Differential Effects of the Enantiomers of α-Hexachlorocyclohexane (α-HCH) on Cytotoxicity and Growth Stimulation in Primary Rat Hepatocytes

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Introduction

Because of its ubiquitous nature and its relative persistance, α-HCH plays an important role in most studies of the pollution of the environment. In Western Europe the application of the technical mixture of the insecticide γ-HCH, which involves other HCH-isomers (e.g. α-HCH 60%-70%), is no longer permitted. In other parts of the world the technical HCH-mixture is still being used thus giving rise to a continuous impact of α-HCH on marine and terrestrial ecosystems.

In brain tissues, concentrations of organic pollutants (e.g. polychlorinated biphenyls) have been found to be a factor ten lower than those found in other organs of the same animals[1)]. This well-known effect is often ascribed to the influence of the so-called blood-brain-barrier. By the way of contrast, α-HCH is accumulated very efficiently in the brain tissue of marine and terrestrial biota[2,3)].

Because of its bioaccumulation in food chains and its preferential permeation through the blood-brain-barrier, α-HCH could be a risk factor for human health. This conjecture is supported by toxicological studies which showed that the α-HCH isomer is carcinogenic in rats[4)]. Furthermore, α-HCH is considered to be a tumor promoter[5)].

In the last two years, α-HCH has received additional attention in environmental process studies, because it is the only of the eight possible HCH-isomers which is chiral and can therefore be studied by chiral gas chromatography. This experimental approach allows a discrimination between enzymatic and non-enzymatic processes[6)]. It was shown that at lower trophic levels of the marine ecosystem (blue mussels, flounders) (-)-α-HCH is preferentially enriched, whereas in liver of common eider ducks and blubber of harbor seals the (-)-enantiomer is largly decomposed[7)]. In contrast to the preferential (+)-α-HCH enrichment at higher trophic levels in marine biota, liver tissues of sheep[8)] and roe-deer[9)] exhibited an enrichment of the (-)-enantiomer of α-HCH. The various degradation pathways of α-HCH may be due to the species-specific expression of liver enzymes. Although studies on the toxicity of racemic α-HCH have been reported, no information on the toxic effects of the respective enantiomers that are accumulated in tissues is avialable. The present study with primary rat hepatocytes aims at gaining first insight into this problem. The cytotoxic effect was determined as a parameter for the acute toxicity of α-HCH. The growth stimulation of hepatocytes may be associated with the chronic toxicity, e.g. tumor promotion.

Materials and Methods

α-HCH was obtained from Riedel-de-Haen (Seelze, Germany). The two enantiomers were accessible by preparative high performance liquid chromatography (HPLC)[10]. The enantiomer separation was carried out on a LiChrocart 250-50 column from Merck, Darmstadt, under following conditions: eluent MeOH/H_2O 75/25 (v/v) isocratic, flow rate 15 mL/min. The purity of separated (+)-α-HCH was 97% and that of (-)-α-HCH 91%. The α-HCH enantiomers were dissolved in dimethyl sulfoxide (DMSO).

Hepatocytes were isolated by collagenase perfusion from adult male Wistar rats and cultured as described[11]. On the second day after cell plating, the mitotic index was determined after 3hr exposure to colchicine (0,1mM). For the cytotoxicity assay hepatocytes were cultured for 24 hours in the presence of (+)- or (-)-α-HCH. After a medium change cells were incubated with neutral red (20μg/ml) for 3 hours. Then dye uptake into the lysosomes of intact cells was determined[12]. The proportion of dead cells was determined by counting at least 400 cells in 5 randomly chosen fields of the culture dishes microscopically.

Results and Discussion

Cytotoxic Effects

In general, the neutral red cytotoxicity assay is being used on the following basis: a reduced dye retention in lysosomes of cells indicates a toxic effect of a respective test compound. However, in the present case, up to concentrations of 3 x 10^{-4} M of the α-HCH enantiomers no toxic effect could be detected with the neutral red assay . Unexpectedly, an increased adsorption of dye was found although a dose-dependent reduction of all viability was observed microscopically.

