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Morphological and stereological effects of the use of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin in the submandibular gland of spontaneously diabetic animals

Victor Augusto Ramos Fernandes[1,2,3]; Camila Mantoani[1]; Raphael Oliveira Franco Netto[1]; Marcelo Rodrigues da Cunha[1,2,3]; Eduardo José Caldeira[1,2]

[1]Researcher of the Laboratory of Tissue Morphology, Department of Morphology and Basic Pathology, Jundiaí Medical School, Brazil.

[2]Professor of Anatomy, Jundiaí Medical School, Associate Professor in Human Anatomy, Brazil.

[3]Professor of Anatomy, Nossa Senhora do Patrocínio University Center – Cruzeiro do Sul University, Itu, São Paulo, Brazil.

***Corresponding Author: Victor Augusto Ramos Fernandes**

Researcher of the Laboratory of Tissue Morphology, Department of Morphology and Basic Pathology, Jundiaí Medical School, Brazil.

Email: victorfernandes@g.fmj.br

Abstract

The substance 2,3,7,8-tetraclodibenzo-p-dioxin, popularly known as TCDD, is widely found in food consumed by humans and in materials widely used by the species, such as sugar cane and papermaking. The ability of dioxin to attenuate the immune-mediated effects related to type 1 diabetes has been studied in an attempt to observe the potential therapeutic aspects of this substance. The present study aimed to verify the morphological and sterological effects of the use of TCDD in spontaneously diabetic animals, precisely in their submandibular gland. The results contrast in part with the available literature, since there was no delay in disease progression, but greater involvement of the gland parenchyma. It is suggested that exposure to TCDD may aggravate the condition of type 1 diabetes mellitus, given the harmful and potentially carcinogenic effects caused by this substance in the animals of this study.

Keywords: 2,3,7,8-tretachlodibenzo-p-dioxin; type 1 diabetes; autoimmune disease; morphometry; stereology; NOD mice.

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Introduction

The condition that favors the development of type 1 diabetes mellitus is still uncertain [1]. It is known that environmental, microbiological, genetic and epigenetic factors are the most likely, however, there are several cases in which there is no proximity of these characteristics and yet different individuals develop the disease. In this sense, some symptoms are frequently observed in patients with type 1 diabetes, loss of body weight with substantial decrease in lean mass, lower physical capacity and fitness for sports activities, lack of glucose metabolism and hyperglycemia that induces a powerful inflammation that generates severe tissue lesions, mainly in the attached glands of the digestive system [2].

The literature shows that pancreatic involvement by type 1 diabetes mellitus occurs initially with an increase of macrophages in the parenchymal tissue, precisely in the islets of Langerhans [3]. The reason for this increase is one of the main doubts that permeate the studies of this disease. However, since these mononuclear phagocytes are found in greater quantity in the pancreatic glandular epithelium, there is known expression of molecules such as interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), chemokine. CXCL8, interleukin 12 (IL-12) and interleukin 6 (IL-6). These small proteins with approximately 25kDa specifically promote increased vascular permeability, lymphocyte and neutrophil recruitment, T cell differentiation, and immune attack causing tissue death [4].

In this way, stroma progress can be verified in areas that were previously parenchymal glandular [4,5]. In fact, the affected individual starts to present deficits in the production of insulin and glucagon, without, however, affecting the synthesis of somatostatin, since the production of this last hormone occurs in other places of the body, besides the pancreatic D cells[6]. Insulin is directly involved in glucose metabolism, and this hormone is primarily responsible for the interaction with insulin receptors at the cellular level, triggering the cascade that culminates in translocation of glucose transporter 4 (GLUT4) to the cytoplasmic membrane and transport of the molecule, into the intracellular environment [7-9]. Thus, impairments in its production due to tissue death and loss of pancreatic B-cell functionality lead the individual to metabolic complications that can cause death if the symptoms of the disease are neglected [10]. An estimate of this aspect can be made by checking the ranking of the leading causes of human death in recent years. Diabetes mellitus is among the ten most common causes, with subtypes 1 and 2 being the predominant ones [12,15].

Several allopathic mediating and natural chemical treatments have been proposed to improve the diabetic condition [16]. Among these, Metformin is one of the most commonly prescribed pharmacists today, and this drug of the biguanide class, which induces increased glucose uptake by muscle cells, has a more suitable applicability in patients with type II diabetes, precisely in obesity and obesity. overweight [17]. On the other hand, few medications are really needed to treat the aggravating progress and tissue loss of type 1 diabetes mellitus, making the patient affected by this disease dependent on insulin and remarkable adaptations of their eating patterns and quality of life [18].

