Cadmium (Cd) is a ubiquitous environmental pollutant that has been associated with male reproductive toxicity in both humans and animal studies. The underlying mechanism of this response, however, is still uncharacterized. To address this issue, we employed a recently developed and optimized three-dimensional primary Sertoli cell-gonocyte coculture system and examined the time- and dose-dependent effects of Cd on morphological alterations, cell viability, activation of stress signaling pathways, and the disruption of the ubiquitin proteasome system (UPS). Our results demonstrated that Cd exposure lead to time- and dose-dependent morphological changes that are associated with the induction of apoptosis. In response to Cd, we also saw a disruption of the UPS as evaluated through the accumulation of high-molecular weight polyubiquitinated proteins (HMW-polyUb) as well as alterations in proteasome activity. Robust activation of cellular stress response, measured through the increased phosphorylation of stress-activated protein kinase/c-jun N-terminal kinase and p38, paralleled the accumulation of HMW-polyUb. In addition, p53, a key regulatory protein, was upregulated and underwent increased ubiquitination in response to Cd. To further characterize the role of the UPS in Cd cellular response, we compared the above changes with two classic proteasomal inhibitors, lactacystin, and MG132. The stress response and the accumulation of HMW-polyUb induced by Cd were consistent with the response seen with MG132 but not with lactacystin. In addition, Cd treatment resulted in a dose- and time-dependent effect on proteasome activity, but the overall Cd-induced proteasomal inhibition was unique as compared to MG132 and lactacystin. Taken together, our studies further characterize Cd-induced in vitro testicular toxicity and highlight the potential role of the UPS in this response.

Key Words: cadmium; Sertoli cell-gonocyte coculture; ubiquitin proteasome system; stress signaling; male reproductive toxicity.

Cadmium (Cd) is a ubiquitous environmental pollutant that has been associated with male reproductive toxicity in both

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hypothesized to Cd (Jungmann et al., 1993). The UPS is a highly conserved pathway that plays an important role in the selective degradation of specific cellular proteins (Marx, 2002). The UPS acts through posttranslational modifications of key transcriptional regulators, impacting various cellular events including cell cycle progression, signal transduction, transcriptional regulation, apoptosis, and DNA repair (DiAntonio et al., 2001; Pagano et al., 1995). Biochemical data indicate that the activity of the UPS is high during spermatogenesis (Rajapurohitam et al., 2002), which is most likely related to the high requirement for massive breakdown of cytoplasmic and nuclear proteins during this process (Baarends et al., 1999; Dickens et al., 2002; Sutovsky et al., 2001a). In addition, defective sperm are found to be ubiquitin tagged and are related to the elimination during mammalian spermatogenesis (Sutovsky et al., 2001b).

Considering the potential role of the UPS in both metal-induced toxicity and the regulation of key cellular events critical to reproductive function, it is evident that further characterization of these processes are needed to help clarify the mechanistic response to environmental Cd exposure.

In our previous studies, we reported the establishment and characterization of a novel three-dimensional primary Sertoli cell-gonocyte coculture (SGC) system (Yu et al., 2005). Here, we further employ this in vitro SGC system to examine whether low levels of Cd affect the development of neonatal testis and to define the role of the UPS in this mechanistic response. To address these questions, we investigated the time- and dose-dependent effect of Cd on morphological alterations, cell viability, the activation of stress signaling proteins, and the disruption of the UPS. The cell cycle regulatory protein, p53, was also evaluated due to its key role within these responses as well as its role as a stress signal in the UPS. We monitored the UPS through the measurement of high–molecular weight polyubiquitinated proteins (HMW-polyUb) accumulation as well as proteasomal activity. To fully understand this response, we compared these measurements with impacts observed using two classic proteasomal inhibitors, lactacystin and MG132. Our results demonstrated that Cd exposure leads to time- and dose-dependent morphological changes as well as a correlated induction of apoptosis. In addition, the accumulation of HMW-polyUb paralleled the robust activation of the stress response as indicated by the phosphorylation of stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK) and p38. Both the accumulation of HMW-polyUb and the activation of the stress response observed with Cd are similar to the response seen with MG132 but not with lactacystin. Cd treatment also leads to a time- and dose-dependent effect on proteasomal activity. This inhibition of the proteasome was different, however, compared to MG132 and lactacystin. Taken together, our studies suggest that UPS dysfunction plays a key role in the underlying mechanism of Cd-induced testicular toxicity.

