on a regular basis. After 7 years, a significant improvement of the inflammatory condition and a decrease in the number of cysts has occurred; however, she will suffer from a life-long disfiguring disease as well as stigmata in the form of a variety of scars and periodically forming abscesses. Patient 2 suffered only from mild facial chloracne, which resolved almost entirely within one year of treatment with topical vitamin A acid (tretinoin) and

mechanical extraction of comedones.
Both patients experienced gastrointestinal symptoms including nausea, vomiting and epigastric pain since late fall 1997. In patient 1, gastroscopy in spring 1998 showed acute helicobacter-negative gastritis. In this patient, a diet resulted in weight loss of about 10 kg prior to hospital admission. Within the following year, in both patients, the abdominal symptoms subsided. Since the presumable time of intoxication (fall 1997) patient 1 had secondary amenorrhoea which resolved spontaneously in the spring of 2002. The initial hormonal status showed slightly decreased estradiol and progesterone levels, and, from May 1999 to 2003, a mild elevation of prolactin, which coincided with the initiation of antidepressive therapy (Paroxetin). The measurement of hormone reserve and regulation by dynamic tests revealed normal values. In both patients, investigations such as ultrasound of the abdomen and heart, chest X-ray, pulmonary function tests, neurological, psychodynamic and electrophysiological investigations were unrevealing.

Laboratory findings

There are no laboratory parameters available from the initial period of acute poisoning. Despite the high TCDD levels, only few routine laboratory parameters monitored on a regular basis during the past 6.5 years were not within the normal range.
In the first patient, moderate elevation of blood lipids, anemia and leukocytosis were the most prominent pathologic changes during the first years after intoxication, the latter more likely due to the extensive inflammatory skin condition and the intermittently given corticosteroids, rather than representing a direct effect of TCDD. In the first three months of observation, the patient had thrombopenia while antiplatelet-antibodies were negative.
In the second patient, apart from marginally elevated values for cholesterol and lipase within the first 3 years, the routine laboratory and immunologic parameters were within the normal range.
In both patients, lymphocyte subset analysis showed a pronounced lymphocytosis and a low percentage of the natural killer cells with normal function of these cells. Details of the laboratory findings were published elsewhere [6]. In both patients, a more extended analysis of both the humoral and cellular arms of the immune system was so far unrevealing, as proven by a controlled study in 2000 with 50 age-matched control subjects [8].

TCDD half-life and attempts to accelerate elimination of TCDD

In both patients, TCDD blood levels were regularly monitored. The last measurement in September 2004 revealed values of 8,300 and 4,300 pg/g blood fat in patient 1 and 2, respectively. Using each of the TCDD values measured over the entire 6.5-year period, overall half-lives of 1.8 and 2.5 years, respectively, can be calculated. These values are not very different from those of 1.5 and 2.9 years, respectively, calculated from the TCDD blood values measured until March 2001 [9]. The half-lives observed in our patients are considerably shorter than those of 7 to 9 years reported in other studies with lower TCDD levels. It can be assumed that this is due to accelerated metabolism (induced by the very high TCDD levels, see below), as the amount of TCDD eliminated via other routes (skin and feces) is not significant [9,10].
During the observation period until 2001, the half-lives of TCDD may additionally have been shortened because of attempts to accelerate its elimination. Since 1998, the administration of olestra-containing chips and olestra as a pure substance, respectively, was effective in increasing the intestinal excretion of TCDD [9,11]. With relatively high doses of olestra intake up to 66 gram per day, excretion was augmented by 8 to 10 fold [11]. The patients continued with the consumption of olestra over a period of almost 3 years, but in decreasing daily amounts, as both patients developed a distaste for it. The estimated overall amounts of olestra consumed were 8.2 kg in patient 1 and 5.5 in patient 2. These amounts contributed to roughly 10 and 15%, respectively, to the overall half-lives of TCDD in the period until 2001 [9]. This relatively low effect of olestra was likely to be due to the relatively high metabolism of TCDD in the patients. The effect of olestra may have been significantly stronger in case of low or moderate TCDD exposure with longer half-lives.
An additional attempt to accelerate the elimination of TCDD from the body was made in patient 1 by lipidapharesis in 1999, where TCDD is eliminated corresponding to the eliminated blood fat. When employed twice a week, the amount of TCDD as parent substance excreted by this method is comparable to that of fecal excretion (without olestra). In view of costs and time involved, lipidapheresis does not seem to be justified for the enhancement of TCDD elimination [9]. Another attempt to increase elimination was made by the application of petrolatum on the skin. Although an increase in the excretion of TCDD via the skin of up to 100% was observed in patient 1, such an approach appears not to be a feasible means of treatment [10].

