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Impact of Glyphosate on the Development of Type-2 Diabetes in Adipose Tissue: Role of Insulin-Like Growth Factor 1 and Tumor Necrosis Factor Alpha Expression

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Abstract

Glyphosate, a commonly used herbicide in agricultural and residential settings, has sparked concerns regarding its potential health impacts. Despite numerous studies exploring potential associations between glyphosate exposure and diabetes, the precise mechanisms remain unclear. Tumor Necrosis Factor-alpha (TNF-α) and Insulin-Like Growth Factor 1 (IGF-1) are implicated in insulin resistance and pancreatic beta cell dysfunction, playing crucial roles in diabetes pathogenesis, especially Type 2 diabetes. This study aimed to assess the effect of glyphosate on TNF-α and IGF-1 expression in male Wistar rats. The results revealed a dose-dependent increase ($p<0.05$) in TNF-α and IGF-1 expression in adipose tissue following glyphosate exposure compared to the control group. These findings suggest that glyphosate exposure may contribute to the development of diabetes by altering the expression of IGF-1 and TNF-α.

Keywords: *Herbicide, IGF-1, Innovative Technology, Novel Method, Type-2 Diabetes, TNF-α.*

Introduction

Glyphosate, an organophosphate compound widely used as an herbicide, exerts its action by inhibiting crucial plant enzymes involved in metabolic processes. Acute exposure studies on male Wistar rats have revealed intriguing molecular effects, including increased aromatase mRNA and protein expression, alongside a decrease in normal sperm count attributed to reduced levels of histone-1 and protamine-1 [1]. Roundup, a glyphosate-based herbicide, has also been investigated for its impact on testicular cells, showing potential necrotic damage to Leydig cells following a 48-hour exposure period [2].

Moreover, high exposure to glyphosate-based herbicides like Roundup has been linked to Leydig cell necrosis in vivo [3]. Shifting focus to diabetes, characterized by impaired regulation of blood glucose levels leading to hyperglycemia, insulin deficiency plays a pivotal role, manifesting in symptoms such as polyuria, polydipsia, weight loss, and blurred vision [4]. Autoimmune destruction of pancreatic beta cells, responsible for insulin production, underlies this deficiency, resulting in hyperglycemia and subsequent diabetes-related complications [5].

Insulin-like Growth Factor 1 (IGF-1), primarily involved in regulating bone formation, is predominantly synthesized by osteoblasts, the body's bone-forming cells. Notably, IGF-1 also influences systemic hormone effects on bone formation and has been implicated in increasing osteoclast

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formation from osteoclast precursors in mice. It is a crucial growth factor stored in the bone matrix, aiding in bone resorption and subsequent coupling of bone formation processes [6].

Tumor Necrosis Factor-alpha (TNF-α), produced by macrophages, plays a pivotal role in tumor cell destruction [7]. However, the precise impact of glyphosate on inflammatory markers remains elusive, despite previous research indicating its association with increased anxiety levels and cognitive deficits in diabetic rats [8-27]. Leveraging our team's expertise and extensive research experience, we aim to fill this gap by analyzing the effect of glyphosate exposure on the expression of IGF-1 and TNF-α in the liver.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents utilized in this study were procured from reputable sources.

Animal Ethics and Maintenance

The experimental procedures involving animals were ethically approved and maintained as per the standard procedure.

Experimental Design

Adult male Wistar albino rats were divided into four groups, each comprising six animals. Group I served as the normal control and was fed a standard diet with access to drinking water. Groups II, III, and IV were orally administered glyphosate dissolved in water at doses of 50 mg/kg, 100 mg/kg, and 250 mg/kg body weight/day, respectively, for 16 weeks. Then, adipose tissue was immediately collected and used for further analysis.

Isolation of Total RNA

Total RNA was isolated from control and experimental samples using the TRIR kit. Briefly, fresh tissue (100 mg) was homogenized with 1 ml TRIR, followed by centrifugation. The resulting supernatant was processed with chloroform and isopropanol to isolate RNA pellets, which were then dissolved in autoclaved Milli-Q water.

Quantification of RNA

RNA samples were quantified spectrophotometrically by measuring absorbance at 260/280 nm. The concentration of RNA was calculated based on absorbance values, with a ratio of absorbance at 260/280 nm > 1.8 indicating good quality RNA.

Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed using the Eurogentec RT kit. First-strand cDNA synthesis was carried out using oligo dT, dNTPs, and reverse transcriptase, followed by PCR amplification using SYBR green master mix in a real-time PCR system. Primer sequences for target genes (IGF-1 and TNF-α) and β-actin as the invariant control were used [28-29]. Primers: Details of primers used in the present study. IGF-1- FW- 5”-CTGAGCTGGTGGATGCTCT- 3”; RW- 5”-CACTCATCCACAATGCCTGT - 3”: TNF-α- FW – 5” –CAG CGG CCG CAA CAC ATC TCC CTC CGG AAA GGA C – 3” RW - 5” – GAC CGC ACA AGT AGG CAA GAG ATG GCG CCG GCG – 3”: β-actin- FW – 5’-TACAGCTTCACCACCACAGC - 3’; RW– 5’- TCTCCAGGGAGGAAGAGGAT - 3’.

Statistical Analysis

Triplicate analyses of control and treated rat experiments were expressed as mean ± standard deviation. Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test. Results with $p<0.05$ were considered statistically significant. Graph Pad Prism version 5 was used for analysis.

