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Inhibition of Rat Testicular Nuclear Kinesins (*krp*2; KIFC5A) by Acrylamide as a Basis for Establishing a Genotoxicity Threshold

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Acrylamide is a toxic substance that induces a variety of cellular responses including neurotoxicity, male reproductive toxicity, tumorigenicity, clastogenicity, and DNA alkylation. Evidence is provided that inhibition of the microtubule motor protein kinesin is responsible for acrylamide-induced clastogenicity and aneuploidy. Two kinesin motors, KIFC5A and KRP2, which are responsible for spindle assembly and disassembly of kinetochore MT, respectively, are inhibited by acrylamide. The inhibitory concentration for a response is below the levels shown to adversely affect the cytogenetic parameters. The relative contribution of these inhibitions compared to DNA alkylation is considered. The implications of inhibition of these kinesins as the site of action of acrylamide with regard to risk assessment are substantial as this event will have a threshold and a safe level of acrylamide can be determined.

KEYWORDS: Acrylamide; kinesin; *krp2*; KIFC5a; DNA; mutagenicity; clastogenicity

INTRODUCTION

Acrylamide is a vinyl monomer used in the manufacture of polymers for dewatering waste, paper manufacture, mining, oil recovery, hospital and research laboratory separations, and many other applications. Historically, acrylamide monomer was manufactured as a crystalline substance, which was then dissolved in water with comonomers and catalysts and used to manufacture polymers. This crystalline monomer generated dust and had a significant vapor pressure due to sublimation. As a result, some people working with this monomer became overexposed. The clinical consequences of this overexposure were a characteristic neurotoxicity (*1*). Clinical findings and research have identified the loss of sensation in the peripheral nerves, which progressed proximally up the nerve, as well as ataxia and progressive muscular weakness and eventual paralysis as the neurological outcomes. The consistency of this response across species made acrylamide a valuable substance with which to study common mechanisms of neurotoxicity; acrylamide continues to be a prototype chemical for these studies. With the discovery of acrylamide in fried carbohydrate-rich foods, the estimated number of people exposed to acrylamide increased enormously.

Acrylamide is tumorigenic to rats, inducing tumors of the mammary and thyroid glands in females and the tunica vaginalis mesothelium and thyroid in males (*2, 3*). The tumor sites are associated with organs under hormone control, suggesting that the involvement of alteration of hormone levels might be a causal factor in the induction or promotion of these tumors (*4*). Acrylamide acts as a partial dopamine agonist, which can provide an alternative explanation for these responses (*5*). As a result of this dopamine agonist activity, a precipitous drop in prolactin levels was observed in male rats, which was followed by testicular atrophy (*4*). In females, there is a drop in progesterone levels, which results in alteration of pseudopregnancy observed in aged rats (*4*). This activity has been postulated to be responsible for the tumorigenicity as the tumors to not appear to be of a genotoxic nature (*4*).

Acrylamide was found to cause chromosomal aberrations and aneuploidy in vitro and in vivo in mice but not in rats (*6*). Acrylamide also induces genetically transmitted chromosome damage in mice and rats as measured by heritable translocation test, dominant lethal test, and specific locus test. However, acrylamide does not cause gene mutations in enzyme-mediated bacterial tests.

Acrylamide is metabolized to an epoxide, glycidamide, by cytochrome P450 2E1 (cyp2E1) (*7*). The areas under the serum concentration curve (AUC) for acrylamide and glycidamide are proportional to the levels of hemoglobin adducts formed by both substances and reflective of conversion of acrylamide to glycidamide (*8*). Mice produce considerably more glycidamide than rats, which in turn produce more than humans (*7*). When

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urinary metabolites from acrylamide are compared, 59% of acrylamide is converted to glycidamide in the mouse, compared to 33% in the rat and 15% in humans (*8*). Similarly, *N*-7-glycidamide−guanine DNA adducts are produced in mouse liver at more than twice the frequency that is observed in rat liver (*9*). This species difference becomes particularly relevant when one considers that virtually all somatic cell studies have been conducted in mice.

