Insulin-like growth factor-I (IGF-I) serum concentrations in healthy children and adolescents: Relationship to level of contamination by DDT-derivative pesticides

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Abstract

Context: Serum levels of Insulin-like growth factor I (IGF-I) play a critical role in children growth and in the pathogenesis of several diseases. In addition, recent studies suggest that DDT-derivative organochlorine pesticides (OC–DDTs) could influence IGF levels in human beings.

Objective and design: Because it has been suggested that IGF-I peak levels at puberty could determine IGF-I levels in adulthood, we developed a cross-sectional study of the potential association between serum levels of OC–DDTs and IGF system in 160 serum samples from young people (81 boys and 79 girls) living in the Canary Islands (Spain).

Results: Multivariate tests were used adjusting for confounding variables (age, height, and weight) and stratifying by gender and age: IGF-I serum levels were significantly lower in pre-pubertal male children (6–15 years) who showed detectable values of p,p′-DDE, and p,p′-DDD than in pre-pubertal male children with undetectable levels of these OC–DDTs-metabolites (p = 0.023 and p = 0.049, respectively). In addition, in this multivariate model, a non-linear dose–response curve was observed between Total DDT body burden (sum of the three DDT-derivatives measured: p,p′-DDT, p,p′-DDE, and p,p′-DDD) and IGF-I in pre-pubertal male children (6–15 years; p = 0.043).

Conclusion: These findings suggest that OC–DDTs could modulate the IGF-system in a way that is highly influenced by gender and age. Improvements in our understanding of exogenous determinants of the IGF-system may provide new insights into the role played by environmental contaminants in IGF-related diseases.

1. Introduction

Insulin-like growth factor-I (IGF-I) is an amino acid peptide, synthesized in the liver in response to growth hormone (GH) and is considered to mediate various GH actions [1,2]. In fact, IGF-I has been established as a useful marker for monitoring the status of the GH–IGF axis [3]. IGF-I circulates in two stages, namely free and bound to one of six binding proteins, with >90% bound to the IGF binding protein 3 (IGFBP-3) [4].

IGF-I serum values are low at birth, rise during childhood with peak levels during puberty, and decline gradually with age thereafter, reaching a plateau in early adulthood [5–8]. Puberty has been suggested as a sensitive period for the programming of adult IGF-I levels. Indeed, IGF-I peak levels at puberty seem to determine IGF-I levels in adulthood [2]. An inverse relationship between age and IGF-I has been described in adults [4,9,10], the same as the existence of a sexual dimorphism with respect to IGF-I levels, with...
men having higher levels than women, especially in older people [1,11].

In adults, IGF-I has been involved in the development of several human diseases: diabetes, osteoporosis, renal diseases, cancer (including prostate, breast, lung, colorectal, and cervical cancer), dementia and cognitive diseases, and cardiovascular diseases [1,12–16]. In children alterations in the levels of IGF-I have also been related to alterations in growth [17,18].

Epidemiological studies have demonstrated the influence of height and weight and dietary and lifestyle factors on serum levels of IGF-I and several of its binding proteins [1,4,19]. Other factors, such as environmental and dietary contamination by chemicals should also be taken into account. The possibility exists that environmental contaminants could influence IGF system. Thus, studies in animals and in human subjects have demonstrated that environmental pollutants, such as benzopyrene, dioxins, dibenzofurans, and hexachlorobenzene could alter the normal synthesis and/or secretion of IGF-I [19–22]. Recently, other authors have established the possibility that occupational and environmental exposure to environmentally persistent pollutants could cause an alteration on IGF-I levels in adults [22,23].

Regarding diet and contaminants present in food, it is well known that foods are the main source of human exposure to environmentally persistent contaminants, and that the group of OC–DDTs is one of the groups of pollutants that are usually detected in foods all over the world [24]. Dietary exposure to OC–DDTs results in the bioaccumulation of these chemicals in the human body [25].

Nowadays, the human body burden of OC–DDTs is a matter of public health concern because of their action as endocrine disrupters and as suspected carcinogens [24,26,27]. In children, OC–DDTs have been linked with several deleterious health effects, such as asthma or growth disorders [28,29].

As children seem to be a subgroup of population with high susceptibility to suffer the potential deleterious health effects exerted by OC–DDTs [28,29], and because IGF-I levels in childhood and adolescence could play a critical role in the function of the GH–IGF axis later in life [2], the aim of this work has been to explore the potential relationship between OCs’ exposure and IGF-I serum levels among youngsters in a setting with relatively high levels of exposure to these contaminants [30].

