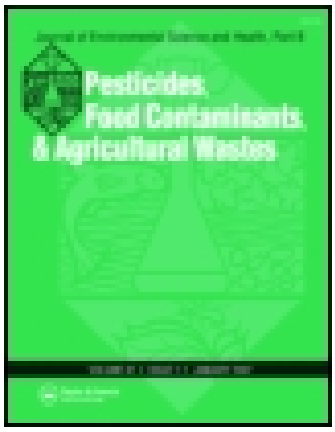

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Eun-Kee Park [a] , Sally K. Mak [b] , Dietmar Kültz [b] & Bruce D. Hammock [a]

[a] Department of Entomology and Cancer Research Center , University of California , Davis, California, USA

[b] Physiological Genomics Group, Department of Animal Science , University of California , Davis, California, USA

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Determination of cytotoxicity of nephrotoxins on murine and human kidney cell lines

EUN-KEE PARK[1], SALLY K. MAK[2], DIETMAR KÜLTZ[2] and BRUCE D. HAMMOCK[1]

[1] *Department of Entomology and Cancer Research Center, University of California, Davis, California, USA*
[2] *Physiological Genomics Group, Department of Animal Science, University of California, Davis, California, USA*

The present study investigates renal inner medullary collecting duct (mIMCD3) cells and human embryonic kidney cells (HEK293) for evaluation of cytotoxicity of nephrotoxic compounds. The 24 h LC_{50} values for cisplatin, paraquat and ibuprofen in mIMCD3 cells were 135, 155 and 3600 μM, respectively. The 24 h LC_{50} values for paraquat and ibuprofen in HEK293 cells were 180 and 1000 μM, respectively. Effects of hyperosmolality on cytotoxicity of paraquat were additive in mIMCD3 cells. These data demonstrate that renal hyperosmolality has an additive effect on cytoxicity of paraquat.

Keywords: mIMCD3 cells; HEK293 cells; paraquat; hyperosmolality.

Introduction

Excessive use of anticancer drugs, nonsteroidal anti-inflammatory drugs (NSAIDs), pesticide and metals can cause renal failure.[1–5] Renal inner medullary cells are often exposed to high concentrations of common nephrotoxic substances and also frequently face hyperosmotic and ischemic stress.[6–7] Renal inner medullary collecting duct (mIMCD3) cells, which are an immortalized cell line derived from mouse renal inner medulla, have been previously investigated for effects of the NSAIDs.[8] However, little is known about cytotoxic effects of drugs and toxicants on mIMCD3 cells despite their wide spread use as a good model for studying renal cell function.

Paraquat, a cationic bipyridylium class herbicide, is a non-selective and non-systemic herbicide known to cause renal failure.[9] Cisplatin[a] platinum-derived chemotherapeutic agent, is widely used in the treatment of various cancers with side effects of renal damage.[10–11] The present study investigated cytotoxicity of nephrotoxins in mIMCD3 and HEK 293 cells. We also tested for additive effects of hyperosmolality in mIMCD3 cells because renal inner medullary collecting duct cells are routinely exposed to hyperosmotic stress *in vivo*.

Address correspondence to Eun-Kee Park, Research and Education Unit, Workers' Compensation Dust Diseases Board, Level 14, 82 Elizabeth St., Sydney, NSW 2000, Australia. E-mail: eunkee.park@ddb.nsw.gov.au
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Materials and methods

Cultures of mIMCD3 and HEK 293 cells

Mouse renal inner medullary collecting duct (mIMCD3) cells of passage 19 and human embryonic kidney (HEK293) cells of passage 35 were used for all experiments. All reagents for cell cultures were purchased from Invitrogen (Carlsbad, CA). Cell culture medium consisted of 45% Ham's F-12, 45% Dulbecco's modified Eagle's medium, 10% fetal bovine serum, 10 milliunits/ml penicillin and 10 μg/ml streptomycin for mIMCD3 cells; for HEK293 cells, 10% heated inactivated fetal bovine serum was used instead of 10% fetal bovine serum. Cells were grown at 37°C in 5% CO_2. Final medium osmolality of the isosmotic medium was 300 ± 5 mosmol/kg medium. An appropriate amount of NaCl was added to the isosmotic medium to make hyperosmotic media of 540 and 605 mosmol/kg. Final osmolality of these media was confirmed by a microosmometer (Model 3300, Advanced Instruments, Norwood, MA).