Methods and materials

SGC and treatment of Cd. The SGC was followed as previously described (Yu et al., 2005). Briefly, male pups were obtained by mating Sprague-Dawley rats (Harlan, Kent, OH). Testes were dissected from 5-day-old rats, and a cell suspension containing primarily Sertoli cells and type A spermatogonia was isolated with the multiple digestion steps. Cells were resuspended in hormone- and serum-free Eagle’s Minimal Essential Medium (Life Technologies, Inc., Gaithersburg, MD) containing 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 3 mM sodium lactate, 1% ITS+™ premix (a culture supplement containing insulin, transferrin, selenium, linoleic acid, and bovine serum albumin; BD Biosciences, Bedford, MA). Cells were plated in 35-mm tissue culture–treated dishes at 2.4 × 10⁶ density in serum-free medium. Immediately after seeding, an ice-cold extracellular matrix (ECM) medium (5 μg/ml BD Biosciences) was applied to these dishes at a final concentration of 200 μg/ml. Serial dilutions were prepared from a stock solution of Cd and added directly to the culture medium 48 h after the addition of ECM overlay. The final concentrations tested were 0.5, 2.5, 5, 10, 20, and 40 μg/ml. Separate treatments with MG132 and lactacystin were conducted for 24 h from stock solutions of these agents in dimethyl sulfoxide, and final concentration was 2.5 μM.

Morphology and viability. All cultures were viewed with a Nikon inverted microscope equipped with phase-contrast optics (Nikon, Tokyo, Japan) at intervals during culture to assess their general appearance. Resultant images were captured and digitized using a CoolSnap Camera (Roper Scientific, Inc., Duluth, GA). The digitized image was processed using Adobe Photoshop. Neutral red (NR) assay was used to determine viability of the cultured cells after treatment as previously reported (Borenfreund and Puerner, 1985). Briefly, cells were treated with different concentrations of Cd. The media with treatment was removed, and fresh media containing 50 μg/ml NR was added to the dish. After incubation for 3 h at 37°C, 5% CO₂, the cells were washed with PBS and NR was eluted with 1% acetic acid/50% ethanol solution. Finally, 200 μl of the resulted NR solution was added to 96 plates and measured at 490 nm.

Assessment of apoptosis. For the evaluation of the apoptotic morphological changes, cells were fixed and stained with Hoechst 33342 (0.1 mg/ml in PBS) after Cd treatment. The stained cocultures were viewed with appropriate filter under fluorescence microscope. Images were captured and digitized using Spot Camera (Diagnostic Instrument, Inc.) equipped with MetaMorph software. Apoptosis–associated end points were further determined in cell extracts by measuring functional activities associated with caspase-3/7 using caspase-specific fluorogenic substrates. The activity of caspase-3/7 was measured with a fluorometric assay, using N-acetyl-Asp-Glu-Val-Asp-AMC (7-amino-4-methylcoumarin) as the specific substrates as previously reported (Nicholson et al., 1995; Shi et al., 2000). Briefly, 10 μg of cell extract was added in duplicate in 96-well plate format. Reaction buffer containing the fluorogenic substrate enzyme-catalyzed release of 7-amino-4-methyl coumarin (AMC) was added to initiate the reaction which was incubated at 37°C for 2 h and enzyme-catalyzed release of AMC measured by a fluorescence microplate reader at excitation 360 nm and emission 460 nm. Fluorescent units were converted to p mol of AMC released per μg of protein and incubation time (h) using a standard curve generated with known serial dilutions of AMC.

Western blot analysis and immunoprecipitation. At the appropriate time points, cultured cells were rinsed twice with ice-cold PBS. Cell lysis buffer (Cell Signaling Technology, Inc., Beverly, MA) was added to each dish, and cells were scraped with a rubber policeman. Harvested cells were then sonicated at 40 W for 15 s. Resultant cell lysates were centrifuged at 16000 × g for 15 min at 4°C. Supernatant fractions were collected, and the concentration of protein was determined with a commercially available kit (Protein Assay kit, Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as a standard. All samples were subsequently stored at −80°C until assayed.

