CHLOROBENZENE COMPOUNDS AS POSSIBLE IMMUNO-DISRUPTOR AGENTS

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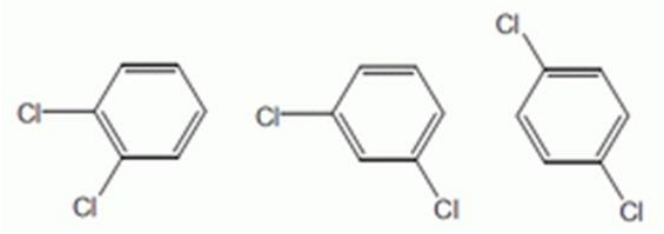
Abstract

Dichlorobenzenes are lipophilic, depositable, colorless liquids that appear as an exposure factor because they are continuously present in households, but are also used in agriculture in large quantities in e.g. insecticides and fungicides. As there is a constant interaction between the living systems and its environment and the internal organizational stability of biological systems is controlled by homeostasis, these agents may disrupt the homeostasis, therefore it is especially important to study the effect of these compounds on the immune system.

Introduction

Dichlorobenzenes (DClB) are lipophilic, depositable, colourless liquids (at T = 25 °C, p = 1 atm) with 3 known isomerization states: ortho-dichlorobenzene (1,2-dichlorobenzene; 1,2-DClB), meta-dichlorobenzene (1,3-dichlorobenzene, 1,3-DClB) and para-dichlorobenzene (1,4-dichlorobenzene, 1,4-DClB) [1,2].



Ortho-DClB meta-DClB para-DClB

Figure 1. Structural formula of ortho-DClB, meta-DClB and para- DClB [1, 2]

DClB can be metabolized in living systems in several ways, e.g. rate of metabolic in human or rat liver: 1,3-DClB → 1,2-DClB → 1,4-DClB. Oral administration of 1,4-DClB, the metabolites in serum were 2,5-dichlorophenyl-methyl-sulfoxide and 2,5-dichlorophenyl-methyl-sulfone [3]. During degradation, para-DClB (PDClB) is hydrolysed to (nephrotoxic) dichlorophenol and then oxidized to dichloro-catechol and dichloro-hydroquinone, which can be further conjugated to glutathione, glucuronic acid and sulfate. These are all hepatotoxic components. 1,4-DClB is less genotoxic [4].

PDClB is a weak antiestrogen via the aryl-hydrocarbon receptor due to estrogen modulation [5]. But sperm destruction, production-reducing and as well as androgenic effects are also known in rats and mice [6]. According to these effects, xenobiotic DClB agents are endocrine disruptor compounds (EDCs). As a fact of exposure, they are very strong because they are constantly present in households (fragrances, fresheners, etc.), but agriculture also uses them in large quantities in insecticides and fungicides, and it is also a raw material in the production of industry and some plastics. The other chlorobenzene (ClB) derivatives are also present in large amounts in the environmental elements, deposited as a function of their stability.

PDClB has become a standard compound of Life Cycle Analysis (LCA) standards, which are the most important basis for environmental safety, and has been used as reference agents in of

Ecotoxicological (ETP) and Human Toxicological Potential (HTP) in impact analyse. These toxicological potentials consistently affect the homeostasis of organisms. The maintenance of human homeostasis, the systemic regulation of psycho-neuroendocrino-immune functions is realised. The dominant element of this is cellular immune function, which is affected by ClB exposures as factors. In this regard, ClBs may be the focus of attention as immune disruptor compounds (IDCs).

Aims

In the present work, we investigated the effects of DClB isomers and hexa-ClB (HClB) on T cell-mediated immunity. We sought to answer the question of whether ClB compounds carry a possible IDC character. Furthermore, did it seem interesting to study why PDClB was chosen by the International Standards as the reference compound?