2,3,7,8-tetrachlorodibenzo-p-dioxin, popularly known as Seveso Dioxin or TCDD is a colorless, odorless substance known to act on the aryl hydrocarbon receptor pathway or AHR, which may be a factor. of transcription of several genes, including those involved in T lymphocyte differentiation and activation of these cells in immunomediated attacks [19,20]. Few studies have sought to understand the effects of using this substance on type 1 diabetic conditions, only research conducted by Kerkvliet et al [20]. In this groundbreaking study, the researchers used low doses of TCDD in Non Obese Diabetic (NOD) mice, which are considered the standard gold for the study of this disease in experimental models, and verified the morphological and gene expression effects of pancreatic cells. The results indicated a moderate decrease in disease progression while dioxin was being administered, as well as increased expression of the foxp3 + gene involved in T lymphocyte differentiation and activation. However, after the treatment period the animals similarly developed disease [20].

Although the efficacy of dioxin was doubtful in the study presented above, it is recognized that treatment has improved during its administration [20]. However, the study focused on the pancreas, due to its importance in the development of type 1 diabetes, but other glands that are also affected by the disease have not been investigated, so little is known about the effects of TCDD treatment in other regions.

The digestive system includes the attached glands and the alimentary canal [21,22]. The first mentioned structures are involved in the metabolism of macronutrients ingested and absorbed by the food channel [22]. The liver, pancreas, biliary tract and salivary glands constitute the attached glands [22]. The pancreatic involvement of type 1 diabetes is already well consoled, as well as the effects of this disease on liver prospects [23]. However, there are many gaps regarding the understanding of this disease in the salivary glands and how the gland tissue behaves through differentiated treatments, eg TCDD.

The salivary glands can be characterized as larger or smaller [22]. The former are the most important and involved in saliva production, being the parotid, submandibular and sublingual. The parotid gland has an acinous glandular morphology, whose secretory portion is exclusively serous, filled with saliva secretory ducts and amylase enzyme producing cells, responsible for cleaving starch and glycogen at the oral level through the contact of food with saliva. The submandibular gland, in turn, also produces the enzyme amylase, however it is the main producer of saliva in the human body, having as morphological characteristic a mucous tissue. The sublingual gland, however, has mixed mucous and serous gland tissue [22].

In this sense, the tissue behavior of salivary glands in relation to the involvement of type 1 diabetes is differentiated from pancreatic behavior, consisting of mechanisms of specific immunological and morphological responses. Therefore, the use of drugs and treatments in salivary gland tissues will also present different responses, therefore, the investigation of these aspects is necessary.

Objective

To verify the morphological and stereological effects of the use of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the submandibular gland of spontaneously diabetic animals.

Material and methods

The present study was approved by the ethics committee (opinion number 4426) and was carried out from the following experimental design. Female NOD (Non Obese Diabetic) animals and BalbC / Unib mice, aged 16 weeks, were selected for

the present research. The 45 total animals were organized into three groups, 15 control animals, without the development of type 1 diabetes, represented exclusively by BalbC / Unib mice, 15 NOD animals that developed type 1 diabetes, representing the sick group and affected by inflammation from of diabetes, a third group, called experimental, consisting of 15 NOD animals undergoing treatment with TCDD at dosages 100 l / 10gr in oily vehicle for 5 weeks and a fourth group with 15 NOD animals receiving oily vehicle treatment only.

After the experimental period, 32 weeks, the animals were euthanized, with doses of thiopental 10 mg/body weight and cervical dislocation, and sequentially the submandibular glands were extracted and embedded in paraffin, following a 70% alcohol wash and then dehydration in a growing series of alcohols (80% alcohol - 2 times, absolute alcohol - 3 times; 1 to 2 hours each). Subsequently, the fragments were diaphanized in xylol for 1 to 2 hours until they became translucent. The fragments were then embedded in paraffin and plastic polymers (Paraplast Plus, Polysciences, Niles, IL) at 56°C for approximately 1 hour; then passed to new paraffin at the same temperature. The tissues were carefully oriented and positioned at the bottom of plastic vats, aiming to obtain transverse histological sections. The blocks were trimmed to obtain flat surfaces and were sectioned with five micrometers thick. The fragments were then placed on albuminized slides and brought to the oven at 60°C. After preparation of the sections, they were stained with hematoxylin / eosin (HE) for general morphological study.