Glycidamide is an extremely stable epoxide, so stable that it is found in mouse, rat, and human urine (*8*). This epoxide reacts slowly with guanine to produce *N*-7-(2-carbamoyl-2-hydroxyethyl)guanine and, to a lesser extent, *N*-3-(2-carbamoyl-2-hydroxyethyl)adenine (*9*).

When conversion of acrylamide to glycidamide is inhibited either chemically using aminobenzotriazole (*10*) or by using cyp2E1 knockout mice (*11*), the genotoxicity of acrylamide is inhibited. This supports the conclusion that glycidamide is the genotoxic form of acrylamide. However, acrylamide treatment induces oxidative stress in vitro and in vivo (*12*), which could account for much of the genotoxic response to acrylamide. The metabolism of acrylamide to glycidamide produces reactive oxygen. This production of reactive oxygen, coupled with the depletion of glutathione due to conjugation of acrylamide and glycidamide, would represent the genesis of this oxidative stress. Acrylamide metabolism would be expected to induce genotoxicity, not only by producing glycidamide but also by reducing glutathione levels and producing reactive oxygen.

The mechanism by which acrylamide induces neurotoxicity is now well established (*13*). Acrylamide and its metabolite glycidamide are highly reactive with sulfhydryl groups in proteins (*14*). There does not appear to be a great deal of specificity as to which proteins are the substrate for this reaction, although some studies single out neurofilament-associated proteins. Alternatively, the sensitivity of specific proteins to sulfhydryl binding may determine the toxicological specificity of the acrylamide response. Therefore, concentration responses become essential to identifying critical site(s) of action of this toxicant.

For the remainder of the manuscript, we restrict our comments to the following topics: (1) data that suggest that DNA binding is not the basis for genetic anomalies, (2) studies that demonstrate that kinesin binding is the mode of action in the peripheral nervous system, (3) analogous kinesin studies conducted on genetic material, and (4) implications for these observations on risk assessment.

DNA ALKYLATION AND GENOTOXICITY

Acrylamide binding to DNA is extremely slow; however, the epoxide metabolite glycidamide does bind DNA. In addition, the adducts formed by binding of glycidamide to DNA are not in the base-pairing region (**Figure 1**) (*15*). The low genotoxic potential of the N-7 adduct is supported by the data presented by Jenkins et al. (*16*), which indicate that N-7 alkyl adducts are effectively repaired, and thus they show threshold dose−response relationships for mutation induction. Others have shown that alkylation of the N-7 and N-3 sites by methylmethane sulfonate is associated with thresholds for its mutagenic potential (*17*). Therefore, the alkylation of DNA does not, a priori, dictate a mutagenic mechanism of action, and genotoxic outcomes can vary depending upon relative alkylation versus repair rate.

There appears to be an inconsistency between the time course of appearance and loss of DNA adducts with the occurrence of genotoxic damage. The repair of DNA appears to be rapid as

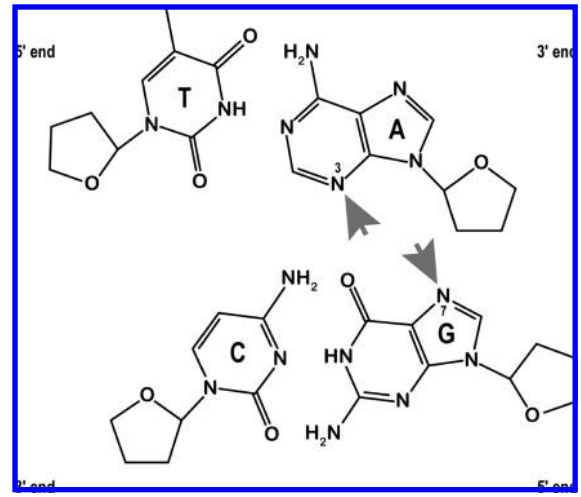

Figure 1. Double-stranded DNA showing the sites of DNA adduct formation including the important targets for alkylators (N7G, N3A, O6G, etc.) in a graphic representation of DNA base-binding sites and their involvement in base pairing.