Compounds

Cisplatin (cis-platinum(II) diammine dichloride) and ibuprofen (2-[4-(2-methylpropyl) phenyl] propanoic acid) were purchased from Sigma (St. Louis, MO). Paraquat dichloride tetrahydrate (1,1′-dimethyl-4,4′ bipyridinium dichloride) was obtained from Chem Service (West Chester, PA). Paraquat was dissolved in deionoized water. Cisplatin and ibuprofen were dissolved in dimethyl sulfoxide (DMSO). Final DMSO concentration did not exceed 1% in

the medium. The solutions of all compounds were prepared just before use.

Determination of cytotoxicity

Cytotoxicity was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) cell survival assay (Roche Applied Science, IN). mIMCD3 and HEK293 cells were grown in 10 cm diameter culture dishes, trypsinized, and seeded evenly with 100 μL of medium into each well of a flat-bottomed 96-well cell culture plate (Nalge-Nunc, Rochester, NY). After they had reached confluence, each compound diluted from a 100-fold stock solution was added to the cell line and incubated in a humidified atmosphere of 5% CO_2 at 37°C, for 24 h. The MTT assay was then performed according to the manufacture's instructions. Briefly, 10 μL final prepared MTT reagent was added to each well and incubated for 4 h, followed by addition of 100 μL of detergent solution. After 24 h incubation, the ratio of absorbance at 560 nm versus 750 nm was measured with a SpectraFluor Plus microplate reader (Tecan, Durham, NC). This ratio represented a measure of viable cells in each well and we normalized this ratio to controls set at 100% that were run in parallel in each 96-well plate. Each condition was repeated in eight wells and experiments were independently replicated between one and four times. Controls consisted of treating cells with the same stock solution used for delivering compounds without any toxins added. Control concentration was 1% in the medium. The concentration at which half of the cells died after 24 h for each of the toxins tested (LC_{50}) was determined.

Statistical analysis

The results are expressed as per cent of cell survival compared to the appropriate control. The results are also presented as mean ± S.E.M. Significance was analyzed with the use of analysis of variance (ANOVA) followed by Bonferroni multiple comparisons post-test. All statistical analyses were performed in GraphPad Prism version 4 (San Diego, CA). A P-value < 0.05 is considered significant.

Results

There was no significant effect of controls on survival of mIMCD3 or HEK293 cells. The 24 h LC_{50} values for cisplatin, paraquat and ibuprofen in mIMCD3 cells presented in Figure 1 were 135, 155 and 3600 μM, respectively. Cisplatin was tolerated by mIMCD3 cells at concentrations up to 100 μM. Only when the ibuprofen concentration exceeded 100 μM, a cytotoxic effect was observed on mIMCD3 cells. The 24 h LC_{50} values for paraquat and ibuprofen in HEK 293 cells presented in Figure 2 were 180 and 1000 μM, respectively, illustrating similar trends in cytotoxicity in the murine mIMCD3 and human kidney cell lines.