Western blot analysis for the selected proteins was performed according to the previously described method (Yu et al., 2001, 2005). Briefly, equal amounts of protein (20 μg) were separated on Criterion™ 10–20% Tris-HCl
proteasome activities and Western blot bands’ densitometric quantification are using a standard curve generated with known serial dilutions of AMC. p emission 460 nm. Fluorescent units were converted to measured by a fluorescence microplate reader at excitation 360 nm and tetraacetic acid, for 4 h, respectively. Enzyme-catalyzed release of AMC was ethanesulfonic acid, pH 8, and 5mM ethyleneglycol-bis(aminoethylether)- ison tests. A to Cd treatment, we observed a dose-dependent disruption in dimensional structure 24 h after plating (Fig. 1A). In response to Cd, we observed a dose-dependent disruption in cell morphology (Figs. 1B–D). An increase in the number of the round-up gonocytes was observed beginning at the low Cd concentration of 5μM (Fig. 1B) and was significantly present at 20μM Cd (Fig. 1D). Approximately 90% of the cells became rounded with 40μM of Cd (not shown), but the rounded cells still adhered to the dish. The disruption of the SGC structure in response to MG132 (2.5μM) began 8 h after treatment (Fig. 1E) and was significant at 24 h (Fig. 1F). There were a few morphological changes in treatment with 2.5μM lactacystin at 8 h (Fig. 1G), with no further morphological changes observed at the 24-h time point (Fig. 1H).

In the morphological examinations of cells with Hoechst 33342 nuclear staining, we observed significant and consistent dose-dependent morphological changes as indicated by the heavily condensed nuclei, nuclear shrinkage, and subsequent chromatin condensation into the periphery of the nuclei (Fig. 2). MG132 (2.5μM) induced morphological alterations indicative of apoptosis at 8 and 24 h, while lactacystin did not induce obvious changes at 2.5μM (data not shown). Cell viability, measured by NR Assay (Fig. 3), illustrates a dose-dependent cytotoxicity in response to Cd with approximately 80% cell viability at 5μM Cd and an LC50 approximately at 10μM Cd.

**Activation of Caspase-3/7-like activity after Cd treatment**

The caspase family of proteases plays a crucial role in apoptotic germ cell death. To examine the apoptotic mechanism involved in Cd-induced cell death within the primary neonatal SGCs, we measured caspase-3/7-like activity (Fig. 4). A dose-dependent increase in caspase-3/7-like activity 24 h after treatment was observed in response to Cd. At 24 h, significant increase in caspase-3/7-like activity was seen at 5μM and above. MG132 (2.5μM) significantly induced caspase-3-like activity at both 8 and 24 h after treatment, while lactacystin (2.5μM) did not activate the caspase-3-like activity.

**Cd-induced Accumulation of HMW-polyUb in SGC**

Recent studies have suggested a role for the UPS in mediating metal toxicity (Figueiredo-Pereira and Cohen, 1999; Figueiredo-Pereira et al., 1998). We explored whether Cd exposure perturbed UPS function within the SGC. Cd treatment resulted in a dose- and time-dependent upregulation of HMW-polyUb proteins (Fig. 5). The HMW-polyUb levels in the 10 and 20μM Cd treatments were already highest at 8-h time point (See Fig. 5). In a time-dependent manner, MG132 increased the level of HMW-polyUb proteins at all time points observed. Lactacystin increased these proteins both at the 4- and 8-h time points and returned to the control level at the 24-h time point.

**Cd-induced Changes of Proteasome Activity in SGCs**

In order to determine whether the accumulation of HMW-polyUb by Cd is due to the alteration of proteasomal activity as...
observed with MG132, we also measured proteasomal activity through the fluorogenic substrates Suc-LLVY-AMC and Z-LLE-AMC. The substrates Suc-LLVY-AMC and Z-LLE-AMC are associated with the chymotryptic and PGPH of the proteasome, respectively. Using this method, we found that the inhibition of the proteasomal activity varied toward the two substrates. There were no dose-dependent alterations in the chymotrypsin-like proteasomal activity following Cd treatments (Fig. 6A). MG132 significantly inhibited the chymotrypsin-like activity of the proteasome throughout the...
24-h treatment. Lactacystin also significantly inhibited the chymotrypsin-like activity at both 4 and 8 h but returned to the level comparable to the control by 24 h. Treatment with Cd at both 10 and 20 μM activated the PGPH-like activity of the proteasome at the early time point (4 h) and then decreased significantly after 24 h (Fig. 6B). MG132 significantly decreased PGPH-like activity only at 8 h after treatment, while no significant changes of PGPH-like activity in lactacystin-treated cells were observed (Fig. 6B).