Table 1. Temporal Relationship between DNA Adducts and DNA Damage[a]

time after treatment[b] (h)	bone marrow Comet[c]	liver Comet[c]	brain Comet[c]	liver 7-GA-GUA[d]	brain 7-GA-GUA[d]
2	113	143	86		
5	229	206	186	5.1	3.2
24	102	142	236	6.3	2.8
48				2.9	2.6

[a] Data from ref (*18*). [b] Groups of rats were treated with 54 mg/kg acrylamide po and killed at the times indicated. [c] Olive tail moment as percent of concurrent control. [d] Expressed as molecules per 10^8 nucleotides. These data are estimated from a graph.

measured in the alkaline Comet assay (*18*). When the DNA damage in the Comet assay at 5 and 24 h is recalculated as a percent of control [to account for differences in control (background)], the damage (Comet assay) in the bone marrow and liver is decreased, whereas the DNA adduct levels in the liver are increased (**Table 1**). In contrast, brain adducts decreased over this time period, whereas the DNA damage in the brain increased (*18*). Therefore, DNA adducts and DNA damage, as measured by the Comet assay, are not temporally correlated.

Human lymphocytes treated with acrylamide at concentrations ranging from 0.5 to 50 μM for 1 h showed double-strand breaks in DNA similar to those produced by hydrogen peroxide, the positive control (*19*). These were repaired within an hour. This repair is in marked contrast to the pattern of DNA alkylation. The loss of DNA alkylated bases takes a great deal longer.

Treatment of 8-week-old rats with 15 mg of acrylamide/kg in drinking water induced no increase in DNA strand breaks in liver (*20, 21*). In contrast, there was a significant increase in DNA breaks/labile sites in both the thyroid (50% over control) and adrenal (80% over control) glands as measured using the Comet assay. Despite this disparity, the levels of DNA alkylation in all three organs are virtually the same (*22*).

In contrast, acrylamide caused an increase in mutant frequency in the TK assay and MN test at a dose of 14 mM while showing no DNA damage as measured by the Comet assay (*23*). In a loss of heterozygosity assay, this increase in mutant frequency was accounted for by chromosomal deletions. Collectively, these data suggest caution in extrapolation of DNA adduct levels to the default conclusion that acrylamide or its

glycidamide adduct produces genotoxicity through a direct DNA reactive pathway. Additional data are required to determine if the DNA adducts are the cause of the genotoxicity seen in vitro and in vivo.

There is extensive literature on the genetic toxicity of acrylamide and glycidamide, some of which is summarized by Dearfield et al. (*24*). It is not the intent of this section to provide a comprehensive review of the literature and the author's conclusions, but to summarize recent, pertinent data on the mechanisms for genetic damage induction by non-glycidamide−DNA adduct mechanisms.

Acrylamide is a weak in vitro and in vivo somatic cell genotoxicant. Prokaryotic cells are not mutated by acrylamide with or without metabolic activation; it was not mutagenic in *Salmonella* at 10 mg/plate in one study (*26*) and was considered to be equivocal at 10 mg/plate in a parallel study despite the fact that its metabolite, glycidamide, is active at 50 μg/plate (*28*). Studies using *Escherichia coli* did not yield a mutagenic response when tested up to 50 mg/plate (*24*). One possible explanation is that acrylamide is not converted to glycidamide in vitro. Emmert et al. (*27*) transfected a methyl-deficient strain of *Salmonella typhimurium* with a plasmid containing cytochrome P450 reductase, cytochrome b5, and cytochrome P450 2E1. Allyl chloride and 2-aminoanthracene, both of which are substrates of cyp2e1, were genotoxic in this strain, but acrylamide, also a substrate for this cyp, was toxic, but not mutagenic, at concentrations up to 10 mg/plate, showing that the cells did not respond even under conditions that supported the metabolism of acrylamide. No satisfactory explanation for this phenomenon has been proposed.