After obtaining the histological slides, they were photographed and will be analyzed, prioritizing the 20x, 40x and 100x objectives on the NIKON Eclipse E100 microscope (equipped with SONY DSC-W120 Imaging System (SONY, Tokyo, Japan) in the Department of Morphology and Basic Pathology of the Jundiaí School of Medicine All sections were used to quantify cytoplasmic and nuclear volumes of acinar cells of the submandibular glands, and where the core diameters were measured in each of the obtained samples. 150 nuclei from each animal, totaling 750 nuclei in each group studied.The choice of nuclei was random, prioritizing the defined cellular limits.Eliptic or spherical nuclei with defined limits and flat cuts were chosen. 10X eyepiece graduated with ruler and attached to light microscope NIKON Eclipse E10 0, fixing the observations with 100X objective. For the measurements of these structures, the eyepiece was previously calibrated with a special slide, with divisions of 0.01mm (µm), aiming to transform the ocular units into micrometers. From these values, the mean volume of nuclei was calculated using: $V = 4/3\ \pi\ r^3$. For spherical nuclei, where "r" is the radius of the nucleus and $V = 4/3\ \pi\ (d / 2)^2\ D / 2$. For elliptical cores, where "d" is the smallest diameter and "D" is the largest diameter. In addition, the volume fractions (Vv) occupied by the nucleus and cytoplasm of acinar cells of the salivary glands were measured for quantitative determination. These measurements were performed using a 10X = eyepiece containing a 100-point quadrilateral integration reticulum attached to the NIKON Eclipse E100 light microscope and a 100X objective. We counted the points located on the nucleus and cytoplasm of four previously defined fields. The volume fraction occupied by the nucleus relative to the cytoplasm was calculated using the following formula: Vv = p / P. Where Vv equals volume density or volume fraction (%), where P equals number of points on nucleus, and P equals total number of points or sum of points on nucleus and cytoplasm in different fields Cytoplasmic volume was calculated from the relationship between cytoplasm Vv, nucleus and nucleus Vv. The volume determination was made from the sum of the values obtained for the nuclear and cytoplasmic volumes, called cell volume. Statistical analysis was performed for the following variables: nuclear volume (μm^3) and cytoplasmic volume (μm^3) of the acinar cells of the submandibular glands. To obtain the mean profiles between the groups, we used the Kruskal-Wallis test involving the pairs of each group, representing the non-parametric test. The entire study was conducted with at least 5% significance.

Results

Initially, the results regarding the stereological analysis of the submandibular gland tissue of the animals of the present study will be presented. Sequentially, the results of the areas and length of the gland ducts, as well as the morphometric modifications of the vessels will be presented.

In a stereological analysis, it was typically observed the presence of inflammatory infiltrate in the glandular tissue of group two animals, NOD, as well as pleomorphic nuclei with cytoplasms in atypical morphology. Also, in control animals, BalbC / Unib, there were no apparent and substantial morphological changes. The group treated with TCDD and an oily vehicle for dioxin cell absorption also had pleomorphic nuclei, as well as a large area filled with inflammatory infiltrate and cytoplasmic morphology compromise. Group four, treated only with the oily vehicle, obtained similar stereological results to group two, however with a large area of inflammatory infiltration when compared to more groups in the experiment (Figure 1).

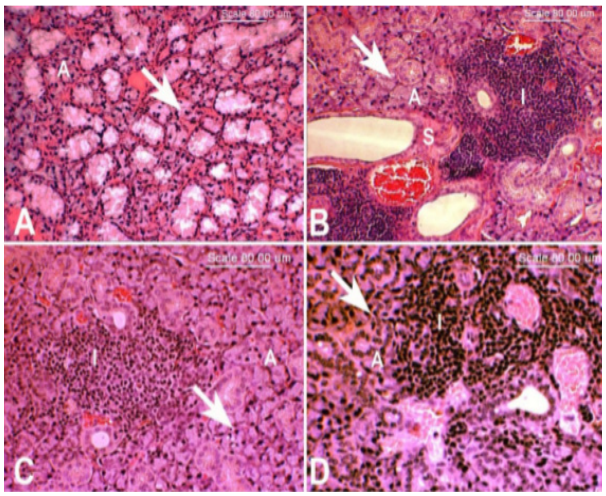

Figure 1: Photomicrograph of histological sections of the submandibular gland of BalbC and NOD animals under different conditions, according to the experimental protocol. A: Normal seromucous acines (A) and normal nuclei (arrow) .B: Accino (A) and pleomorphic nuclei (arrow), enlarged stroma (S), Inflammatory infiltrate reduction (I) C: Accino (A) and pleomorphic nuclei (arrow), Inflammatory infiltrate (I). D: Acino (A) and pleomorphic nuclei (arrow), Inflammatory infiltrate (I).

