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The role of NF-κB signaling in impaired liver tissue repair in thioacetamide-treated type 1 diabetic rats ☆

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Abstract

Previously we reported that an ordinarily nonlethal dose of thioacetamide (300 mg/kg) causes liver failure and 90% mortality in type 1 diabetic rats, primarily because of inhibited tissue repair. On the other hand, the diabetic rats receiving 30 mg thioacetamide/kg exhibited equal initial liver injury and delayed tissue repair compared to nondiabetic rats receiving 300 mg thioacetamide/kg, resulting in a delay in recovery from that liver injury and survival. These data indicate that impaired tissue repair in diabetes is a dose-dependent function of diabetes. The objective of the present study was to test the hypothesis that disrupted nuclear factor-κB (NF-κB)-regulated cyclin D1 signaling may explain dose-dependent impaired tissue repair in the thioacetamide-treated diabetic rats. Administration of 300 mg thioacetamide/kg to nondiabetic rats led to sustained NF-κB-regulated cyclin D1 signaling, explaining prompt compensatory tissue repair and survival. For the first time, we report that NF-κB–DNA binding is dependent on the dose of thioacetamide in the liver tissue of the diabetic rats. Administration of 300 mg thioacetamide/kg to diabetic rats inhibited NF-κB-regulated cyclin D1 signaling, explaining inhibited tissue repair, liver failure and death, whereas remarkably higher NF-κB–DNA binding but transient down regulation of cyclin D1 expression explains delayed tissue repair in the diabetic rats receiving 30 mg thioacetamide/kg. These data suggest that dose-dependent NF-κB-regulated cyclin D1 signaling explains inhibited versus delayed tissue repair observed in the diabetic rats receiving 300 and 30 mg thioacetamide/kg, respectively.

Keywords: Diabetes; Liver; NF-κB; Thioacetamide; Tissue repair

1. Introduction

Type 1 as well as type 2 diabetes are known to potentiate the hepatotoxicity of numerous structurally and mechanistically diverse hepatotoxicants such as thioacetamide, $CHCl_3$, and CCl_4 (El-Hawari and Plaa, 1983; Hanasono et al., 1975; Sawant et al., 2004). A recent epidemiological study shows that diabetic patients are at higher risk of acute liver failure (El-Serag and Everhart, 2002). Previously, our laboratory reported that exposure of type 1 diabetic rats to an ordinarily nonlethal dose of thioacetamide (300 mg/kg) exhibited higher initial liver injury and 90% mortality, primarily due to inhibited tissue repair (Wang et al., 2000a). It could be argued that inhibited compensatory tissue repair observed in the diabetic rats receiving 300 mg thioacetamide/kg was simply due to CYP2E1-mediated higher initial liver injury (Wang et al., 2000b) and lower number of healthy hepatocytes available to divide. To investigate this possibility, 30 mg thioacetamide/kg was administered to diabetic rats, which exhibited initial liver injury equal to the nondiabetic rats receiving 300 mg thioacetamide/kg (Wang et al., 2000a) (Fig. 1A). In

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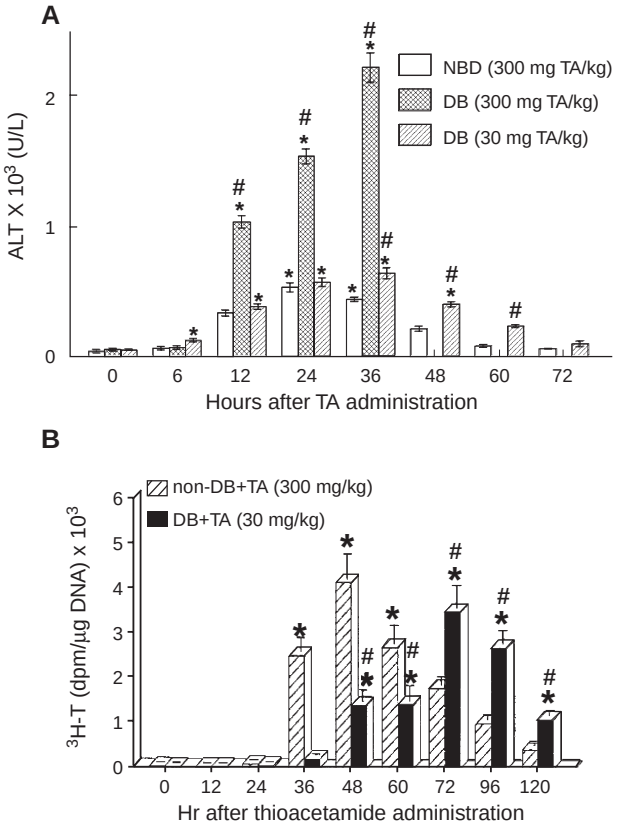