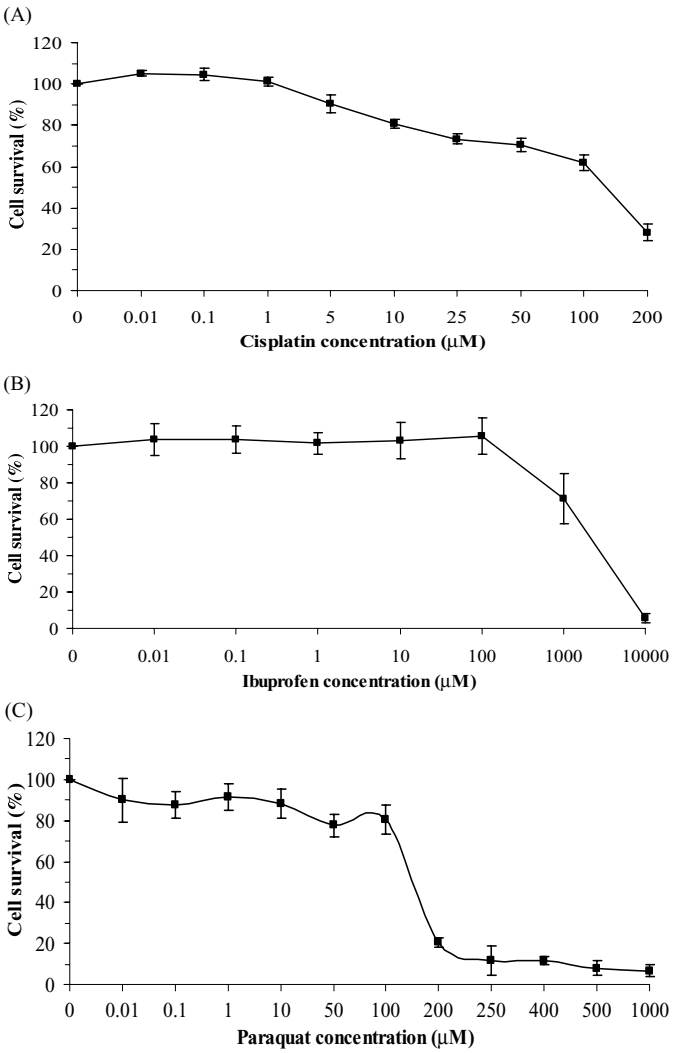

Fig. 1. Cytotoxicity of (A) cisplatin, (B) ibuprofen and (C) paraquat in mIMCD3 cells in normal isosmotic (300 mosmol/kg) medium. Data are expressed as% cell survival compared to control (n = 8, 2–4 independent experiments).

Because *in vivo* kidney cells are exposed to additive toxicant and osmotic stress, the combined effect of hyperosmolality (540 and 605 mosmol/kg) stress and paraquat stress in mIMCD3 cells are presented in Figure 3. Increasing osmolality was shown to reduce cell survival even further, demonstrating that stress caused by hyperosmolality and nephrotoxin are additive ($P < 0.05$).

Discussion

The present study was designed to characterize the response of renal inner medullary cells to clinically prominent nephrotoxins, such as cisplatin (anticancer drug), ibuprofen

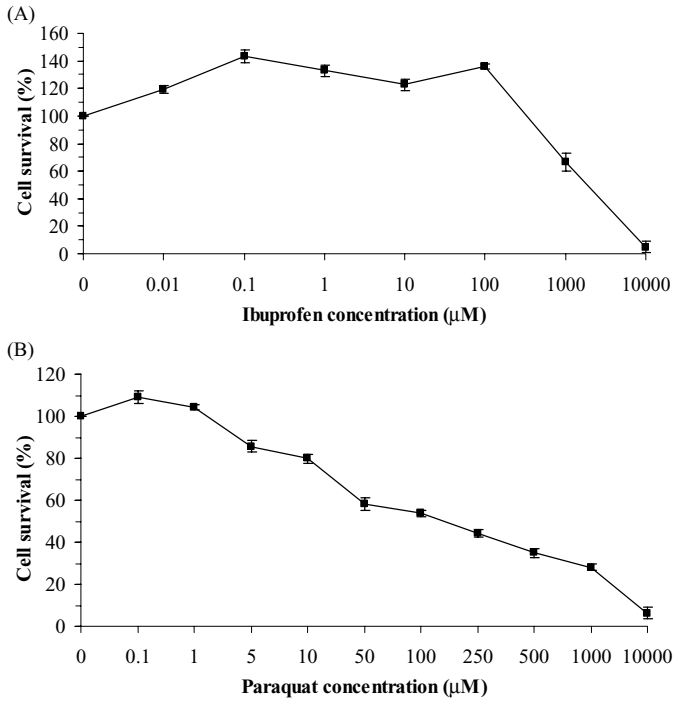

Fig. 2. Cytotoxicity of (A) ibuprofen and (B) paraquat in HEK293 cells in normal isosmotic (300 mosmol/kg) medium. Data are expressed as % cell survival compared to control (n = 8, 1 independent experiment).

(NSAID) and paraquat (herbicide). The colorimetric cell survival assay using the tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a useful method for measuring cell proliferation and survival.[12] The MTT assay measures the reduction of a tetrazolium component (MTT) into an insoluble purple formazan product by the mitochondria of viable cell. This method has been widely used to assess effects of chemotherapeutic agents and pesticides.[13–15]

Cisplatin, a platinum-derived chemotherapeutic agent, had a toxic effect (135 μM of LC_{50}) in mIMCD3 cells in this study. Cisplatin caused apoptosis by generating DNA and protein adducts,[16] and mainly accumulated and promoted the damage of the proximal tubular epithelial cells.[17–18]

Paraquat had the 24 h LC_{50} values of 155 μM in mIMCD3 cells and 180 μM in HEK293 cells. To compare relative toxicity of these toxicants in a different cell line with our data on mIMCD3 cells, HEK293 cells were used. HEK293 cells showed similar paraquat toxicity profile compared to mIMCD3 cells. Toxicity mechanisms underlying paraquat were reported including (1) generation of reactive oxygen species (ROS); (2) the oxidation of NADPH (nicotinamide adenine dinucleotide phosphate) and (3) lipid peroxidation.[19] Direct cellular toxicity of paraquat was examined in other types of kidney cells with 24 h LC_{50} values for proximal renal epithelial cells (LLC-PK1 cells) and Madin Darby Canine Kidney cells (MDCK cells) of 24 μM and 417 μM, respectively.[20]