2. Methods

2.1. Study group and sample collection

The study was based on 160 healthy subjects (81 boys and 79 girls) aged between 6 and 19 years old enrolled in the Canary Islands’ nutritional survey (1998). The whole sample from this Nutritional Survey was representative of the population of these Islands’ nutritional survey (1998). The local ethics committee approved the design of the study, and informed consent was obtained from the study participants or their legal tutors.

2.2. Materials and analytical methods

As previously reported by others, serum IGF-I and IGFBP-3 were measured through commercially available enzyme-linked immunosorbent assay (ELISA) kits (Diagnostic Systems Laboratories, Webster, TX, USA). The ELISA methodology, commonly employed in epidemiologic studies, is an useful and convenient system for quantifying IGF-I and IGFBP-3 [4,23]. The overall intra-batch coefficients of variation were lower than 10% for both proteins. The average inter-batch coefficients of variation were 9% for IGF-I and 12% for IGFBP-3.

The OC–DDTs measured were: DDT isomers (p,p'-DDT and o,p'- DDT) and DDT-metabolites (p,p'-DDE, o,p'-DDE, p,p'-DDD and o,p'- DDD). As previously published, OC–DDTs were measured in serum by gas chromatography electron capture detection [30]. The analytical limit of detection (LOD) was 1 ppb (ng/g fat) for all the analytes tested. In this study we only have considered those DDT-derivatives that were present at measurable levels in more than 20% of the samples analyzed in any age/sex-group. In fact, throughout this work only p,p'-isomers of DDT-derivatives reached such percentage and were included in the study. Table 2 shows the concentrations found in serum samples from young people from the Canary Islands. Additionally, we have considered Total DDT body

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Male children</th>
<th>Female children</th>
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<tbody>
<tr>
<td>Sex</td>
<td>n</td>
<td>Median (p25–p75)</td>
</tr>
<tr>
<td>IGF-I (ng/l)</td>
<td>62</td>
<td>249 (175–361)</td>
</tr>
<tr>
<td>IGFBP-3 (ng/l)</td>
<td>60</td>
<td>4665 (4059–5165)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62</td>
<td>11.2 ± 2.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>62</td>
<td>147.2 ± 17.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62</td>
<td>36.4 ± 12.7</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>OCs (ng/g fat)</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Median (p25–p75)</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>35.5</td>
<td>0.0 (0.0–214.8)</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>66.1</td>
<td>60.40 (0.0–76.2)</td>
</tr>
<tr>
<td>p,p'-DDD</td>
<td>24.2</td>
<td>0.0 (0.0–0.0)</td>
</tr>
<tr>
<td>Total DDT</td>
<td>96.8</td>
<td>192.9 (65.61–643.5)</td>
</tr>
<tr>
<td>T1 (Total DDT)</td>
<td>38.3</td>
<td>61.3 (90.7–66.6)</td>
</tr>
<tr>
<td>T2 (Total DDT)</td>
<td>33.3</td>
<td>209.8 (175.7–254.0)</td>
</tr>
<tr>
<td>T3 (Total DDT)</td>
<td>31.6</td>
<td>806.9 (686.9–1010.3)</td>
</tr>
</tbody>
</table>

p < 0.001. Mean (SD) = mean and standard deviation. Median (p25–p75) = median and percentile 25 and 75 values.
burden (Total DDT) as the sum of the three \( p,p' \)-isomers of DDT-derivatives more frequently measured (\( p,p' \)-DDT plus \( p,p' \)-DDE plus \( p,p' \)-DDD).

2.3. Statistical analysis

Taking into account that the IGF-I level in childhood and adolescence is subjected to important variations with age [32,33], we analyzed the population-sample fitting separate models for males and females. Following the previously described results of population-based studies [34], the age of 13 years old for girls and 15 years old for boys were selected to separate adolescents from pre-pubertal children. Thus, we stratified the population-sample in four groups: male children (6–15 years), female children (6–13 years), male adolescents (15–19 years) and female adolescents (13–19 years).

An initial assessment was made of the baseline characteristics of the population studied. The Kolmogorov–Smirnoff normality test showed non-normal distribution of OC–DDTs, IGF-I, and IGFBP-3 serum levels in this population-sample. For this reason non-parametric tests were employed. Mann–Whitney tests were used to compare serum levels of IGF-I and OCs between gender and age-groups.

