

Short Communication

A possible critical dosing period of p-cumylphenol for development of cystic kidneys in rat neonates

Tomomi Nakazawa^{*}, Yuko Yamaguchi², Yachiyo Fukunaga¹, and Kazutoshi Tamura²

¹ Pathology Division, Tsukuba Research Institute, Bozo Research Center Inc., 8 Okubo, Tsukuba-shi, Ibaraki 300-2611, Japan

² Pathology Division, Gotemba Research Institute, Bozo Research Center Inc., 1284 Kamado, Gotemba, Shizuoka 412-0039, Japan

Abstract: In accordance with a previous report on cystic kidneys induced in rat neonates when dosed with p-cumylphenol (PCP) for 18 days from postnatal day (PND) 4, 3 rat neonates were dosed with PCP once a day for 14 days, either from PND 14, 21, 28, 35, or 42 as W2, W3, W4, W5, and W6 groups, respectively, to investigate whether dosing periods in different PNDs influenced the development of cystic renal tubules. The lesion was striking in the W2 group and at a lesser magnitude in the W3 group, whereas either kidney was unaffected when dosing was initiated beyond PND 28. These findings, together with the results from the previous study, suggested that PND 14–28 is a critical dosing period for PCP to develop cystic kidneys in rat neonates. The lining epithelium of the cystic tubules was immunohistochemically positive for AQP2. This finding and the anatomical location indicated that the cystic tubules were of collecting duct origin. Either obstruction, fluid accumulation, or reparative hyperplasia of the lining epithelium was unlikely to be involved in the formation of cystic tubules lined with a monolayer of cuboidal or columnar epithelium with a high nuclear density. Thus, the follow-up investigation on PCP suggested a critical dosing period of PND 14–28 in rat neonates for the development of cystic dilation of renal collecting ducts. This study further supports that additive hyperplasia of the lining epithelium is a fundamental basis of this unique lesion. (DOI: 10.1293/tox.2021-0010; J Toxicol Pathol 2022; 35: 123–127)

Key words: cystic kidney, rat, critical dosing period, p-cumylphenol

Cystic kidney diseases in humans encompass diverse conditions of cyst formation in glomeruli and tubules with a wide range of classifications based on genetic alterations¹. Autosomal recessive polycystic kidney disease, autosomal dominant polycystic kidney disease, and autosomal dominant tubulointerstitial kidney disease belong to the hereditary group^{2, 3}. Acquired cystic kidney diseases are known to occur in patients with chronic renal disease⁴. Chemically induced cystic kidneys, another type of cystic kidney disease, is found in animal species^{5, 6}. They are associated with a wide spectrum of chemical groups, including p-cumylphenol (PCP), a chemical used as lubricants, surfactants, insecticides and phenolic resins⁷. A previous investigation focused on morphologically characterizing cystic kidneys induced in rat neonates when dosed with PCP for 18 days from postnatal day (PND) 4 revealed that treatment with PCP resulted in the sequential formation of cystic tubules in rat neonates⁸. A follow-up investigation was carried out

to determine the critical dosing period for PCP to develop cystic kidneys in rat neonates.

Eleven male and 11 female Crl:(CD)SD rats purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan) were mated, and 46 male neonates were obtained from the same birth date. The date of birth was defined as a PND of 0. On PND 7, all the neonates were separated from dams, and then selected 10 each were fostered by 4 dams, respectively, to standardize body weights. Finally, 25 neonates were further selected and allocated to 5 groups of 5 each by stratified random sampling based on body weight. All neonates were weaned on PND 21.

In these 5 study groups, 3 animals each were orally dosed with 300 mg/kg/day of PCP (Sun Techno Chemical Inc., Tokyo, Japan) dissolved in olive oil and the remaining 2 animals each with olive oil alone to serve as the vehicle controls at a dose volume of 10 mL/kg once a day for 14 days, either from PND 14, 21, 28, 35, or 42. Thus, these 5 test groups were designated as the W2, W3, W4, W5, and W6 groups, respectively (Fig. 1). The dose level and dosing schedule of PCP were designed in reference to the results obtained from the previous study where histopathological examinations on PND 8, 12, 19, and 22 revealed that the development of cystic kidneys was confined to PND 19 and thereafter following daily PCP dosing from PND 4 at 300 mg/kg/day⁸. All the animals were housed in an environmentally controlled room and were allowed free access to a sterilized basal diet (CRF-1, Oriental Yeast Co. Ltd., Tokyo,

Received: 22 June 2021, Accepted: 30 August 2021

Published online in J-STAGE: 24 September 2021

*Corresponding author: T Nakazawa

(e-mail: nakazawa-tomomi@bozo.co.jp)

©2022 The Japanese Society of Toxicologic Pathology

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives

(by-nc-nd) License. (CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>).