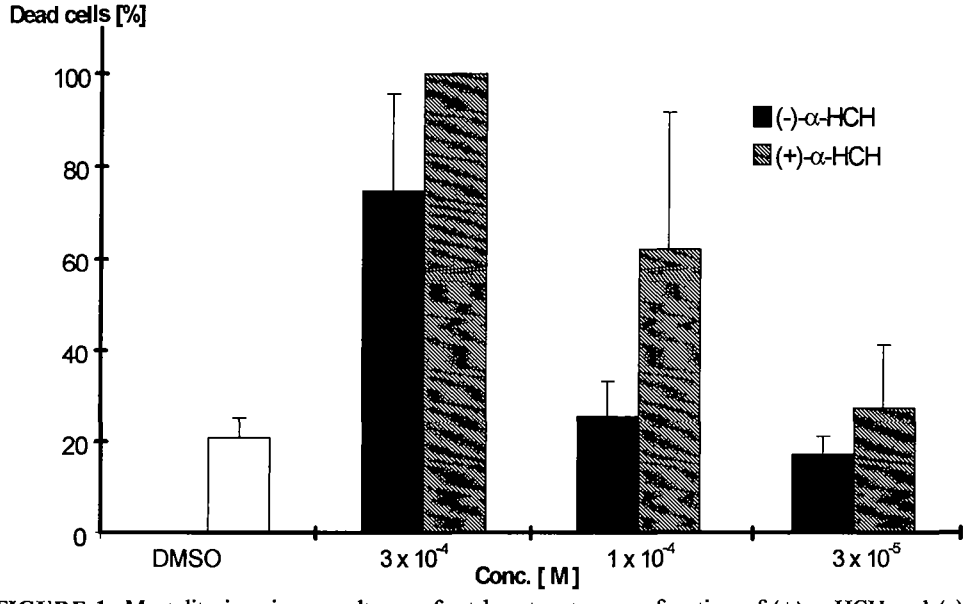

FIGURE 1: Mortality in primary cultures of rat hepatocytes as a function of (+)-α-HCH and (-)-α-HCH concentrations. Data represent the mean ± SD (triplicate cultures from 3 male animals)

Therefore, the numbers of viable and dead cells were counted microscopically. It turned out that a concentration of 3 x 10^{-4} M (+)-α-HCH leads to a mortality of 100 % , while at the same concentration of (-)-α-HCH 75 % dead cells were observed (Figure 1). At a lower concentration, i.e., 1 x 10^{-4} M no cytotoxic effect of the (-)-enantiomer of α-HCH was found, whereas the (+)-enantiomer still showed a significant mortality of 62 %. These results make evident that caution has to be applied when inferring cytotoxic effects exclusively from the neutral red assay.

Growth Stimulation

The mitotic rates of primary rat hepatocytes were stimulated by both enantiomers at concentrations of 5 x 10^{-5} M and 3 x 10^{-5} M (Figure 2) compared with DMSO controls. In the presence of (+)-α-HCH [5 x 10^{-5} M] significantly higher rates of mitosis (factor 2,4) compared with the stimulation of (-)-α-HCH (factor 1.7) were observed. At a concentration of 1 x 10^{-5} M the (+)-enantiomer stimulated the mitosis whereas the (-)-enantiomer was uneffective.

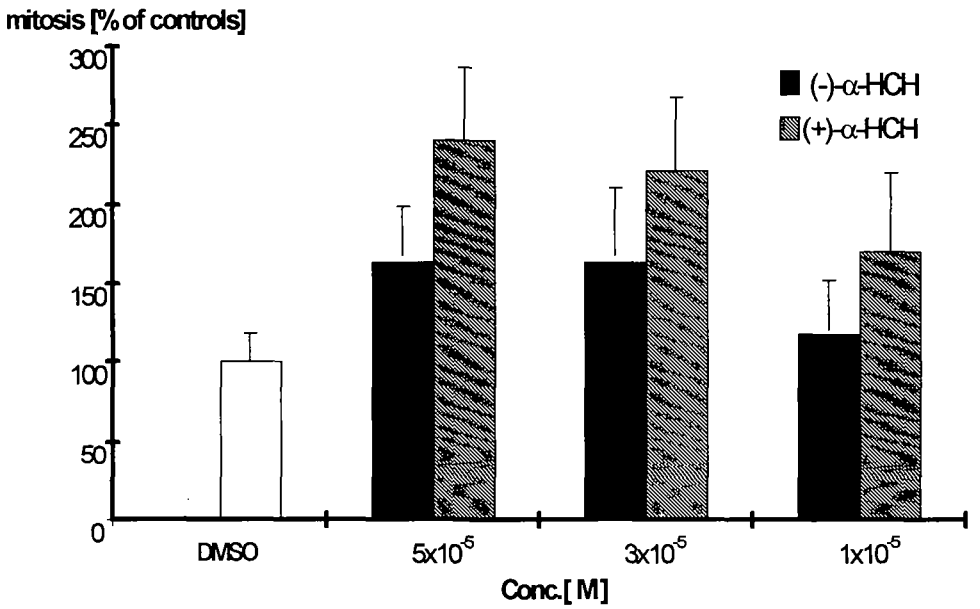

Figure 2: Stimulation of mitosis in primary rat hepatocytes by (+)-α-HCH and (-)-α-HCH. The DMSO controls were set as 100%. Data represent the mean ± SD (triplicate cultures of 4 male rats).

In summary, a significant difference between the α-HCH enantiomers was observed concerning the cytotoxic effect as well as the growth stimulation. In both assays the (+)-α-HCH was more effective than the (-)-enantiomer. It may be concluded from our results that an enatioselective enrichment of (-)-α-HCH is associated with a lower risk factor than the accumulation of (+)-α-HCH. Thus, the quantitative determination of α-HCH as a mixture of both enantiomers may lead to an overestimation of the toxicity of this compound in those animal tissues (e.g. the liver of sheep[10] and roe-deer [9]) in which the more toxic (+)-enantiomer is preferentially degraded.

TOX (po)

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