Ibuprofen had a more toxic effect (1000 μM of LC_{50}) in HEK293 cells than in mIMCD3 cells (3600 μM of LC_{50}). Ibuprofen did not cause any harmful effect on the mIMCD3 cell model in this study. Our results suggest that mIMCD3 cells are not the most appropriate model system for elucidating the physiological mechanism of nephrotoxicity of ibuprofen at least not when the cells are dosed only with this compound.

Our data also show that additive hyperosmotic and toxicant stress (paraquat) is more harmful to renal cells than either stress alone. Santos et al.[21] and Michea et al.[22] reported that acute increases in osmolality to 650mosmol/kg or above caused mIMCD3 cell death This observation is significant because kidney cells are routinely exposed to hyperosmolality *in vivo* and combinations

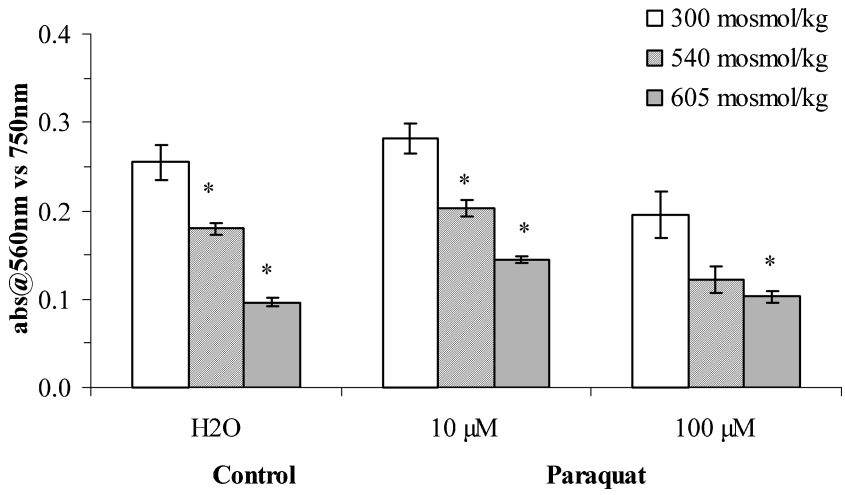

Fig. 3. Effect of combing hyperosmotic stress on cytotoxicity of paraquat in mIMCD3 cells. Data are expressed as the mean ± S.E.M. (n = 8, 1 independent experiment). *, $P < 0.05$ compared to values in 300 mosmol/kg.

of osmotic and toxicant stress are more physiologically relevant than toxicant stress alone. Thus, *in vivo*, kidney cells are likely to be more susceptible to nephrotoxins than in cell culture models.

Taken together, the results of the present study demonstrate that mIMCD3 cells are shown to be a valuable model for studying as the mechanism of other clinically important nephrotoxins (cisplatin, ibuprofen, paraquat). Finally, additive effects of hyperosmolality and toxicant stress on renal cells underline the importance of studying effects of nephrotoxic compounds in cell culture models under conditions that approximate the physiological milieu in the kidney as closely as possible.