Japan) and tap water. Clinical signs and body weight were checked periodically throughout the experimental period.

The animals were euthanized by exsanguination under deep ether anesthesia on the day following the 14th daily administration. The kidneys were sampled, weighed, fixed in 10% neutrally buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin using a standard procedure for microscopic examination. The sections were also immunohistochemically stained for AQP2 (LifeSpan BioSciences Inc., Seattle, WA, USA) to investigate cytotogenesis of the cystic tubules.

The experiment was approved by the Institutional Animal Care and Use Committee, BoZo Research Center Inc., and was carried out in full compliance with pertinent public laws and guidelines, as well as the institutional standard of Code of Conduct in Animal Experiment at BoZo Research Center Inc. to meet the concept of animal welfare.

All the animals survived to the scheduled necropsy. No abnormal clinical signs were observed in either the control or PCP-treated animals throughout the experimental period. Body weight gain was slightly suppressed in PCP-treated animals in the W2 and W3 groups. Absolute and relative kidney weights increased compared to the controls, and multiple cysts were found at necropsy in 1 of 3 PCP-treated animals in the W2 group.

The histopathological findings observed in the PCP-treated animals are shown in Table 1 and Figs. 2 and 4.

In the W2 group dosed during PND 14–28, cystic dilation was striking in a number of tubules primarily within

the outer medulla, yielding a polycystic structure that replaced nearly the entire outer medulla (Fig. 2). Immunohistochemical staining for AQP2 was positive in these cystic tubules (Fig. 2). This finding and the anatomical location within the kidneys indicated that the dilated tubules were of the collecting duct origin^{9, 10}. The cystic ducts were lined with a monolayer of epithelia resting on the basement membrane. Examinations of serial sections clarified that the cystic ducts were connected to the non-cystic ducts (Fig. 3); therefore, they shared the lumen where findings suggestive of obstruction, cellular debris, or urinary casts were absent, as were in any other regions of the kidney. The cysts did not grow, compressing the surrounding non-cystic tubules (Fig. 3). A few single-cell necroses and mitosis were scattered in the cystic epithelium. The nuclear density of the lining epithelium was far greater in the cystic ducts than in the non-cystic ducts (Fig. 3). Consistent with the high nuclear density, PCNA immunohistochemistry was strongly positive in the cystic epithelium as compared to the adjacent tubules⁸. However, no polypoid or papillary proliferation was noted, and the hyperplastic lining epithelium remained in a cuboidal to columnar form within a simple monolayer. The other segments of the nephron were spared from cystic lesions.

In the W3 group dosed during PND 21–35, cystic collecting ducts were also observed (Fig. 4). The ducts were decreased in number, lined in part by a flattened epithelial layer, and had a rounded contour compared to the W2 group. More significantly, these morphological features resembled kidney findings following 1 week cessation of dosing with PCP⁸. This suggested that the last 1 week during PND 21–35 was consistent with a non-dosing period.

In the W4 and W5 groups, all the kidneys were morphologically not remarkable, and cystic tubular changes observed in the W2 and W3 groups were no longer observed. These results were in agreement with the concept that PCP dosing was ineffective during PND 28–35 (last 1 week) in the W3 group.

The W6 group was excluded from histopathological examinations because of the lack of cystic lesions in the W4 and W5 groups.

Therefore, administration of PCP resulted in cystic dilation of the collecting ducts, leading to a polycystic appearance in the kidneys of rat neonates. This morphological alteration was most evident when dosed during PND 14–28

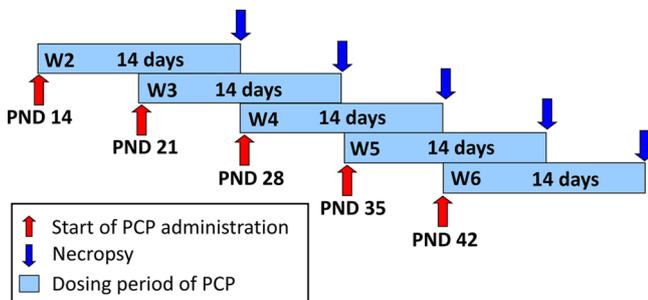


Fig. 1. Study design. PCP was administered at 300 mg/kg/day once a day for 14 days, either from postnatal week 2, 3, 4, 5, or 6 for the W2, W3, W4, W5, and W6 groups, respectively.