In this sense, when comparing the use of TCDD and the vehicle, only with the use of the vehicle, results are observed that do not differ in a stereological perspective. Also, when comparing the untreated NOD and the TCDD treatment groups, the effects of dioxin can be verified as shown in Figure 2.

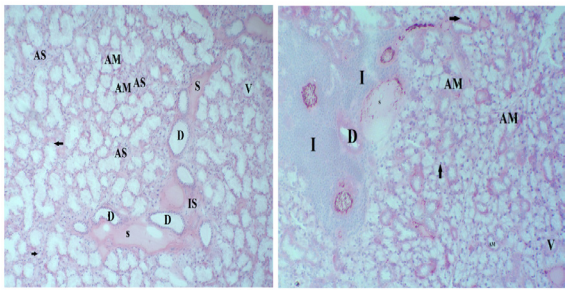

Figure 2 : Comparison of stereological analysis of submandibular gland tissue of TCDD-treated animals versus untreated NOD animals. The abbreviations AM indicate mucosal acini, AS serous acini, D indicate ducts, S shows the advancement of tissue stroma, IS the relation of inflammatory infiltration in the stromal tissue and V the local vessels. Letter I shows the inflammatory infiltrate in isolation. The arrows indicate the nuclei, which in both Figures are pleomorphic. The right Figure shows group three (NOD + TCDD), while the left Figure indicates group two (NOD).

The following graphs express in morphometric perspectives the comparisons between area and length of ducts and vessels present in the submandibular gland of the animals of this experiment (Figure 3).

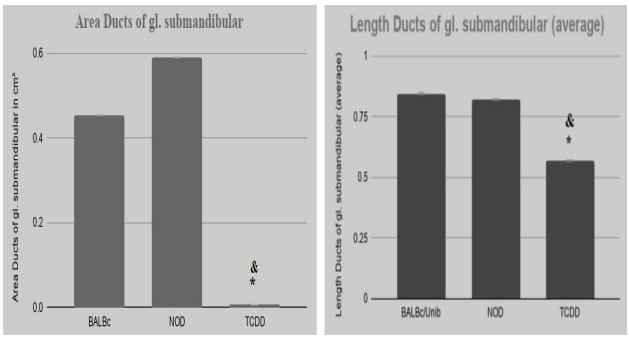

Figure 3 : Comparison of morphometric analyzes of submandibular gland tissue in relation to the mean area and length of the ducts. The presence of the & sign above the column indicates a statistical difference in relation to the NOD (diabetic) group. . The presence of * indicates statistical difference in relation to the control group (one-way test with $p < 0.05$).

Invasion of mononuclear phagocytic cells during the inflammatory infiltration characteristic of type 1 diabetes is known to occur through vessels that penetrate the affected glands. In this sense, understanding the relationships of endothelial tissue, as well as the morphometry of local vessels, was also one of the objectives of this work.

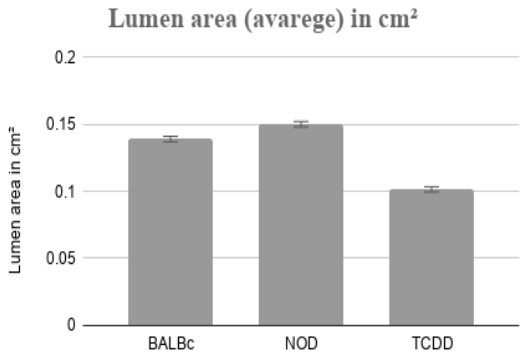

Figure 3 : Comparative graph between the areas of the vessels that vascularize the submandibular gland of the three groups. The results showed no statistical differences (one way anova test with $p < 0.05$).

Discussion

The event that provided remarkable public recognition of TCDD's potential took place in the city of Seveso, Italy [20,21] . Following the disruption of one of the boilers at the chemical company that worked with large quantities of this dioxin, directly affecting rivers and local soil, killing more than 12,000 fish indirectly, and indirectly more than 100,000 animals, preventing dioxinda from entering the plant. human food chain in the region. This precaution has been taken to avoid the potential effects of the substance [21].