Acrylamide was clastogenic to TK6 and L5178Y cells in culture and did not produce point mutations when tested without liver S-9 (*28, 29*). It was not tested with S9 in L5178Y cells. Measurement of colony size in L5178Y cells revealed that acrylamide induced both large and small colonies, which are presumptive indicators of gene and chromosome mutations, respectively. However, molecular analysis of the mutant colonies showed that they were all deletions (*29*). In human TK6 cells, acrylamide produced an increase in mutant frequency at the TK locus resulting from deletions and MN at a dose of 14 mM, while showing no DNA damage, as measured by the Comet assay (*28*). Glycidamide was mutagenic, producing exclusively deletions in the Mei et al. (*29*) study and primarily point mutations in the Koyama et al. (*23*) study. An unpublished NTP study of acrylamide in CHO cells resulted in induction of chromosome aberrations with S9; the response without S9 was judged to be "questionable" (*30*). Results showing chromosomal aberrations and mitotic spindle effects, and not point mutation, are a recurring theme in acrylamide genetic toxicology (*24, 31*). These lesions are of a threshold nature (*32–34*).

Although there appears to be no organ specificity to DNA adduction, the DNA damage as measured in the Comet assay is organ specific. Treatment of 8-week-old rats with 15 mg of acrylamide/kg in drinking water induced no increase in DNA strand breaks in liver (*18, 21*). In contrast, there was a significant increase in DNA breaks/labile sites in both the thyroid (50% over control) and adrenal (80% over control) glands as measured using the Comet assay. The levels of DNA alkylation in all three organs are the same (*22*). At a single oral dose of 54 mg/kg, only the brain, not the liver, bone marrow, testes, or adrenals, showed an increase in the Comet response as measured by tail moment (*36*). At 36 mg/kg and lower, no significant responses were observed. There is evidence to support the conclusion that the effects on DNA were the result of oxidative stress (*12*). This is supported by studies in vitro. The Comet assay response was minimal in human lymphocytes exposed to glycidamide using the standard alkaline elution procedure, but strongly positive when the cells were treated with formamidopyrimidine−DNA−glycosylase (FPG), which recognizes and removes the oxidative 8-OH-dG adducts (*37*).

The conclusion from these studies is that acrylamide in vitro primarily causes deletions and other structural chromosome damage rather than point mutations and that these effects appear to be associated with oxidative damage or interference with mitosis.

KINESIN BINDING IS THE MODE OF ACTION IN THE PERIPHERAL NERVOUS SYSTEM

Kinesins are a superfamily of proteins with a common 340 amino acid motor domain sequence. This globular domain possesses both microtubule (MT) binding sequences and cargo binding sequences and ATPase activity, which accounts for the ability (of most kinesins) to locomote along MT. Variability in amino acid sequence in the nonmotor domain as well as the position of the motor domain in relationship to the molecules backbone affects the directionality of movement along MT, and the binding to different cargos as well as participation in different functions. The kinesins isolated from adult brain are predominantly plus-end directed motors involved in intracellular transport toward nerve terminals. The motor domain is in the N-terminal end of the kinesin (*37, 38*). Two different kinesins are considered here: KIFC5A is a C-terminal motor, which belongs to a family of kinesins that move toward the minus end of the MT. These motors possess a second MT binding domain that collectively works together to align MT into bundles necessary for spindle formation and maintenance. KRP2 is a kinesin with the motor domain in the middle of the complete sequence and belongs to a family that disassembles MT rather than locomoting along them. This family is attributed to disassembly of kinetochore MT during anaphase A. Additional kinesins are located in the mitotic/meiotic spindles such that there is both redundancy for protection of the spindle and balance forces for fidelity of genomic segregation (*3*).

The relationship between kinesin inhibition and neurotoxicity has been recently reviewed (*13*). Using several indices of transport, acrylamide inhibits fast anterograde axonal transport and retrograde axonal transport (*40*). These have been evaluated in vivo and in vitro. Because acrylamide induces a distal-to-proximal dying back of neurons, this mode of action is logical. The mechanism by which acrylamide induces this response has been the major subject of research. Candidate sites of action have included neurofilament binding, energy/ATP production, and kinesin activity (*41, 42*). Neurofilament (NF) binding has been eliminated as a candidate due to the neurotoxicity of acrylamide in crayfish, a model lacking neurofilaments (*43*). Tail injections of acrylamide produced ataxia and paralysis of the pincher as well as morphological expression of axonal degeneration in a spatial and temporal pattern of progression comparable to mammalian species possessing NFs. This finding was extended to a transgenic mouse model (*40, 44*) lacking axonal NFs using both acrylamide and a similarly acting diketone model. Subchronic acrylamide exposure resulted in progressive and cumulative increases in sensorimotor deficits. Neurobehavioral tests demonstrated similar expressions of neurotoxicity in transgenic (T) mice and their nontransgenic (NT) litter mates (containing normal numbers of axonal NFs). Quantitation of acrylamide-induced axonal lesions demonstrated the distal location of pathology and equal susceptibility of T

