MUTAGENICITY AND CARCINOGENICITY OF BIOLOGICAL REACTIVE INTERMEDIATE’S DERIVED FROM A “NON-GENOTOXIC” CARCINOGEN

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INTRODUCTION

Hydroquinone (HQ) is used as a developer in the photographic industry, as an antioxidant in the rubber industry, and as an intermediate in the manufacturing of food antioxidants. HQ is also an important metabolite of benzene (1,2). HQ has been identified in relatively high concentrations in the smoke of unfiltered cigarettes (up to 155 μg per cigarette) (3) and was chosen for study by the National Cancer Institute because it is produced in large quantities, humans are frequently exposed to it, and there is little adequate carcinogenicity data on it (4). Smoking unfiltered cigarettes and long-term cigarette smoking (~30 years) correlate with a high incidence of renal cell carcinoma in men (5-7). Although the basis for the increased incidence of renal tumors in cigarette smokers is not known, cigarette smoke contains high concentrations of oxidants and free radicals, the principal radical in the tar phase being the 1,4-benzoquinone/hydroquinone redox couple (8).

Although HQ is generally not mutagenic in short-term bacterial mutagenicity assays (9,10), and no mutagenic activity has been found in mouse cells in vivo (11), HQ causes base-pair changes in the TA1535 Salmonella test strain (12) and is mutagenic in oxidant-sensitive (TA104 and TA2637) Salmonella test strains (13), consistent with the mutagenicity of 1,4-benzoquinone in several Ames bacterial test strains (14). HQ is also clastogenic and induces sister chromatid exchange (4,12,15), catalyzes the in vitro formation of 8-oxo-deoxyguanosine (16,17) and causes single-strand DNA breaks in isolated hepatocytes (18). In addition, HQ causes renal tubular cell degeneration in the renal cortex of male Fischer 344 rats, and at high doses, markedly increases the number of tubular cell adenomas (4). Renal cell tumors also arise in animals exposed to HQ, but only at nephrotoxic doses and only in male rats (4,19) and mice (19). HQ also acts as a tumor promoter dependent on the target organ and on the initiation protocol used (20). Neither the mechanism of HQ-mediated nephrocarcinogenicity in male rats, nor the basis for the species and sex differences are known.

The acute nephrotoxicity of HQ is dependent upon the activity of γ-glutamyl transpeptidase (γ-GT), indicating that the toxicity of HQ is dependent upon the formation of metabolites that are substrates for this enzyme (21). Consistent with this view, glutathione (GSH) conjugates of HQ, in particular 2,3,5-tris-(glutathion-S-yl)HQ (TGHQ), are potent nephrotoxicants (21,22), and HQ is metabolized in vivo to GSH conjugates in amounts sufficient to support their role in the acute nephrotoxic effects of HQ (23).

Spontaneous renal Cell carcinoma (RCC) is rare in rats, occurring in most strains with a frequency of <0.05% (24). However, Eker rats (25) carry a single autosomal
mutation that predisposes them to the development of spontaneous renal cell tumors at a high incidence. A germline insertion of an endogenous retrovirus in the tuberous sclerosis 2 (Tsc-2) tumor suppressor gene is responsible for the predisposing Eker mutation (26,27). The Tsc-2 gene has been primarily associated with the development of RCC in rats (28-30), although humans with tuberous sclerosis are at increased risk for the development of benign and malignant renal tumors (28,31). In rats carrying the Eker mutation, preneoplastic lesions in the renal tubules begin to appear at about 2-3 months of age, and by the age of 1 yr, the incidence of renal cell tumors in gene carriers approaches 100%. Most renal cell tumors in Eker rats originate from the renal proximal tubules and are histologically similar to renal tumors in humans (28,32).

After exposure to known renal carcinogens, the number of renal tumors in susceptible Eker rats (i.e., those carrying a germline Tsc-2 mutation) is greatly increased (33,34), those carrying the mutation (Tsc-2EER+) being more than 70-fold more susceptible to the induction of RCC than their homozygous wild-type (Tsc-2+/+) littermates (33). Loss of the second allele of this gene as a somatic event leads to the development of RCC in these animals, supporting Knudson's two-hit hypothesis for the loss of tumor suppressor gene function in tumorigenesis (24). Thus, the Eker rat model system offers a unique opportunity to investigate mechanisms of chemical-induced renal carcinogenesis in kidney epithelial cells that are predisposed to tumor development. We therefore used Eker rats to test the hypothesis that a quantitatively minor metabolite of HQ plays an important, if not essential, role in "nongenotoxic" carcinogen-mediated carcinogenesis (34).

RESULTS AND DISCUSSION

Cell Proliferation Following TGHQ Treatment in the Eker Rat

Increased cell proliferation in response to TGHQ (2.5 μmol/kg for 4 mo. followed by 3.5 μmol/kg for additional 6 mo.; i.p.; 5 days/week) was evident after 4 months treatment and was predominantly located in a band-like region of the OSOM extending to the medullary rays (Figure 1A), as previously reported for acute HQ exposure (21). The labeling index was increased 19-fold in kidneys of Eker rats treated with TGHQ compared with saline-treated controls. Figure 1B shows characteristic BrdU immunostaining, which was localized to the regions of toxicity. Exfoliated necrotic cells were evident within dilated tubules lined by cuboidal epithelium, and many of the cells were in S phase and were strongly immunoreactive (Figure 1B arrowhead). In contrast, kidney sections from control animals showed a distinct absence of cells in S phase (Figure 1C).

The correlation appears clear between TGHQ-induced nephrotoxicity and nephrocarcinogenicity. Acute toxicity after both HQ and TGHQ exposure occurs in the outer stripe of the outer medulla (OSOM) of the kidney and progresses with time along the medullary rays (21). This region is the site of the vast majority of preneoplastic and neoplastic lesions observed in TGHQ-exposed kidneys. The site-selectivity of this toxicity may be a consequence of the susceptibility of this area to oxidative stress and of the high concentrations of γ-GT in the brush-border membrane of proximal tubular cells (35). Pretreatment of rats with acivicin to inhibit γ-GT completely prevents HQ-mediated nephrotoxicity (21), implying that metabolism of HQ to metabolites requiring processing by this enzyme is a prerequisite for toxicity (22). The site-selective toxicity is also reflected in the rapid excretion of γ-GT into urine, in the absence of gross kidney dysfunction or accumulation of blood urea nitrogen (21). Moreover, reactive electrophilic metabolites of TGHQ become covalently adducted to proteins in the same region of the kidney that ultimately give rise to tumors (36). Thus, the proteins necessary for both the transport and bioactivation of TGHQ (37) reside at the site of the initial tissue injury and cell proliferation.

Histological Changes Induced by TGHQ in the Eker Rat

TGHQ-treated rats (10 months treatment) developed numerous toxic tubular dysplasias (Figure 1D) of a form not observed in age-matched untreated Eker rats. Toxic tubules differ from the normal proliferative lesions of the Eker rat in that they exhibit a thick circumscribing peritubular fibrosis surrounding a dilated tubular profile. These preneoplastic lesions were found as early as 4 months of TGHQ treatment, and are believed to represent early transformation within tubules undergoing regeneration in response to