Table 1. Severity of Histopathological Findings of the Kidney

Region	Findings	Group				
		W2	W3	W4	W5	W6*
Outer medulla	Cystic dilation, collecting duct, lined with hyperplastic epithelium	4	2	0	0	/
	Single cell necrosis collecting duct	1	1	0	0	/
	Mitosis, collecting duct	1	1	0	0	/
Inner medulla	Dilation, collecting duct	1	0	0	0	/
Papilla	Dilation, papillary duct	2	1	0	0	/

Grades: 0=No remarkable change, 1=minimal, 2=mild, 3=moderate, 4=severe.

*: W6 group was not examined because no remarkable change was observed in W4 and 5 groups.

and induced to a lesser extent during PND 21-35. In contrast, all the kidneys remained unaffected when dosing was initiated beyond PND 28. These findings imply a critical dosing period for PCP to develop cystic kidneys in rat neonates. Taken together, the following results of the dosing period

with PCP in relation to development of cystic kidneys, PND 4-12 = no development⁸; PND 14-28 = most prominent; PND 21-35 = minor degree with similar morphology to the kidney at 1 week post-dosing period; and beyond PND 28 = no development, the critical dosing period likely resides during PND 14-28 (Fig. 5).

The rat kidneys keep growing after birth, during which many renal events of anatomical and functional development are known to occur. The kidneys continue to differentiate into morphologically more mature kidneys up until PND 11-15¹¹, while tubular differentiation still continues un-

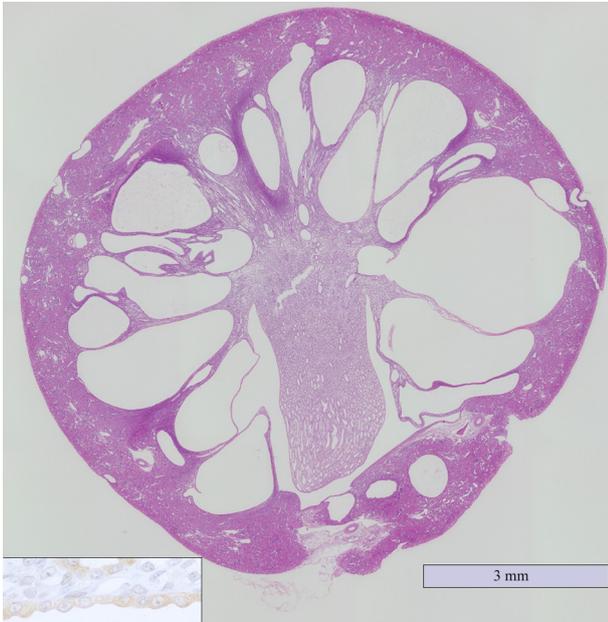


Fig. 2. A PCP-treated kidney in the W2 group. Hematoxylin and eosin. Distinctive and striking cystic appearance. Inset (Lower left): Immunohistochemistry for AQP2. The lining epithelium of the cyst is positive for AQP2.

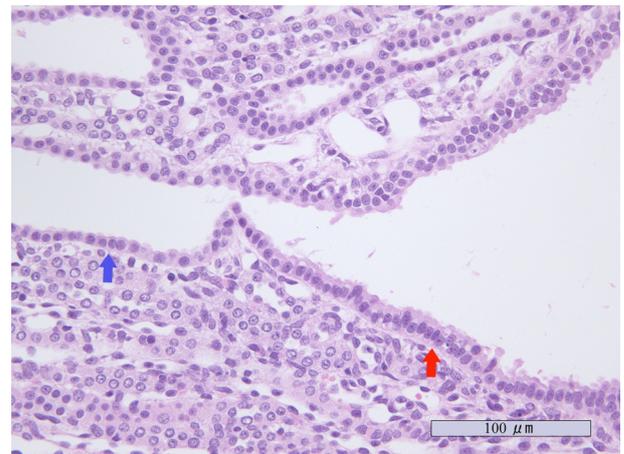


Fig. 3. A PCP-treated kidney in the W2 group. A junction of the cystic duct and non-cystic duct. Hematoxylin and eosin. Red arrow: the lining epithelium of a cystic duct with a high nuclear density. Blue arrow: the lining epithelium of a non-cystic duct.



Fig. 4. A PCP-treated kidney in the W3 group. Hematoxylin and eosin. Scattered cyst formation.

