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Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: Evidence consistent with a glycidamide-mediated effect

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Abstract

Acrylamide, an animal carcinogen and germ cell mutagen present at low (ppm) levels in heated carbohydrate-containing foodstuffs, is oxidized by cytochrome P4502E1 (CYP2E1) to the epoxide glycidamide, which is believed to be responsible for the mutagenic and carcinogenic activity of acrylamide. We recently reported a comparison of the effects of acrylamide on the genetic integrity of germ cells of male wild-type and CYP2E1-null mice [B.I. Ghanayem, K.L. Witt, L. El-Hadri, U. Hoffler, G.E. Kissling, M.D. Shelby, J.B. Bishop, Comparison of germ-cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect, Biol. Reprod. 72 (2005) 157–163]. In those experiments, dose-related increases in dominant lethal mutations were detected in uterine contents of female mice mated to acrylamide-treated wild-type males but not CYP2E1-null males, clearly implicating CYP2E1-mediated formation of glycidamide in the induction of genetic damage in male germ cells. We hypothesized that acrylamide-induced somatic cell damage is also caused by glycidamide. Therefore, to examine this hypothesis, female wild-type and CYP2E1-null mice were administered acrylamide (0, 25, 50 mg/kg) by intraperitoneal injection once daily for 5 consecutive days. Twenty-four hours after the final treatment, blood and tissue samples were collected. Erythrocyte micronucleus frequencies were determined using flow cytometry and DNA damage was assessed in leukocytes, liver, and lung using the alkaline (pH > 13) single cell gel electrophoresis (Comet) assay. Results were consistent with the earlier observations in male germ cells: significant dose-related increases in micronucleated erythrocytes and DNA damage in somatic cells were induced in acrylamide-treated wild-type but not in the CYP2E1-null mice. These results support the hypothesis that genetic damage in somatic and germ cells of mice-treated with acrylamide is dependent upon metabolism of the parent compound by CYP2E1. This dependency on metabolism has implications for the assessment

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of human risks resulting from occupational or dietary exposure to acrylamide. CYP2E1 polymorphisms and variability in CYP2E1 activity associated with, for example, diabetes, obesity, starvation, and alcohol consumption, may result in altered metabolic efficiencies leading to differential susceptibilities to acrylamide toxicities in humans.
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Keywords: Acrylamide; Glycidamide; Micronuclei; DNA damage; Comet assay; Genotoxicity; CYP2E1-null mice

1. Introduction

Acrylamide, a rodent carcinogen and probable human carcinogen [2,3], has been reported to be mutagenic in somatic cells in vitro and in vivo, particularly in assays such as the micronucleus and chromosomal aberration tests that detect chromosomal damage [2,4–6]. Acrylamide, or a metabolite, also has a strong affinity for mammalian sperm cells, inducing genetic damage with great efficiency in late spermatids through epididymal spermatozoa, maturation stages in which protamines have replaced histones in the highly condensed chromatin [1,7–11]; some genetic damage has also been reported in non-differentiated spermatogonial stem cells of mice following acrylamide exposure [12].

Human exposure to acrylamide is known to occur through a number of different manufacturing processes as well as from cigarette smoking [3]. Recently, low levels of acrylamide have been discovered in carbohydrate-rich foodstuffs subjected to high heat during processing [13–15]. This new source of acrylamide has expanded the concern from acute and/or high level occupational exposures of adults, which can be measured and controlled, to much lower but less easily quantified or regulated chronic or sporadic dietary exposures among the general population, including children.

Acrylamide is metabolized either via direct glutathione conjugation followed by excretion of mercapturic acids or via oxidative pathways catalyzed by cytochrome P450 enzymes to yield glycidamide, believed to be involved in the carcinogenic and mutagenic effects of acrylamide. Sumner et al. [16] investigated the oxidative metabolism of acrylamide in mice using ^{13}C NMR to compare the urinary metabolite profile in cytochrome P4502E1 (CYP2E1)−/− (CYP2E1-null) mice to the metabolite profile in CYP2E1+/+ (wild-type) mice after treatment with ^{13}C-acrylamide. They demonstrated that acrylamide is metabolized to the DNA reactive epoxide glycidamide in wild-type CYP2E1-competent mice. In contrast, no metabolites resulting from acrylamide epoxidation were detected in the urine of CYP2E1-null mice. Sumner et al. [16] concluded that acrylamide was metabolized exclusively via direct glutathione conjugation in CYP2E1-null mice. Therefore, this mouse model is ideal for investigating the mutagenic and carcinogenic effects of acrylamide that are directly linked to a competent CYP2E1 metabolic pathway that generates the reactive metabolite glycidamide.

Glycidamide is a stable DNA-reactive epoxide that is evenly distributed throughout the tissues of laboratory rodents [17,18]; it has a half-life of approximately 1.5 h in rats [19] and mice [18], depending upon the tissue analyzed. Glycidamide is detectable in the urine of humans following oral administration of acrylamide, although at lower levels than are seen in rodents administered equivalent doses [20]. Glycidamide is mutagenic in bacterial and mammalian cells [21] and it induces micronuclei when administered by intraperitoneal (IP) injection to mice and rats [22]. When administered orally, glycidamide also induces dominant lethal mutations in germ cells of male mice [23]. It was recently reported that although both acrylamide and glycidamide form DNA adducts in vitro, comparisons of the mutagenicity of acrylamide and glycidamide in human and mouse cell lines showed higher levels of DNA adducts and gene mutations in defined targets after glycidamide treatment compared to acrylamide [24].

Recently, using CYP2E1-null mice, Ghanayem et al. [1] demonstrated that induction of dominant lethal mutations in germ cells of male mice following exposure to acrylamide requires metabolically functional CYP2E1 enzyme. The induction of genetic damage in somatic cells following exposure to acrylamide is presumed to have the same metabolic dependency, but the requirement for functional CYP2E1 enzyme activity has never been directly demonstrated in somatic

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