Cd-induced Upregulation of Phosphorylation of SAPK/JNK and p38 MAPK

Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine protein kinases that are involved in many cellular pathways such as cell proliferation, differentiation, movement, and death and have also activated in response to cellular stress. Treatment with Cd resulted in a time- and dose-dependent upregulation of the phosphorylated forms of SAPK/JNK (p-SAPK/JNK, Figs. 7A, B) and p38 (p-p38, Fig. 7C). Significant upregulation of p-SAPK was observed at 10 μM Cd (24 h), while significant upregulation of p-p38 was observed at the higher doses of 20 and 40 μM, 4 and 8 h after Cd treatment. At the 24-h treatment, p38 was significantly activated at 10 μM. MG132 (2.5 μM) also upregulated p-SAPK and p-JNK throughout the observation period, while lactacystin only upregulated p-SAPK/JNK at 4 and 8 h.

Cd-induced Upregulation of p53 and Its Ubiquitination in the SGC

The expression of p53 and the activation of p53 through phosphorylation at serine-15 in response to Cd were examined (Fig. 8A). A significant increase of the total p53 and phospho-p53 was evident at 24 h, 20 μM. Similarly, MG132 (2.5 μM) increased the p53 and phospho-p53 in a time-dependent manner with peak levels occurring at 24 h, a time of peak apoptosis. Lactacystin increased p53 to a lesser extent at the 4- and 8-h time points and returned to the control level by the 24-h time point. By using immunoprecipitation, we first precipitated with p53 antibody, then probed with either anti-ubiquitin antibody or anti-p53 antibody, found a significant increase in ubiquitinated p53 proteins in Cd, MG132, and lactacystin-treated cells (Fig. 8B), and the specific band around 50 kDa was further confirmed by using the p53 monoclonal antibody (Fig. 8C).

DISCUSSION

In vitro models for testicular toxicity provide important tools for investigating specific mechanisms of toxicity (Yu et al., 2005). We applied this in vitro model to further characterize the mechanism of Cd-induced testicular toxicity. To this end, we investigated the time- and dose-dependent effect of Cd on
morphological alterations, disruption of the UPS, and activation of stress signaling proteins. Our results demonstrated that Cd exposure leads to time- and dose-dependent morphological changes, a response that is supported by the induction of apoptosis. Cd exposure also resulted in a dose-dependent accumulation of HMW-polyUb paralleling the activation of cellular stress responses. Cd appears to act more like a nonspecific proteasomal inhibitor, demonstrating similar cellular responses to MG132 as compared to the specific proteasomal inhibitor, lactacystin. Treatment of Cd also leads to a time- and dose-dependent effect on proteasomal activity; however, this response was distinctly different from both MG132 and lactacystin. Taken together, our studies demonstrate that Cd exposure leads to UPS dysfunction and cellular stress, suggesting an important role for this mechanistic response in testicular toxicity.

The UPS pathway has emerged as an important mechanism for regulating a variety of cellular processes including DNA repair, cell cycle control, oncogenesis, abnormal protein catabolism, antigen processing, ribosome biogenesis, transcription, viral infection, neural and muscular degeneration, and stress response (Ben-Neriah, 2002; DiAntonio et al., 2001; Pagano et al., 1995; Schulman et al., 2000). Biochemical data indicate that the UPS pathway (Ub, E1, E2, E3, and deubiquitination enzymes) is highly active in the testis due to the specific requirements associated with the proliferation and massive breakdown of cytoplasmatic and nuclear proteins during the last phase of spermatogenesis (Kon et al., 1999; Rajapurohitam et al., 2002; Sutovsky et al., 2001c). Spermatogenesis, the specific process of proliferation and differentiation of germ cells, is a highly regulated process where defective sperms are ubiquitin tagged and eliminated (Sutovsky et al., 2001b). Although the molecular mechanism of ubiquitination of sperm is not clear, the assay for detecting ubiquitin-tagged sperm has been proposed as a biomarker for male infertility (Rawe et al., 2002; Sutovsky, 2003; Sutovsky et al., 2001a, 2003). In spermatogenesis, there appears to be a special requirement for certain components of the UPS, as exemplified in humans and mice by the mutation of USP9Y and HR6B, respectively (Baarends et al., 1999; Ng et al., 2002). Both genes encode for proteins that take part in the UPS and are ubiquitously expressed, but their mutation generates no apparent phenotype other than male infertility (Roest et al., 1996). A recent study found that a deletion within a gene encoding for one of the key deubiquitin enzymes, ubiquitin carboxyterminal hydrolase-1 (Uch-L1), caused shrinking of seminiferous tubules, decreasing total number of cells and enlargement of remaining cells in seminiferous tubules in 25-week-old mice (Kwon et al., 2003, 2004). Immunohistochemical studies have shown that Uch-L1 is localized in both spermatogonia and Sertoli cells in the testis (Kon et al., 1999). Clearly, the UPS is a critical pathway within the testis and is important for normal spermatogenesis. Disruption of this pathway, therefore,
by environmental toxicants such as Cd may play a significant role in the underlying mechanism of testicular toxicity.