Induction of hepatic cytochrome P450 1A2 (CYP1A2)

One of the best investigated and most sensitive biochemical effect of TCDD in laboratory animals is the induction of the cytochrome P450 system. It is already observed in the low ng/kg body weight range and shows a typical dose-

response curve [12]. With the extremely high contamination in our patients (in the low µg/kg body weight range), an induction of this enzyme system was to be expected and confirmed, using an indirect method for measurement, which involves caffeine (oral application of 3 mg caffeine per kg body weight). Its metabolism due to CYP1A2 was quantified using different methods (^{13}C-breath test, measurements of caffeine and its metabolites in serum and urine). The CYP1A2 activity measured in the two patients was compared to that of 50 age-matched control subjects (30 non-smokers and 20 heavy smokers). In both patients, the CYP1A2 activity initially measured in 1998 was about 8- to 10-times higher than the mean of non-smokers [13]. Over the years, the activity decreased, but is still clearly above of that of smokers who have higher on average values than non-smokers. So far, the CYP1A2 activity indirectly measured using the caffeine test is the only laboratory parameter indicating a clear-cut difference in comparison to uncontaminated subjects, and is therefore appropriate for the use as a biomarker in response to TCDD exposure. However, in man, TCDD blood levels of at least several hundred pg/g blood lipids are necessary to have a respective effect. It is unknown as whether the hepatic CYP1A2 induction observed in our patients is responsible for accelerated TCDD metabolism.

Conclusion

In conclusion, apart from significant skin manifestations, there are only a few clinical symptoms present in our patients and no parameter indicative of TCDD intoxication. In our first patient, the disfiguring course of chloracne, unresponsive to isotretinoin, was most impressive, whereas the second patient despite the very high TCDD blood level revealed only a mild expression of chloracne. Olestra is effective in acceleration of intestinal TCDD excretion. In patients with high level TCDD contamination, significantly shorter half lives than expected were observed.

Literature

1. van den Berg M., de Jongh J., Poiger H., Olson JR. (1994) *Crit Reviews in Toxicol*. 24: 1- 74.
2. Crow KD. (1981) *Clin and Exp Dermatol.* 6: 243-257.
3. Tindall JP. (1985) *J Am Acad Dermatol.* 13: 539-558.
4. Suskind RR. (1985) *Scand J Work Environ Health.* 11:165-171.
5. Geusau A., Khorchide M., Mildner M., Pammer J., Eckhart L., Tschachler E. (2005) *J Invest Dermatol.* 124: 275-277.
6. Geusau A., Abraham K., Geissler K., Sator MO., Stingl G., Tschachler E. (2001) *Environ Health Perspect.* 109: 865-869.
7. Geusau A., Jurecka W., Nahavandi H., Schmidt JB., Stingl G., Tschachler E. (2000) *Br J Dermatol*. 143: 1067-1071.
8. Abraham K., Geusau A., Stingl G., Bauer S., Brockmöller J., Wahn U., Helge H. (2002) O*rganohalogen Compounds.* 55:399-400.
9. Geusau A., Schmaldienst S., Derfler K., Päpke O., Abraham K. (2002) *Arch Toxicol*. 76: 316-325.
10. Geusau A., Tschachler E., Meixner M., Päpke O., Stingl G., McLachlan M. (2001) *Br J Dermatol.* 145: 938-943.
11. Geusau A., Tschachler E., Meixner M., Sandermann S., Päpke O., Wolf C., Valic E., Stingl G., McLachlan M. (1999) *Lancet.* 354: 1266-1267.
12. Abraham K., Krowke R., Neubert D. (1988) *Arch Toxicol*. 62: 359-368.
13. Abraham K., Geusau A., Tosun Y., Helge H., Bauer S., Brockmöller J. (2002) *Clin Pharmacol Ther.* 72: 163-174.