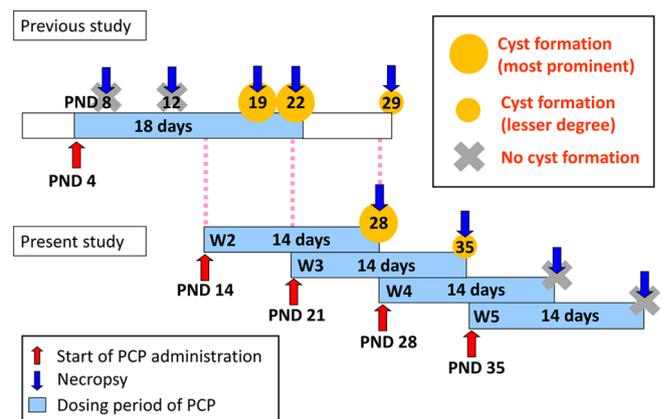


Fig. 5. Schematic results of the previous and present studies. PCP was administered at 300 mg/kg/day once a day for 18 days from PND 4 in the previous study, and for 14 days from PND 14, 21, 28, or 35, for the W2, W3, W4, and W5 groups, respectively, in the present study. Cystic kidneys were most prominent on PND 19 and 22 in the previous study, and on PND 28 in the present study. In comparison, they were absent on PND 8 and 12 in the previous study, or on PND 42 and thereafter in the present study.

til the time of weaning on PND 21, yielding a well-defined structure of the inner and outer medullas¹¹. Renal vasculogenesis is not completed until PND 17-19¹². Renal blood flow reaches full maturation during PND 16-24¹². The glomerular filtration rate and tubular secretion are also functionally developed by PND 21¹³. These periods of postnatal nephrogenesis coincide with the critical dosing period for the development of cystic kidneys in rat neonates treated with PCP.

The lining epithelium of cystic ducts remained cuboidal and columnar with a high nuclear density in the W2 group, indicating that the cysts were unlikely to be formed due to fluid accumulation, leading to expansion of the ductal lumen. Various sized polypoid hyperplasia preceded by cell proliferation of the tubular epithelium may obstruct the lumen, and the resultant increase in resistance to the outflow of tubular urine leads to cyst formation¹⁴. This process was unlikely in the PCP-induced cystic kidneys in rat neonates, since hyperplastic duct epithelia remained as a monolayer that never projected into the ductal lumen.

Acquired cystic kidney is associated with chronic nephropathy and end-stage renal disease in humans^{4, 15}, where cell proliferation is an intriguing finding¹⁶. Epithelial proliferation is a major element in the development of cystic kidneys¹⁷. Kidney development occurs as a unifying process through interactions between the ureteric bud and metanephric mesenchyme, and epithelial growth factor (EGF) plays an integral role in maintaining collecting duct morphogenesis¹⁸. Estrogen receptors are expressed and contribute to the regulation of several physiological functions in kidneys¹⁹. Crosstalk between estrogen receptors and EGF receptors may augment estrogen and growth factor action²⁰. Ciliopathy is implicated in the development of cystic kidneys, and aberrant cell proliferation is involved in cystic dilation of renal tubules²¹. All these facts and results showing the cystic epithelium with a high nuclear density and positive PCNA immunohistochemistry⁸ support the notion that cell proliferation plays a critical role in the development of cystic kidneys in rat neonates treated with PCP. Reparative cell proliferation is unlikely to be a major element since single-cell necrosis was of minor degree and no cell debris was found in any of the cystic lumen. Thus, the cell kinetics of additive cell proliferation mediated by growth factors may be a target of investigation to elucidate the pathway of PCP-induced cystic kidneys in rat neonates.

In conclusion, the present study suggested that in rat neonates, the development of cystic kidneys associated with PCP dosing was closely linked to the critical dosing period of PND 14-28, and that the cystic tubules were of the collecting duct origin, further strengthening the hypothesis that additive cell proliferation constitutes a fundamental basis for the development of this unique lesion.

Disclosure of Potential Conflicts of Interest: The authors declare no conflicts of interest.

Acknowledgments: The authors are grateful to Megumi Yahata, Masanobu Uematsu, and Akihito Watanabe for their technical support.