Methods

In our experiments we used human (♂: 22-34 years) 0 Rh (+) blood group castle samples with healthy physiological parameters, from a portion of heparin (7 IU) anticoagulated blood.
From another part of the heparin blood samples, T lymphocyte transformation activity was tested in whole blood culture. Homogeneous blood samples diluted 10 x in supplemented RPMI-1640 medium were used under sterile conditions in a 96-well plate (p = 5% CO_2, 37 °C). A 180 µl diluted blood sample + 20 µl mitogenic mix (0.1 µg/ml CONA + 1: 1000 PHAP + 0.1 µg/ml PWM) was used as a control. Spontaneous cell transformation was examined in the 180 µl diluted blood sample + 20 µl RPMI-1640 (+suppl.) system. For exposure samples, in the 180 µl RPMI-1640 (+suppl.) diluted blood sample, the test substances (ortho-DClB, meta-DClB, PDClB, HClB) were already present at doses of 0.01 and 0.1 µg/ml, which was supplemented with the +20 µl mitogenic mixture. After 12 and 24 hours of incubation in the treatment protocol, 20 µl of ^{3}H-Thymidine (20 µCi/ml ^{3}H-Thymidine in RPMI-1640) was added to each experimental system for an additional 18 hours.
Evaluation of results:

LySi = stLy cpm / spLY cpm,

in wich:

- Lymphocyte (LY) stimulation index= LySi
- Stimulated Ly transformation cpm (radioactivity)= stLy cpm
- Spontaneous Ly transformation cpm (radioactivity)= spLY cpm

Data were evaluated by ANOVA.

Results

As can be seen from the data in Figure 2, the dose of DClB treatment used inhibited blast transformation.

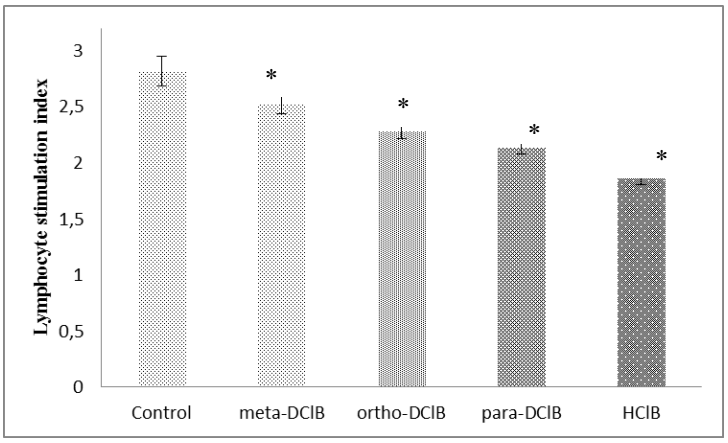


Figure 2. Effects of 0.01 µg/ml DClB isomers on immune function over a 12-hour treatment period (n=5, means±SD, ∗: P<0.001)

It can be seen in the Figure 3, that DClB treatments at a dose of 0.01 µg/ml resulted in a decrease in the lymphocyte stimulation index.

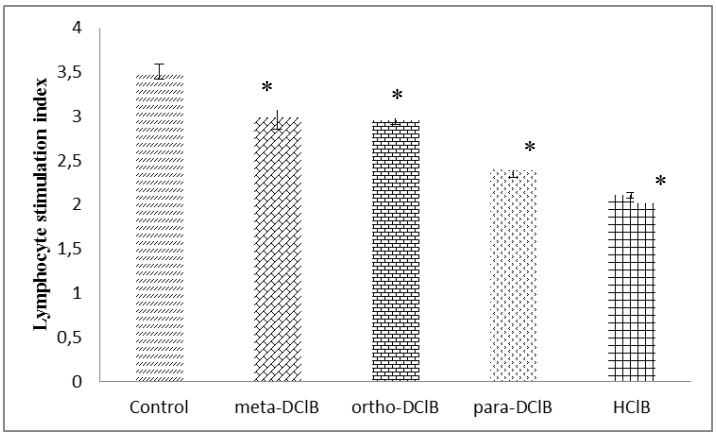


Figure 3. Effects of 0.01 µg/ml DClB isomers on immune function over a 24-hour treatment period (n=5, means±SD, ∗: P<0.001)

In the set experimental protocol, the applied 0.1 µg/ml dose of DClB treatment modulated the blast transformation.

