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Nitrofurazone-induced gene expressions in rat hepatocytes and their modification by *N*-acetylcysteine

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Abstract

The antibiotic nitrofurazone (NF) at a subtoxic dose has been shown to increase hepatocyte DNA synthesis with no preceding cell damage or necrosis. This was suppressed by concomitant administration of the antioxidant *N*-acetylcysteine (NAC), which suggests that free radical production is involved in the process. In this study, male F344 rats were given a single oral subtoxic dose of NF to investigate the changes in genes implicated in hepatocyte proliferation between 1 and 20 h postdose by real-time PCR. Some rats were also given NAC to examine the involvement of free radicals. There were transient and sequential increases in mRNA levels of c-myc and c-jun shortly after the administration, followed by tumor necrosis factor-α (TNF-α), transforming growth factor-α (TGF-α), c-Ha-ras, and cyclin E. The increases were blocked by concomitant administration of NAC. In contrast, there were no NF-specific increases in c-fos, hepatocyte growth factor, epidermal growth factor or cyclin D1 mRNAs. These results indicate that the induction of hepatocyte proliferation by NF is triggered by free radicals, with a pathway involving increases in c-jun, c-myc, TNF-α, TGF-α, c-Ha-ras, and cyclin E. The results also indicate that NF-induced proliferation resembles that of other mitogens.

Keywords: Nitrofurazone; Rat; Hepatocyte; Gene expression; Proliferation; *N*-acetylcysteine; c-fos; c-myc; c-jun; c-Ha-ras; Tumor necrosis factor-α (TNF-α); Transforming growth factor-α (TGF-α); Hepatocyte growth factor (HGF); Epidermal growth factor (EGF); cyclin D1; cyclin E

Introduction

Nitrofurazone (5-nitro-2-furaldehyde semicarbazone; NF), a broad-spectrum antibiotic, has been known to elicit toxic effects, including convulsive seizures, testicular degeneration, and degenerative arthropathy in rodents (Hagenäs et al., 1978; Kari et al., 1989). It has, in addition, been reported to induce necrotic changes in the liver and adrenal gland at high doses (Ito et al., 2004). In contrast, NF at low doses elicits hepatocyte proliferation without the preceding loss of hepatocytes, i.e. it acts as a mitogen (Ito et al., 2002). Indeed, in mice fed NF for 13 weeks, liver to body weight ratios were moderately increased (Kari et al., 1989), apparently without histopathological changes. The mitogenic effect is abrogated by concomitant administration of antioxidants *N*-acetylcysteine (NAC) or cyanidanol, indicating the involvement of free radical production (Ito et al., 2003). It is noteworthy that NF behaves as a

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hepatotoxicant at higher doses and as a mitogen at lower, subtoxic doses. Such is also the case with agents which are generally better known for hepatotoxicity such as thioacetamide (Mangipudy et al., 1995a, b) and carbon tetrachloride (CCl_4, Rao et al., 1997).

Proliferation of hepatocytes occurs very infrequently in normal adult rodents, the vast majority of cells being in the resting phase of the cell cycle (G_0). Hepatocytes, however, are equipped with mechanisms which allow them to quickly modulate the rate should the need arise. Two different types of proliferation are currently known, compensatory proliferation and mitogen-induced proliferation (Ledda-Columbano et al., 1993; Columbano and Shinozuka, 1996). Compensatory proliferation follows the loss of hepatocytes after partial hepatectomy or ''chemical hepatectomy'' by hepatotoxicants such as CCl_4. Mitogen-induced proliferation, in contrast, entails no overt damage or loss of hepatocytes. ''Mitogens'' include a broad spectrum of chemically unrelated substances such as lead nitrate (Columbano et al., 1983), cyproterone acetate (CPA, Roberts et al., 1995), cyclosporine (Masuhara et al., 1993), ethylene dibromide (EDB, Nachtomi and Farber, 1978), and hypolipidemic drugs (e.g. Wy-14,643, Rusyn et al., 2000), as well as a large number of stimuli. These include free radicals, decreases in protective enzymes, glutathione depletion, and sustained accumulation of normally low endogenous products (Iatropoulos and Williams, 1996). Although the two modes of proliferation share some of the events following the trigger, there are also differences in the series of changes in growth factors and early response genes. Compensatory proliferation involves increases in hepatocyte growth factor (HGF), transforming growth factor-α (TGF-α), epidermal growth factor (EGF), tumor necrosis factor-α (TNF-α), c-fos, c-myc, c-jun, and c-Ha-ras (Fausto and Mead, 1989; Fausto et al., 1995; Fausto, 1996), whereas some of these are unaltered in mitogen-induced proliferation (Coni et al., 1990, 1993; Masuhara et al., 1993; Goldsworthy et al., 1994; Shinozuka et al., 1994).

Much attention has recently been devoted to the mechanism involving free radicals. Free radicals at high concentrations inflict damage on cells, but at low doses are believed to act as a mediator of various signaling pathways within a cell (Remacle et al., 1995). The antioxidant NAC is widely used as an experimental tool in biological and pathological processes. Its mode of action is two-fold, as a free radical scavenger by directly reacting with them, and, additionally, it elevates intracellular glutathione content by providing a precursor (Zafarullah et al., 2003), thereby enhancing the cellular defense system. It has been used to counteract the effects of free radicals, and the mitogenic effect of NF has been prevented by its concomitant administration (Ito et al., 2003), suggesting the involvement of free radicals in the process.

This study examines the changes in gene expressions generally associated with hepatocyte proliferation in order to further characterize the nature of NF's mitogenic effect. The effect of NAC, which blocks free radicals and NF-induced proliferation, was concurrently examined for the modification of NF-induced genes in an attempt to elucidate to what extent free radicals contribute to the sequence of events following the use of NF.

Materials and methods

Animals

SPF male Fischer rats were purchased from Charles River Japan Inc. (Kanagawa, Japan), and acclimated until use in a room which was kept at 23 ± 2 °C, with a relative humidity of 55 ± 10%, ventilated 15 times/h, and lighted for 13 h (from 8:00 a.m. to 9:00 p.m.). The animals were given a commercially available diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum. The rats were 10–11 weeks old at the start of treatment. All animals received humane care throughout the experiment in compliance with the institutional guidelines for the care and use of laboratory animals.

Nitrofurazone and *N*-acetylcysteine administration and sample collection

NF (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was suspended at a concentration of 16 mg/mL in a 0.5% methylcellulose solution. NAC (Wako Pure Chemical Industries, Ltd.) was dissolved in saline at a concentration of 20 mg/mL.

Rats were given a single dose of NF at 80 mg/kg (5 mL/kg) by gavage using a stomach tube. Five animals were sacrificed at 1, 2, 5, 8, 12, 16, and 20 h postdose, respectively (NF rats). In addition, five rats each were given an intraperitoneal injection of NAC at 50 mg/kg (2.5 mL/kg) 30 min before and, where applicable, 5 h after NF administration, and sacrificed as above (NAC + NF rats). Five rats given a 0.5% methylcellulose solution alone served as controls at each time point.

Rats were sacrificed by exsanguination under ether anesthesia. The liver was excised for total RNA extraction.

RNA extraction and reverse transcription

The liver was homogenized in approximately 1 mL of ISOGEN (Nippon Gene, Tokyo, Japan) per 100 mg of tissue, and total RNA was extracted according to the manufacture's instructions. Total RNA was quantified