Multivariate models (ANCOVA) were used, adjusting for a number of well-known confounding variables such as age, height, weight and IGFBP-3 [4,23,32], which were introduced in the model as continuous variables. Because of the large number of zeros (serum samples which present OC–DDTs below the LOD), the OC group variables were classified as non-detectable samples (ND-samples) or as detectable samples (D-samples), and introduced in the multivariate model as a categorical factor. However, given the large number of samples that showed Total DDT above the LOD, this variable was categorized in gender- and age-specific tertiles (lowest Total DDT levels in 1st tertile, medium levels in 2nd tertile and the highest levels in 3rd tertile) and introduced in the model as a categorical factor [23].

The database management and statistical analysis were performed with SPSS v 17.0 (SPSS Inc., Chicago, IL, USA). A \( p \)-value of less than 0.05 (two tailed) was considered to be statistically significant.

3. Results

As shown in Table 1, with respect to IGF-I and IGFBP-3 there were no statistically significant differences between genders neither in the youngest group (male children between 6 and 15 years and female children between 6 and 13 years) nor in the groups of boys and girls (15–19, and 13–19 years, respectively).

Regarding OC–DDTs only one OC–DDT showed statistically significant differences between the age- and sex-groups analyzed. Thus, serum level of the main DDT-metabolite, \( p,p' \)-DDE, was higher in boys than in male children (\( p = 0.028 \)) (Table 2).

In addition, multivariate analysis showed that the presence of detectable values of \( p,p' \)-DDE and \( p,p' \)-DDD in the group of pre-pubertal male children between 6 and 15 years old was inversely associated to IGF-I levels (\( p = 0.023 \) and \( p = 0.049 \), respectively) (Table 3).

Although, DDT-metabolites, \( p,p' \)-DDE and \( p,p' \)-DDD, seemed to exert a negative effect on IGF-I levels in boys, the effect exerted by the combination of the three \( p,p' \)-DDT-derivatives (Total DDT) seemed to be very different. In the multivariate analysis, the relationship between IGF-I and Total DDT in pre-pubertal male children (6–15 years) showed a non-linear dose–response curve: IGF-I levels decreased from the 1st to the 2nd tertile of Total DDT but then an increase was observed in the 3rd Total DDT tertile (\( p = 0.043 \)) (Table 4).

On the contrary, OC–DDTs exposure did not show any relationship with IGF-I or IGFBP-3 levels in boys (15–19) (Table 3) nor in females (both, pre-pubertal children or girls) (Table 4).

4. Discussion

To our knowledge, this is the first study to date assessing the possible association of IGF-I with OCs' exposure in children and adolescents. Considering that the principal confounding factors were excluded and that the investigated population was stratified by gender and age, our data suggest the possibility that OC–DDTs could have an influence on IGF-I serum levels in young people. It is well known that there is a close relationship between exogenous factors, such as diet, and IGF-I. Thus, many studies seem to indicate that IGF-I may mediate the association between protein intake and growth in children, and evidence suggest that protein restriction results in low IGF-I concentrations in healthy children.
[32]. Therefore, dietary restriction decreases the serum concentration of IGF in both human and animals [35]. In addition, the presence of environmental contaminants in food is a well-known circumstance all over the world, and this circumstance should be borne in mind in any study regarding exogenous and dietary factors related to the IGF system. It has been suggested by others the possibility that exposure to environmental pollutants, such as organochlorine compounds, could be an exogenous factor capable of modulating the IGF system [22,23,36,37]. Interestingly, in vivo and in vitro studies have demonstrated that the most ubiquitous DDT-derivative, i.e. p,p'-DDE, could alter the expression of IGF-I [38].

In this work, we analyzed a young population group (children and adolescents), so we have to take into account the physiological pattern of GH–IGF axis during this developmental stage. IGF-I serum levels are low in the newborn, then rise during childhood, reaching peak levels during puberty, firstly in females, and decline with age thereafter [6,18,33,34]. This pattern is attributed to the age-related decline in growth hormone secretion [33]. The complex interaction between sex steroids and GH–IGF axis is little known [39]. Nevertheless, physiologically, there is a positive association between androgens and IGF-I, and the contrary for estrogens [18]. Since the IGF-system shows these characteristics, our analytical approaches included stratification by gender and age.