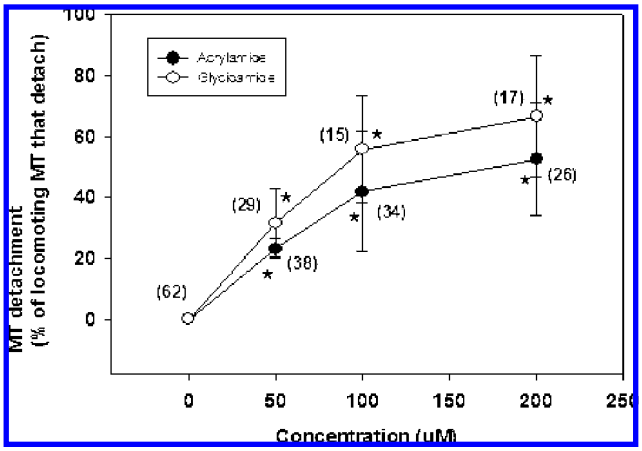

Figure 2. Effects of acrylamide and glycidamide on kinesin-based microtubule motility [from Sickles et al. (*48*)]. Bovine brain kinesin was incubated with various concentrations of acrylamide and glycidamide and the activity assessed. Results are expressed as percent inhibition versus concurrent control.

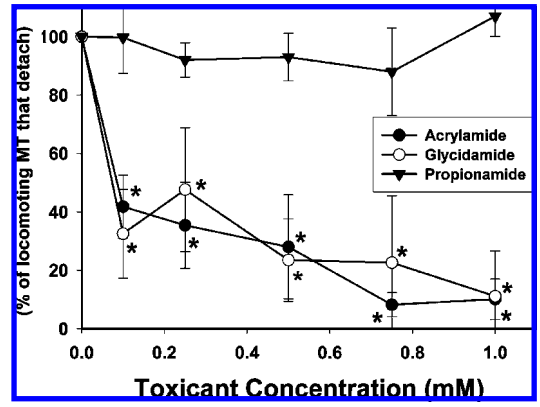

Figure 3. Effects of acrylamide, glycidamide, and propionamide on KIFC5A. Acrylamide and glycidamide were incubated with purified rat testicular KIFC5A, and the activity was assessed. Results are expressed as percent inhibition versus concurrent control.

and NT axons. With regard to energy production, there is extensive literature on a wide variety of enzymes involved in neuron energy metabolism [reviewed in Sickles (*13*)]. Whereas many of these enzymes are inhibited at high concentrations of acrylamide, there appears to be little if any correlation to ATP production (*44, 45*). In contrast, bovine brain kinesin was extremely sensitive to acrylamide (*46*). As can be seen in **Figure 2**, significant kinesin inhibition is observed at acrylamide concentrations as low as 100 μM. Further investigations have shown inhibition at 50 μM acrylamide and comparable inhibition by glycidamide (unpublished data, see **Figure 2**). This is 5−10-fold less than the daily dose used to produce neurotoxicity in a variety of animals.

EFFECT OF ACRYLAMIDE ON *KRP* ACTIVITY AS THE MODE OF GENOTOXIC ACTIVITY

Toxicant action on meiotic and mitotic spindle microtubules is a longstanding cause of defects in cell division, some of which lead to structural chromosome damage and to carcinogenicity. Whereas acrylamide binds to tubulin, there are no significant effects on MT assembly and dynamics. In the absence of a MT effect and because acrylamide inhibited neuronal kinesins, it was hypothesized that other kinesin family members associated with cell division might also be inhibited. We had also previously identified mitotic arrest in cultured HT1080 cells by exposure to acrylamide at concentrations ranging from 1 to 10 mM for 4 h (*44*). The number of mitoses was increased as much as 4.5-fold, similar to that produced by 30 μM colchicine. With both colchicine and acrylamide, chromosomes were observed either free of the mitotic spindle or locked in metaphase. These data demonstrated an effect on the spindle apparatus in these cells and further suggested kinesins as a potential site of action (*45*).