Acknowledgments

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References

[1] Kintzel, P.E. Anticancer drug-induced kidney disorders. Drug Saf. **2001**, *24* (1), 19–38.
[2] Arany, I.; Safirstein, R.L. Cisplatin nephrotoxicity. Semin. Nephrol. **2003**, *23* (5), 460–464.
[3] Perazella, M.A. Drug-induced renal failure: Update on new medications and unique mechanisms of nephrotoxicity. Am. J. Med. Sci. **2003**, *325* (6), 349–362.
[4] Barbier, O.; Jacquillet, G.; Tauc, M.; Cougnon, M.; Poujeol, P. Effect of heavy metals on, and handling by, the kidney. Nephron Physiol. **2005**, *99* (4), 105–110.
[5] Rahman, A.; White, R.M. Cytotoxic anticancer agents and renal impairment study: The challenge remains. J. Clin. Oncol. **2006**, *24* (4), 533–536.
[6] Burg, M.B. Response of renal inner medullary epithelial cells to osmotic stress. Comp. Biochem. Physiol. Part A. Mol. Integr. Physiol. **2002**, *133* (3), 661–666.
[7] Lee, W.Y.; Jang, S.B.; Cha, S.H.; Lee, J.H.; Lee, K.H.; Kim, J.; Jo, Y.H.; Endou, H. Different sensitivity to nephrotoxic agents and osmotic stress in proximal tubular and collecting duct cell lines derived from transgenic mice. Toxicol. In Vitro **2002**, *16* (1), 55–62.
[8] Rocha, G.M.; Michea, L.F.; Peters, E.M.; Kirby, M.; Xu, Y.; Ferguson, D.R.; Burg, M.B. Direct toxicity of nonsteroidal antinflammatory drugs for renal medullary cells. Proc. Natl. Acad. Sci. USA **2001**, *98* (9), 5317–5322.
[9] Houze, P.; Baud, F.J.; Mouy, R.; Bismuth, C.; Bourdon, R.; Scherrmann, J.M. Toxicokinetics of paraquat in Humans. Human Exp. Toxicol. **1990**, *9* (1), 5–12.
[10] Mollman, J.E.; Glover, D.J.; Hogan, W.M.; Furman, R.E. Cisplatin neuropathy. Risk factors, prognosis, and protection by WR-2721. Cancer **1988**, *61* (11), 2192–2195.
[11] Gogas, H.; Shapiro, F.; Aghajanian, C.; Fennelly, D.; Almadrones, L.; Hoskins, W.J.; Spriggs, D.R. The impact of diabetes mellitus on the toxicity of therapy for advanced ovarian cancer. Gynecol. Oncol. **1996**, *61* (1), 22–26.
[12] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods **1983**, *65* (1–2), 55–63.
[13] Carmichael, J.; Mitchell, J.B.; Degraff, W.G.; Gamson, J.; Gazdar, A.F.; Johnson, B.E.; Glatstein, E.; Minna, J.D. Chemosensitivity testing of human lung cancer cell lines using the MTT assay. Br. J. Cancer **1988**, *57* (6), 540–547.
[14] Arnould, R.; Dubios, J.; Abikhalil, F.; Libert, A.; Ghanem, G.; Atassi, G.; Hanoco, M.; Lejeune, F.J. Comparison of two cytotoxicity assays – tetrazolium derivate reduction (MTT) and tritiated thymidine uptake – on three malignant mouse cell lines using chemotherapeutic agents and investigational drugs. Anticancer Res. **1990**, *10* (1), 145–154.
[15] Schaaf, A.; Sagi, S.; Langbein, S.; Trojan, L.; Alken, P.; Michel, M.S. Cytotoxicity of cisplatin in bladder cancer is significantly enhanced by application of bcl-2 antisense oligonucleotides. Urol. Oncol. **2004**, *22* (3), 188–192.
[16] Chu, G. Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. J. Biol. Chem. **1994**, *269* (2), 787–790.
[17] Safirstein, R.; Winston, J.; Goldstein, M.; Moel, D.; Dikman, S.; Guttenplan, J. Cisplatin nephrotoxicity. Am. J. Kidney Dis. **1986**, *8* (5), 356–367.
[18] Safirstein, R.; Winston, J.; Moel, D.; Dikman, S.; Guttenplan, J. Cisplatin nephrotoxicity: Insights into mechanism. Int. J. Androl. **1987**, *10* (1), 325–346.
[19] Suntres, Z.E. Role of antioxidants in paraquat toxicity. Toxicology **2002**, *180* (1), 65–77. Thadhani, R.; Pascual, M.; Bonventre, J.V. Acute renal failure. N. Engl. J. Med. **1996**, *334* (22), 1448–1460.
[20] Chan, B.S.; Lazzaro, V.A.; Seale, J.P.; Duggin, G.G. The renal excretory mechanisms and the role of organic cations in modulating the renal handling of paraquat. Pharmacol. Ther. **1998**, *79* (3), 193–203.
[21] Santos, B.C.; Chevaile, A.; Hebert, M.J.; Zagajeski, J.; Gullans, S.R. A combination of NaCl and urea enhances survival of IMCD cells to hyperosmolality. Am. J. Physiol. **1998**, *274* (6), F1167–F1173.
[22] Michea, L.; Ferguson, D.R.; Peters, E.M.; Andrews, P.M.; Kirby, M.R.; Burg, M.B. Cell cycle delay and apoptosis are induced by high salt and urea in renal medullary cells. Am. J. Physiol. Renal Physiol. **2000**, *278* (2), F209–F218.