References

1. Bisceglia M, Galliani CA, Senger C, Stallone C, and Sessa A. Renal cystic diseases: a review. *Adv Anat Pathol*. **13**: 26–56. 2006. [Medline] [CrossRef]
2. Sweeney WE Jr, and Avner ED. Molecular and cellular pathophysiology of autosomal recessive polycystic kidney disease (ARPKD). *Cell Tissue Res*. **326**: 671–685. 2006. [Medline] [CrossRef]
3. Eckardt K-U, Alper SL, Antignac C, Bleyer AJ, Chauveau D, Dahan K, Deltas C, Hosking A, Kmoch S, Rampoldi L, Wiesener M, Wolf MT, Devuyst O. Kidney Disease: Improving Global Outcomes Autosomal dominant tubulointerstitial kidney disease: diagnosis, classification, and management—a KDIGO consensus report. *Kidney Int*. **88**: 676–683. 2015. [Medline] [CrossRef]
4. Nahm A-M, and Ritz E. Acquired renal cysts. *Nephrol Dial Transplant*. **16**: 1506–1508. 2001. [Medline] [CrossRef]
5. Doby DC, Hill D, Lewis T, and Bulger RE. Cyst formation in rat kidney induced by cis-platinum administration. *Lab Invest*. **45**: 260–268. 1981. [Medline]
6. Ito F, Toma H, Yamaguchi Y, Nakazawa H, Onitsuka S, and Hashimoto Y. A rat model of chemical-induced polycystic kidney disease with multistage tumors. *Nephron*. **79**: 73–79. 1998. [Medline] [CrossRef]
7. National Institutes of Health: Compound Summary 4-Cumylphenol. PubChem: <https://pubchem.ncbi.nlm.nih.gov/>
8. Nakazawa T, Kasahara K, Ikezaki S, Yamaguchi Y, Edamoto H, Nishimura N, Yahata M, Tamura K, Kamata E, Ema M, and Hasegawa R. Renal tubular cyst formation in newborn rats treated with p-cumylphenol. *J Toxicol Pathol*. **22**: 125–131. 2009. [Medline] [CrossRef]
9. Rojek A, Führtbauer EM, Kwon TH, Frøkiaer J, and Nielsen S. Severe urinary concentrating defect in renal collecting duct-selective AQP2 conditional-knockout mice. *Proc Natl Acad Sci USA*. **103**: 6037–6042. 2006. [Medline] [CrossRef]
10. Loffing J, and Korbmayer C. Regulated sodium transport in the renal connecting tubule (CNT) via the epithelial sodium channel (ENaC). *Pflugers Arch*. **458**: 111–135. 2009. [Medline] [CrossRef]
11. Zoetis T, and Hurtt ME. Species comparison of anatomical and functional renal development. *Birth Defects Res B Dev Reprod Toxicol*. **68**: 111–120. 2003; (Part B). [Medline] [CrossRef]
12. Bueters R, Bael A, Gasthuys E, Chen C, Schreuder MF, and Frazier KS. Ontogeny and cross-species comparison of pathways involved in drug absorption, distribution, metabolism, and excretion in neonates (review): kidney. *Drug Metab Dispos*. **48**: 353–367. 2020. [Medline] [CrossRef]
13. Snodgrass WR. Physiological and biochemical differences between children and adults as determinants of toxic response to environmental pollutants. In: *Similarities and Differences between Children and Adults, Implications for Risk Assessment*. PS Guzelian, CJ Henry, and SS Olin. (eds), ILSI Press, Washington DC. 35–42. 1992.

14. Evan AP, Gardner KD Jr, and Bernstein J. Polypoid and papillary epithelial hyperplasia: a potential cause of ductal obstruction in adult polycystic disease. *Kidney Int.* **16**: 743–750. 1979. [[Medline](#)] [[CrossRef](#)]
15. Chan EYH, and Warady BA. Acquired cystic kidney disease. In: *Pediatric Dialysis Case Studies*. B Warady, F Schaefer, S Alexander (eds). Springer, Cham. 335–341. 2017.
16. Mendley SR, and Toback FG. Cell proliferation in the end-stage kidney. *Am J Kidney Dis.* **16**: 80–84. 1990. [[Medline](#)] [[CrossRef](#)]
17. Fick GM, and Gabow PA. Hereditary and acquired cystic disease of the kidney. *Kidney Int.* **46**: 951–964. 1994. [[Medline](#)] [[CrossRef](#)]
18. Chen J, Zeng F, Forrester SJ, Eguchi S, Zhang M-Z, and Harris RC. Expression and function of the epidermal growth factor receptor in physiology and disease. *Physiol Rev.* **96**: 1025–1069. 2016. [[Medline](#)] [[CrossRef](#)]
19. Yanes LL, Sartori-Valinotti JC, and Reckelhoff JF. Sex steroids and renal disease: lessons from animal studies. *Hypertension.* **51**: 976–981. 2008. [[Medline](#)] [[CrossRef](#)]
20. Levin ER. Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor. *Mol Endocrinol.* **17**: 309–317. 2003. [[Medline](#)] [[CrossRef](#)]
21. Kathem SH, Mohieldin AM, and Nauli SM. The roles of primary cilia in polycystic kidney disease. *AIMS Mol Sci.* **1**: 27–46. 2014. [[Medline](#)] [[CrossRef](#)]