Kinesins were cloned from rat testicular c-DNA and used to transform bacteria for protein expression, followed by purification of the proteins. Two different kinesins with different functions were tested for their inhibition by acrylamide. KIFC5A is a chromosome motor involved in the formation and maintenance of the mitotic spindle. Analysis of the sequence showed that KIFC5A is related to motors involved in stabilizing the spindle pole, including *Xenopus* XCTK2, Drosophila ncd, and hamster CHO2. All are C-terminal motors, and most move toward the microtubule minus-ends. Fluorescent antibody staining with an antibody (provided by Dr. Ryoko Kuriyama) that recognizes CHO2 and KIFC5A localized these proteins to male germ cells, where they were found in multiple microtubule complexes in spermatogenesis (spindle, manchette, flagella). This is consistent with a common microtubule bundling activity in these structures. KIFC5A addition at high concentrations relative to MT caused bundling of the MT. At lower KIFC5A concentrations, MT attached to coverslips in a concentration-dependent manner: the latter activity was used to assay its function.

KIFC5A was significantly and equally inhibited by acrylamide and glycidamide (see **Figure 3**). The dose response was extremely similar to the dose response for inhibition of bovine brain kinesin. Interruption of function of this family of kinesin motors results in decreased number of spindles formed and/or malformed (twisted) spindles. Therefore, one would predict that acrylamide would alter the appearance of the mitotic spindle at relatively high doses. In our previous work, 10 mM acrylamide produced mitotic spindles that were distorted in appearance. We attribute the inhibition of proteins such as KIFC5A to this alteration in spindle morphology. Therefore, just as nervous system kinesin was inhibited by acrylamide, so was this mitotic or meiotic kinesin. Furthermore, inhibition of KIFC5A has a similar sensitivity to neuronal kinesins, leading one to predict similar dose−response effects on dividing cells and neurotoxicity. Factors that may affect this correlation include redundancy in motor proteins, the relative safety factors for each KRP (i.e., the excess in quantity versus the amount required for normal function), and the sensitivity of the assays).

In a similar fashion, the effect of acrylamide was assessed on KRP2 activity (**Figure 4**). KRP2 is a member of the internal motor subfamily (kin I) that destabilizes the ends of microtubules (*48*). Kin I members are microtubule destabilizing enzymes. These motors are not conventional motors and have not been shown to glide microtubules (except in one case). KRP2 is closely related to mitosis centromere-associated kinesin (MCAK). Another kinesin-related protein, called XKCM1, has been identified recently from *Xenopus* with sufficient homology to be placed in the same subfamily with KRP2 (*50*). Walczak et al. had difficulty with purification of the protein and went on to identify a microtubule disassembly function for their protein (*50*). In fact, XKCM1 has been demonstrated by electron microscopy to bind to microtubule ends and effectively strip individual protofilaments from the tubule. The combination of location and function indicates this kinesin is associated with microtubule disassembly, which occurs during anaphase A when

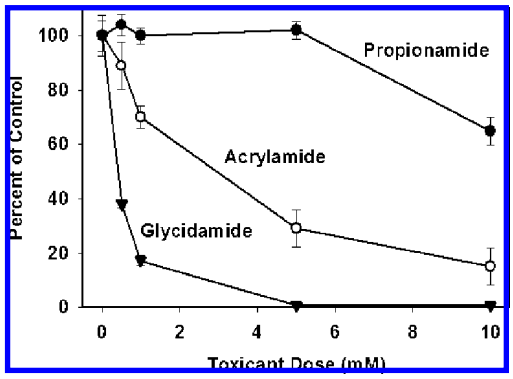

Figure 4. Effect of acrylamide on rat testicular KRP2 activity. Acrylamide and glycidamide were incubated with purified rat testicular KRP2, and the activity was assessed. Results are expressed as percent inhibition versus concurrent control.