Fig. 1. On day 0, male Sprague–Dawley rats (250 to 300 g) received either a single dose of streptozotocin (60 mg/kg, 0.01 M citrate buffer as vehicle, i.p.) to induce diabetes or citrate buffer (0.01 M, pH 4.3, 1 ml/kg, i.p.) as the nondiabetic control. On day 9, plasma glucose level was measured and animals were considered diabetic if plasma glucose level was ≥250 mg/dl (Mean 390±112 mg/dl). On day 10, diabetic rats were divided into two groups. The first group of diabetic rats (n=4 for all time points except 36 h where n=8 was used to obtain n=4 of surviving animals) was treated with thioacetamide (300 mg/kg, i.p.), while the second group of diabetic rats (n=4) was treated with 30 mg thioacetamide/kg i.p. Citrate buffer receiving control group i.e. nondiabetic group (n=4) received 300 mg thioacetamide/kg. At 0, 6, 12, 24, and 36 h after thioacetamide administration, plasma and liver samples were collected and stored at −20 and −80 °C, respectively. A. Plasma alanine aminotransferase activity over the time course of 0 to 72 h. Diabetic rats receiving 300 mg thioacetamide/kg exhibited higher initial liver injury, which is progressive and irreversible. Even though a 10-fold lower dose of thioacetamide (30 mg thioacetamide/kg) causes equal bioactivation-mediated liver injury in diabetic rats as observed in nondiabetic rats receiving 10-fold higher dose, completely divergent outcomes are evident in the time course of injury and recovery. Liver injury is progressive in the diabetic rats whereas in the nondiabetic rats it decreases promptly indicating complete recovery. B. [^{3}H]-T incorporation from 0 to 120 h after thioacetamide administration. [^{3}H]-T (35 µCi i.p.) was given 2 h before animal sacrifice (Wang et al., 2000a). In the same study described in panel A, stimulation of S-phase was measured as hepatonuclear incorporation of [^{3}H]-T in pulse labeling experiments. Prompt stimulation of S-phase in nondiabetic rats peaking at 48 h is indicative of prompt and robust stimulation of tissue repair leading to prompt recovery from injury as evident in panel A. In contrast, stimulation of S-phase was considerably delayed in the diabetic rats even though they received a 10-fold lower dose of thioacetamide (30 mg/kg), leading to progression of liver injury (Panel A). Although delayed, adequate stimulation of tissue repair does occur, peaking at 72 h in the diabetic rats, permitting complete recovery (Panel B).

spite of equal initial bioactivation-mediated liver injury, diabetic rats receiving 30 mg thioacetamide/kg exhibited delayed S phase DNA synthesis as measured by [^{3}H]-thymidine incorporation (Fig. 1B), resulting in delayed recovery from liver injury and survival. These findings indicated that impairment of tissue repair is due to the diabetic condition and plays a determinant role in the final outcome of the injury, i.e. survival or delayed recovery from the injury (Wang et al., 2001; Wang et al., 2000b). Therefore, any understanding of molecular mechanisms responsible for impaired tissue repair response is of continued clinical interest.

Our previous studies with [^{3}H]-thymidine pulse labeling and proliferating cell nuclear antigen (PCNA) identified the inability of thioacetamide-treated diabetic rat hepatocytes to progress from G_0 to S phase of cell division cycle (Wang et al., 2000a). Numerous studies have confirmed the pivotal role of nuclear transcription factor kappa B (NF-κB) signaling in liver regeneration, especially in the early phase of the cell cycle (Baldwin, 1996; FitzGerald et al., 1995; Perkins, 2000). NF-κB is a crucial factor during liver regeneration after partial hepatectomy to trigger cell cycle progression of hepatocytes (Plumpe et al., 2000). NF-κB controls cell growth and differentiation through transcriptional regulation of cyclin D1 (Guttridge et al., 1999). Cyclin D1 promotes mitogen-independent cell cycle progression in hepatocytes (Albrecht and Hansen, 1999). We hypothesized that a dose-dependent perturbation of NF-κB-regulated cyclin D1 signaling in the thioacetamide-treated diabetic rats may offer an explanation for inhibited versus delayed tissue repair observed in the diabetic rats receiving 300 versus 30 mg thioacetamide/kg, respectively.

In a normal adult liver, NF-κB is retained in the cytoplasm by its endogenous inhibitor, IκBα. Cytokines like tumor necrosis factor-α (TNF-α) stimulate IκB kinase (IKK) to phosphorylate IκBα, releasing NF-κB to translocate into the nucleus (Brown et al., 1995; Rothwarf and Karin, 1999). Another signaling pathway, mitogen activated protein kinase (MAPKs), is also known to trigger NF-κB pathway. In the nucleus, NF-κB binds to cognate DNA sequences and activates transcription of the target genes including cell cycle regulatory cyclin D1. We report here that thioacetamide-treated nondiabetic group exhibited sustained NF-κB–DNA binding and cyclin D1 expression. In contrast, NF-κB-regulated cyclin D1 expression was inhibited in the diabetic rats receiving 300 mg thioacetamide/kg, explaining inhibited tissue repair that leads to progression of injury, liver failure and death in this group. On the other hand, in the diabetic rats receiving a 10-fold lower dose of thioacetamide (30 mg/kg) NF-κB–DNA binding was remarkably stimulated, indicating NF-κB–DNA binding exhibits thioacetamide dose-dependent phenomenon in the diabetic condition. Even though NF-κB–DNA binding was increased in the diabetic rats exposed to 30 mg thioacetamide/kg, delayed cyclin D1 expression explains delayed tissue repair and late recovery of the diabetic rats in this group.