Our findings point to the possibility that some OCs could modulate negatively the IGF-system: p,p'-DDE and p,p'-DDD seems to exert a negative effect on IGF-I levels in boys. This negative association agrees with recent studies that suggest that prenatal exposure to DDT-metabolites may induce decreased height and weight in children [29]. Such negative modulation of the GH/IGF system could be related with the estrogenic and anti-androgenic actions (endocrine-disrupting properties) described for DDT-metabolites [24,26,40]. Taking into account that androgens may modulate positively the IGF-system [33], while estrogens may modulate it negatively [41,42], and that the negative association between IGF-I and p,p'-DDE and p,p'-DDD, was only observed in the group of pre-pubertal male children, our findings might well suggest an anti-androgenic effect of these DDT-metabolites that could lead to a significant decrease in IGF-I activity in boys [43].

Due to the fact that it has been suggested that IGF-I peak levels at puberty could determine IGF-I levels in adulthood, this effect could be, therefore, a determinant factor in the modulation of GH–IGF axis with potential consequences in chronic IGF-related disorders later in life [2].

In any case, it is relevant that this effect was evident for p,p'-DDE, the most frequently detected DDT-metabolite. For its ubiquity DDE is the DDT-derivative that has been more studied until now. In fact, most studies have focused on the biological effects exerted by p,p'-DDE [24,25,27,44]. On the contrary, studies focusing on the biological effects exerted by p,p'-DDD are scarce. Perhaps more attention should be aimed at evaluating the biological effects exerted by other emerging environmental pollutants, given that the limited knowledge about the biological effects exerted by most OC–DDTs may hamper the interpretation of our findings.

Nevertheless, as stated previously by others, the mixture of environmental contaminants with agonistic/antagonistic effects (estrogenic, antiestrogenic and/or anti-androgenic) is the most frequent circumstance found in human biological samples [23,30,45]. Thus, the real problem is the biological effect exerted by the simultaneous exposure to many environmental chemicals, since the combination of pollutants in human tissues seems to have a very different biological action to that of chemicals taken individually [45,46]. In this sense our findings showed that, in male children, the combination of the p,p'-isomers of DDT-derivatives (Total DDT) seems to modulate IGF-I serum levels following a non-linear dose–response curve. The term “non-linear” is used to describe dose–response relationships in which the direction of a response changes with increasing or decreasing dose. This non-linear dose–response curve has been described previously in human beings and in animal models for a mixture of organochlorines [23,45,46]. The existence of this type of dose–response curve could indicate that low-dose exposure could be as harmful as high doses for human beings. Nevertheless, investigating the possible biological effects of mixtures is a complex issue because the mechanisms of action for individual compounds are often poorly understood, and some chemicals may act through different routes depending on their concentration.

Although other authors have proposed the possibility that exogenous factors, such as OC–DDTs, may exert modulatory effects on the GH–IGF axis [18], the possible role played by such endocrine-disrupting properties of OC–DDTs on the IGF-system is uncertain and deserves more research. Since the liver is the main source of IGF-I, the possibility exists that the relationships found between OC–DDTs and IGF-I could be an indirect effect of these environmental pollutants on liver tissue [18]. Nevertheless, other unknown hepatic or non-hepatic mechanisms may be involved (such as genetic polymorphisms related to xenobiotic–metabolizing enzymes). In any case, the gender- and age-related differences observed may be linked to differences in interaction between sex steroid hormones and the GH/IGF system during infancy, pubertal transition, and adolescence [39].

There are some limitations to this study. In addition to being a cross-sectional study with associations observed at only one point in time, there were an important number of samples that did not show measurable values of some contaminants. Furthermore, we studied a sample-population of children and adolescents. The large variation in circulating IGF-I levels in adolescence makes it difficult to use IGF-I in the evaluation of the potential modulation of the GH/IGF system by exogenous factors [34]. However, this study is sufficiently large, and a multivariate model (adjustment by potential covariates of serum IGF-I) has been allowed, to permit examination of the IGF–DDTs associations separately by sex and age in youngsters. This is of value since some studies suggest that environmental contaminants could modulate the GH–IGF axis [18,22,23] and because this type of population-based studies are difficult to be allowed.

In summary, we have found several significant associations between serum levels of OC–DDTs and the IGF system in young people. Our results are consistent with previous observations in adults and with the relationship between OC–DDTs exposure and impaired growth in children. Although most OC–DDTs are hormonally active agents, a plausible mechanism for their effects on the IGF system is not readily apparent. As environmental-induced variations in IGF concentrations in childhood and adolescence might have important implications for IGF-I levels in adulthood, the possibility that environmental contaminants could modulate IGF system implies that these contaminants could influence the long-term risk of several chronic diseases related to IGF.

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