The effects of prolonged dioxin exposure are still quite uncertain [20]. However, one of the most attributed symptoms is the appearance of chloracne on the face of the exposed individual, as well as a greater expression of genes involved in lipid metabolism, proto-oncogenesis and tumor suppressors. These factors denote direct TCDD relationships and an ability to alter the expression of nucleic acids, leading to cellular and tissue morphometric modifications, which could ultimately be related to the appearance of tumors or autoimmune diseases [21].

In this sense, some studies have been developed to observe the effects of exposure at different dosages of dioxin TCDD and the effects this could have on human and animal health [23-27]. It has been observed that low dosages of TCDD do not interfere with human health; moreover, people are usually exposed to such low dosages, as dioxin is found in several commonly used products such as fish meat, chicken, beef and pork, in addition to higher doses in sugar cane and during and paper production. However, very high doses of dioxin are potentially carcinogenic, a fact that has been the subject of several studies. TCDD dioxin is lipophilic and is therefore difficult to absorb in the environment. Its accumulation that leads to greater exposure and, consequently, increase of the harms that is speculated to this substance [28].

The present study aimed to observe the morphological and stereological effects of dioxin TCDD in animals sick with type 1 diabetes mellitus, seeking to establish relationships between the presence of the substance and the glandular tissue changes of the digestive system, submandibular gland. This relationship occurred after the verification of studies and hypotheses that emerged before the effects of TCDD in the AHR pathway, acting in a suppressive way, attenuating the cytotoxic effects of CD4 and T CD8 lymphocytes present in the pancreas of NOD animals [29].

According to the results presented in Figure 1, in which the stereological comparison between the different experimental groups is made, the relationship between the progress of a tissue inflammation present in spontaneously diabetic animals and its absence in healthy control animals has been elucidated. Regarding the presence of dioxin diluted in oily medium for cell absorption, there are no substantial differences between tissue inflammation, diabetic animals and diabetic animals treated with TCDD, however, there has been a reduction in the extent of local inflammation. These results cannot confirm the hypothesis that dioxin attenuates the harmful inflammatory effects of type 1 diabetes, but offers greater support for this possibility.

In Figure two, which uniquely compares groups two (NOD) and three (NOD + TCDD), the presence of invasive stroma in the gland parenchyma was easily identifiable, thus altering the cellular activity and production of aqueous material, saliva, in both. the situations. It can be seen from the results discussed so

far that dioxin did not significantly impact the development of the disease in the gland tissue studied in this research. This fact corroborates in part with the findings observed by Kerkuliet et al [20]. The concordant aspects are a decrease in the progress of the disease.

However, unlike Kerkuliet and colleagues' observations, in which they looked in detail at the molecular effects of dioxin at the pancreatic level, this study looked at the morphometric and stereological effects on the submandibular gland [20]. Thus, it was found that the mucous and serous acini of the group that received TCDD dioxin administration for five days had a pleomorphic cell morphology, both the nuclei and the cytoplasms of the cells. This aspect was also observed in the cells of type 1 diabetic animals. Regarding the area of the vessels of the submandibular gland, no statistically significant results were observed between the groups. However, it is speculated that there are clinical effects from the use of dioxin TCDD, considering that These substances have hypertensive effects already documented in the literature [29].

The ducts of animals exposed to the dosages of 100 µl/10 gr dioxin TCDD were substantially reduced, even compared to NOD animals. The same effect was observed in the serous and mucous acini areas of the submandibular gland. This result may be related to the ability of dioxin to alter cellular metabolism, a fact that would cause this reduction in cell volume, which at tissue level, compromises the acini area as well as its length. In this sense, the use of dioxin TCDD led to worsening of the diabetic condition of the animals, given that, in addition to the advancement of mononuclear phagocytic cells, there was also a substantial reduction in the parenchyma still active and producing saliva and its constituents [30].

Nuclear pleomorphism is associated with a modification of gene expression with recognized carcinogenic potential [31]. This corroborates the information about Seveso dioxin and its implications for cellular health. Stroma advancement, in addition to the presence of large mononuclear phagocytic cells in the gland tissue, indicates that TCDD dioxin has low disease progression delay capacity, as well as an already recognized carcinogenic potential. Therefore, its use could cause more tissue damage than beneficial effects, so further studies in this segment are needed to understand the real implications of long-term exposure to this dioxin.

Conclusion

The substance popularly known as TCDD, or Seveso's Dioxin, had harmful morphometric effects on the submandibular gland, and did not stereologically modify the condition caused by type 1 diabetes. Its administration was not efficient, as regards the dosages used in this study, in the fight against the advance of autoimmune diabetes, as well as compromised the parenchymal tissue of the submandibular gland of diseased animals by altering the area of mucous and serous acini.

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