the chromosomes segregate into the daughter cells. As a consequence, inhibition of this subclass of kinesins interferes with cell division. Following expression of KRP2, the proteins could not be purified by microtubule affinity because it would disassemble the MT. Fortunately, the supernatant from the bacterial lysate possessed sufficient KRP2 to be used without purification. As the bioassay for KRP2 function, the amount of soluble tubulin in a high-speed spin of microtubules was measured following incubation of KRP2 with MT. Exposure of KRP2 to acrylamide reduced the quantity of soluble tubulin in the range of 1−10 mM, indicative of an inhibition of the MT disassembly activity (**Figure 4**). KRP2 was even more sensitive to glycidamide, with significant inhibition obtained at 500 μM. It should be noted that the inhibition may be significantly affected at concentrations lower than the lowest (500 μM) concentration tested because a 60% reduction was observed at 500 μM. In addition, these studies were conducted using taxol-stabilized MT, which would strongly counter the action of KRP2. Both of these factors will shift the concentration response curve considerably. It is now apparent that these studies should be repeated at lower acrylamide concentrations and with unstabilized MT to accurately determine the dose−response range. However, both of these methodological adjustments would result in a demonstration that KRP2 is more sensitive than currently observed.

The results of these studies indicate that KRP2 is inhibited by acrylamide at concentrations lower than those required to block mitosis in HT1080 cells in vitro. The retention of chromosomes at the metaphase plate in these cells is consistent with the concept that microtubule-depolymerizing kinesin inhibition by acrylamide leads to failure of the migration of chromosomes from the metaphase plate. Lower concentrations of acrylamide may affect the failure of single chromosomes to migrate properly, resulting in aneuploidy, which may be observed as micronuclei. In addition, mitotic spindle-damaging agents may also result in clastogenicity for reasons unclear at this time. This observation implicates the inhibition of kinesins as having a significant impact on parameters of cell division that can lead to genetic damage and/or tumor formation. Genomic instability and aneuploidy are common mechanisms active in tumor formation. Therefore, the inhibition of this KRP may represent a mechanism by which action on dividing cells can produce abnormal genetic content, carcinogenicity, or altered cell cycle events.

IMPLICATIONS FOR THESE OBSERVATIONS ON RISK ASSESSMENT

Two critical issues relevant to interpretation of the carcinogenic risk potential are the concentration-related responses and a determination of threshold versus nonthreshold mechanisms of action. Implicated mechanisms considered here are DNA reactivity and kinesin motor inhibition. We address these two critical issues with each mode of action and, as well, identify critical information missing from the acrylamide database to clearly identify its mode(s) of action for tumorigenicity.

The mitotic/meiotic kinesin data (in addition to the binding of acrylamide/glycidamide to sperm cell protamines; not addressed here) identify protein targets that can explain most of acrylamide's genetic effects. Disruption in chromosomal segregation can lead to aneuploidy, which is a major effect associated with tumorigenicity. The observation of clastogenicity with mitotic spindle poisons also suggests that acrylamide's clastogenic action may be due in part to its action on kinesin. A kinesin mode of action obviously would have a threshold due to its non-DNA target, a lack of effect at minimal concentrations as well as continual replacement of the target, although in a mitotic/meiotic cells the replacement may be more limited. Concentration-related responses for protein targets are particularly relevant. Kinesins are a sensitive end point; indeed, both the neuronal kinesins associated with neurotoxicity and the mitotic/meiotic kinesins associated with cell division are the most sensitive targets for acrylamide identified to date. These responses are consistent with the in vivo toxicity of acrylamide. Kinesin inhibition will induce a threshold response analogous to non-neurotoxic doses of acrylamide. Considering that the dose−response curves between bovine brain kinesin and rat testicular kinesins are very similar, this provides dose guidance on establishing an appropriate and safe dose for acrylamide monomer. It is likely that the doses inducing neurotoxicity in mammals are indicators of the other toxic responses, just as multiple toxicities may be due to action on a similar and sensitive kinesin target.

In conclusion, additional research addressing the relative contributions of a DNA versus kinesin target in acrylamide genotoxicity and tumorigenicity is required. With a kinesin (protein) mode of action, the default application of a linearized dose−response curve for assessing risk is inappropriate. In the case of toxic agents that produce DNA adducts, concentrations become critical due to the potential of DNA repair and, as a consequence, threshold-based risk assessment may applicable.

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