Results

Impact of Glyphosate on IGF-1 and TNF-α mRNA Expression in Adipose Tissue

The mRNA expression levels of IGF-1 and TNF-α were evaluated via Real-Time PCR to understand the effects of glyphosate exposure. A significant dose-dependent increase ($p<0.05$) in both IGF-1 and TNF-α expression was observed in glyphosate-treated rats compared to the control group, which displayed no alterations in gene expression. Specifically, as glyphosate dosage increased (50 mg, 100 mg, and 250 mg), TNF-α expression escalated, triggering an inflammatory response. Figure 1 illustrates the impact of glyphosate on IGF-1 mRNA expression, with a clear elevation observed with increasing glyphosate exposure. In brief, IGF-1 mRNA expression was a 0.4-fold increase in 50 mg glyphosate-treated rats ($p<0.05$) compared with control while 0.5-fold and 0.7-fold changes were observed in 100 mg, and in 250 mg ($p<0.05$) glyphosate treated rats respectively (Figure 1). Similarly, the impact of glyphosate on TNF-α mRNA expression (Figure 2) revealed a dose-dependent increase in expression levels, indicating heightened inflammatory response with greater glyphosate exposure. In brief, compared to control, TNF-α mRNA expression was 0.4-fold increase in 50mg glyphosate exposed rats ($p<0.05$) whereas in 100 and 250 mg glyphosate exposed rats showed a 0.7- and 0.8-fold increase in the mRNA compared to control and 50mg treated rats. This study clearly indicates that glyphosate exposure has diabetogenic potential via the modulation of IGF-1 and TNF-α signaling in the adipose tissue.

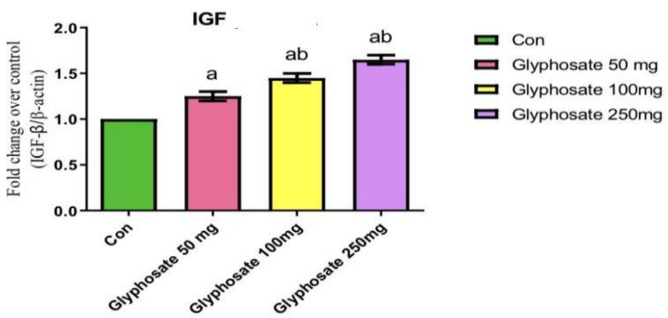

Figure 1. Impact of Glyphosate on mRNA Expression on IGF- 1 In Adipose Tissue of Adult Male Wistar Rats

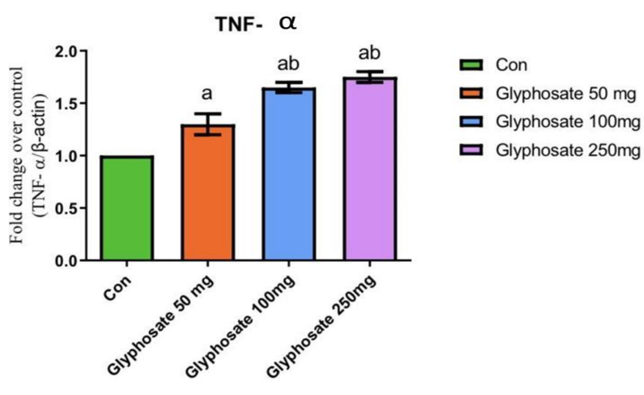

Figure 2. Impact of Glyphosate on mRNA Expression on TNF-alpha. The X-Axis Represents a Controlled Group of Rats in Comparison to Rats Who Are Exposed to Subsequent Doses of Glyphosate

Discussion

This study uncovered significant alterations in IGF-1 and TNF-α gene expression following glyphosate exposure, leading to an inflammatory response associated with potential disorders such as arteriosclerosis in rats [30]. IGF-1, a polypeptide with 70 amino acids, is widely distributed in mammalian tissues. On the other hand, TNF-α, encoded by the human TNF-α gene located on chromosome 6p21.1–21.3, is a cytokine secreted by immune cells implicated in tumour cell necrosis and autoimmune diseases. Dysregulation of TNF-α levels can contribute to various pathological conditions.

TNF-α plays diverse roles in cell survival, signalling, and proliferation, and its aberrant expression is linked to diseases like psoriasis, diabetes, and rheumatoid arthritis [31]. Glyphosate's toxic effects have been implicated in neurodegenerative diseases such as Alzheimer's by increasing TNF-α levels in the brain [7]. Moreover, TNF-α has been associated with ischemic injuries like stroke and can inhibit IGF phosphorylation, contributing to diabetes pathogenesis [32-36].

IGF-1, also known as Insulin-like Growth Factor, exhibited a significant increase upon glyphosate exposure, potentially contributing to diabetes development in rats [32-36]. This finding aligns with previous studies indicating IGF-1's role in bone formation and its therapeutic potential. Additionally, TNF-α expression increased with subsequent glyphosate doses, corroborating its involvement in various diseases and immune responses [37-40].

Further research is warranted to elucidate the mechanisms linking IGF-1 and TNF-α expression with glyphosate exposure and diabetes progression. [41-45]. Exploring downstream signalling pathways of proinflammatory mechanisms could provide insights into glyphosate's diabetogenic potential and aid in the development of novel therapeutic interventions.

Conclusion

In conclusion, glyphosate exposure exacerbates inflammation, potentially contributing to diabetes progression in adipose tissues by altering IGF-1 and TNF-α expression. Future investigations focusing on proinflammatory transcription factors' protein expression in response to glyphosate exposure are essential to delineate its mechanisms of action in diabetes development.

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Conflict of Interest

The author declares that there is no conflict of interest in the present study.

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