Our study demonstrates the time- and dose-dependent disruption of the UPS through the observed accumulation of HMW-polyUb as well as proteasomal inhibition, suggesting that the UPS plays a primary role in the mechanistic response to Cd (Figs. 5 and 6). Alternate studies have similarly linked metals with the accumulation of HMW-polyUb and proteasomal inhibition (Araya et al., 2002; Figueiredo-Pereira and Cohen, 1999; Kirkpatrick et al., 2003). The accumulation of HMW-polyUb that we observed in our study correlated with the response seen with the nonspecific, broad-range proteasomal inhibitor MG132 but not with the specific inhibitor lactacystin (Fig. 5). Supporting this observation, as with the HMW-polyUb, both Cd and MG132 demonstrated a similar, time-dependent increase in the caspase-3/7-like activity or apoptotic response. In the case of lactacystin, the cells were able to recover by 24 h and demonstrated very little caspase-3/7 activity (Fig. 4). The changes in caspase-3 activity support the observed morphological alterations (Figs. 1 and 2).
Within our study, the resultant accumulation of HWM-polyUb in response to Cd exposure paralleled the activation of cellular stress responses (Fig. 7). Previous studies have demonstrated that the disruption of the UPS, as observed with lactacystin and MG132, leads to significant activation of stress signaling, as well as alterations to other cellular pathways (Lopez Salon et al., 2000; Yang and Yu, 2003). In addition, oxidative stress has been implicated in Cd-induced toxicity both in vivo or in vitro studies (Dong et al., 1998; Jurczuk et al., 2004; Shaikh et al., 1999; Yang et al., 1997). Cd specifically has been linked with oxidative stress and the accumulation of ubiquitinated proteins within neuronal cells (Figueiredo-Pereira et al., 1998). A key component of the stress response is the MAPK family which is comprised of vital

FIG. 6. Chymotryptic-like activities (A) and PGPH (B) of the proteasome in the SGCs. Cells were treated with the Cd chloride from 0 to 20 μM or treated with MG132 (2.5 μM) or lactacystin (2.5 μM) for 4, 8 and 24 h and harvested. Aliquots of 25 μg protein extract were incubated in 100 μl reaction buffer containing fluorogenic substrates Suc-LLVY-AMC and Z-LLE-AMC. These substrates are used to measure chymotryptic (A) and PGPH (B) activities associated with the proteasome. The assay was incubated at 37°C, and the fluorescence was monitored with spectrofluorimeter. Fluorescent units were converted to p mol of AMC released per 10 μg of protein and incubation time (h) using a standard curve generated with known serial dilutions of AMC. Data are presented as mean percent ± SE, n ≥ 3.