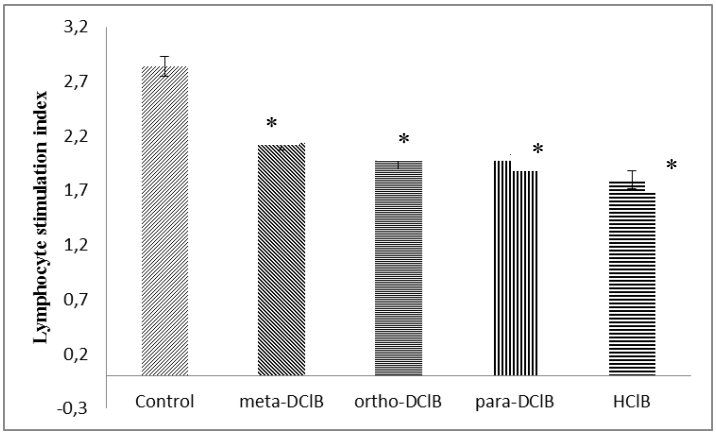


Figure 4. Effect of 0.1 µg/ml DClB isomers on immune function over a 12-hour treatment period (n=5, means±SD, ∗: P<0.001)

Based on the data in Figure 5, the tested DClB isomers significantly reduced the lymphocyte stimulation index during 24-hour treatment.

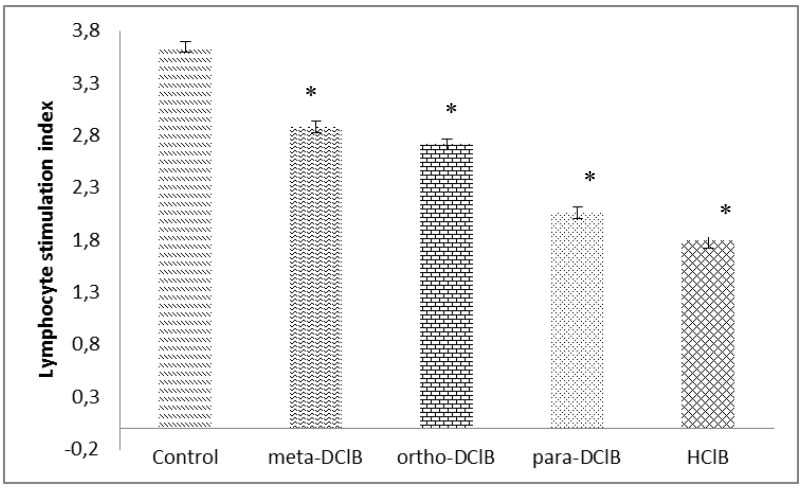


Figure 5. Effect of 0.1 µg/ml DClB isomers on immune function over a 24-hour treatment period (n=5, means±SD, ∗: P<0.001)

Discussion and conclusion

According to our results, in the study of innate immune functions, PDClB proved to be the most potent of the DClB compounds among the DClB isomers, with HClB showing a stronger T-lymphocyte transformation deactivating effect. Because all of the ClB compounds tested were degradative in cellular immunomodulation, these agents could also be treated as IDCs.

Chlorinated benzenes are known to consist of twelve chemicals: one mono-, three di-, three tri-, three tetra-, one penta-, and one hexa-chlorobenzene. Of these, the annual production of 1,4 DClB is the highest in the world [7], and of the DClB compounds, PDClB is the most stable. These two factors: the high xenobiotic presence in society and chemical persistence, combined with lipophilicity, already justify the use of PDClB as a reference compound in the determination of standard toxicity potentials (HTP, ETP).

Acknowledgements

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Reference

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