FIG. 7. Dose- and time-dependent inductions of SAPK/JNK and p38 MAPK in SGC. Sertoli cells and gonocytes were isolated by the sequential enzymatic digestion and cocultured for 48 h. Cells were treated with the Cd chloride from 0 to 40 μM or treated with MG132 (2.5 μM) or lactacystin (2.5 μM) for 4, 8 and 24 h and harvested. Cell extracts prepared and subjected to Western blot analysis of the phosphorylation status of SAPK/JNK and p38 as described in the “Materials and Methods” section. Quantification of resulting band intensities was achieved using the “NIH J-Image” software. Quantitative analysis of the phosphorylation of both bands associated with SAPK/JNK (p46/54) is shown in (A) and (B) and p38 in (C). Data are presented as arbitrary units after internal standard correction with β-actin. Each data point represents the mean ± SE of three separate experiments. Statistical significance was determined by ANOVA followed by Tukey-Kramer multiple comparison (*p < 0.05) as compared with the control for each time point.
Cd-induced upregulation of p53 (A, B) and its ubiquitination (C, D) in the SGCs. Sertoli cells and gonocytes were isolated by the sequential enzymatic digestion and cocultured for 48 h. Cells were treated with the Cd chloride from 0 to 20 µM or treated with MG132 (2.5 µM) or lactacystin (2.5 µM) for 24 h and harvested. Cell extracts prepared and subjected to Western blot analysis of p53 (both phosphorylated and total p53, A, B) as well as immunoprecipitation analysis (C, D) as described in the "Materials and Methods" section. To immunoprecipitate p53, whole cell lysates containing 100 µg of total proteins were incubated with 1 µg/ml of p53 polyclonal antibody for 24 h. The separated proteins were blotted onto the nitrocellulose membranes and probed either ubiquitin polyclonal antibody (C) or p53 (D) monoclonal antibody. Figures are the representatives of the three independent experiments.
signal transducers differentially activated in response to a wide variety of extracellular stimuli (Chuang et al., 2000; Figueiredo-Pereira et al., 1998; Garrington and Johnson, 1999; Nebreda and Porras, 2000). Major subfamilies of MAPKs have been described including extracellular signal–regulated kinase, JNK, and p38. In this study, Cd treatments significantly increased level of p-SAPK/JNK in SGC cells even at low concentration (10 μM) at all time points (Fig. 7). However, a significant increase in p-p38 MAPK was only observed at 24 h at Cd concentrations of 10 and 20 μM. This response paralleled the accumulation of HMW-polyUb and induction of apoptosis. The upregulation of p-SAPK/JNK and p-p38 has previously been associated with low-level Cd exposure and subsequent impacts on cell cycle progression and mitotic arrest (Ding and Templeton, 2000; Iryo et al., 2000). To date, the relationship between MAPK activation and the accumulation of HMW-polyUb induced by Cd is unclear. Our study links these two critical responses of Cd-induced toxicity, cellular stress, and UPS disruption, as indicated by the accumulation of HMW-polyUb and the parallel activation of stress responses.

One pivotal target that responds to stress is the tumor suppressor, p53. Normally, the cellular level of p53 is low due to a short protein half-life. In response to a variety of stimuli, however, it is increased steeply by stabilization. This balance between p53 production and degradation is maintained by the UPS and its components (Li et al., 2002; Maki et al., 1996). Impact to the UPS through classical proteasomal inhibitors have been associated with alterations in p53 regulation leading to apoptosis and cell cycle alterations (Chen et al., 2000). In addition, Cd has previously been associated with increased phosphorylation of p53 (Matsuoka and Igisu, 2001). These characteristics highlighted p53 as a possible key component of the mechanistic response to Cd. Our study demonstrates a significant upregulation of p53 and its ubiquitination at the critical dose of 20 μM (Fig. 8). The results seen with p53 in this study further suggest that both the UPS and the proteins involved in stress responses are critical mechanisms of Cd exposure and testicular toxicity.

In summary, the results of the present study demonstrate unequivocally that low doses (10 μM) of Cd lead to an accumulation of HMW-polyUb in conjunction with cytotoxicity. In addition, the accumulation of HMW-polyUb parallels the stress response. Cd exhibited similar properties to the nonspecific proteasomal inhibitor, MG132, resulting in the activation of stress proteins and apoptosis. However, this response was seen at a higher dose with Cd as compared to MG132. This suggests that Cd-induced cytotoxicity may be only partially associated with proteasomal-like inhibition resulting in the accumulation of HMW-polyUb conjugates. Cd-induced alterations to the UPS, however, provide an integrative mechanism that can explain the critical events leading to alterations in key oxidative stress signaling pathways, disruption in cell cycle regulation, and ultimately in spermatogenesis. The UPS might represent a novel and critical key pathway for male reproductive toxicity.

Characterization of this pathway and understanding its role in metal toxicity will improve our ability to prevent the damage caused by both metals and other toxicants that are mediated through the UPS.

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