Chronic Progressive Nephropathy (CPN) in the Rat: Review of Pathology and Relationship to Renal Tumorigenesis

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Abstract: Chronic progressive nephropathy (CPN) is a rodent-specific, age-related renal disease, particularly of male rats, characterized by a spectrum of distinct histological changes which may begin early in the animal’s life and progress to end-stage renal disease in certain rat strains. Although CPN-related pathology is well known to most toxicological pathologists other features of CPN such as pathogenesis, modulating factors, proliferative nature, response to chemical exposure and relationship to tumorigenesis are less clearly acknowledged. CPN is generally regarded as a degenerative to atrophic disease with compensatory regenerative hyperplasia. The proliferative nature of CPN often becomes problematic in advanced to end-stage renal disease. At this stage, a number of tubule profiles may be mistaken for atypical tubule hyperplasia, the reported precursor lesion of tubule adenoma. CPN associated proliferative tubule profiles must be carefully separated from atypical tubule hyperplasia particularly in studies where chemical exposure has exacerbated CPN. Over the past several years increasing evidence has supported the hypothesis that CPN may be regarded as a type of “mode of action” during renal carcinogenesis in rodent bioassay studies. Retrospective studies of control and treated animals have consistently shown a relationship between the increased severity of CPN and the presence of atypical tubule hyperplasia and small, incipient renal adenomas. Understanding CPN-related tumorigenesis is important for human risk assessment interpretation. Since CPN is a rodent specific disease with no apparent similar human kidney disease condition, evidence that renal tumors may arise from an interaction with CPN could assist regulatory agencies in interpreting data from studies with exacerbated CPN. (J Toxicol Pathol 2008; 21: 199–205)

Key words: rat, kidney, chronic progressive nephropathy, pathology, tumorigenesis, risk assessment

Introduction and Overview

Chronic progressive nephropathy (CPN) is one of the most widely recognized disease entities in rodent preclinical studies. CPN is a common spontaneous age-related disease of rodent kidneys, particularly in rats\(^1\)–\(^3\). Because of the kidney’s central role in maintaining the normal physiologic balance of the body’s internal environment and its importance in drug metabolism and excretion, understanding the basic mechanisms and pathology of CPN is critical. The etiology of CPN remains unknown. In some models of nephropathy, speculation regarding hemodynamic alterations leading to hyperperfusion and hyperfiltration of macromolecules within the glomerulus resulting in mesangial overload and glomerulosclerosis has been postulated\(^4\). However, hemodynamic alterations do not seem to be associated with CPN\(^5\). There are strain, sex, and age differences with respect to the incidence and severity of CPN associated lesions\(^6\)–\(^10\). Sprague-Dawley and F344 rats generally have an earlier onset and higher incidence and severity than the Wistar, Brown Norway and Long-Evans rat strains. Although the term CPN has undergone a number of name changes over the years, currently CPN or “nephropathy” are synonymous terms and recommended by standardized nomenclature classifications.

Pathologists beginning their career in toxicological pathology are quickly exposed to the spectrum of CPN associated lesions such as regenerative (basophilic) tubules surrounded by thickened basement membranes, hyaline proteinaceous casts, glomerulosclerosis, interstitial fibrosis and infiltration of mononuclear inflammatory cells which can begin as early as 2–3 months of age and progress continually through the life-span of the animal. Histological changes associated with CPN also result in a number of functional changes that result in increased proteinuria and decreased urine concentration ability\(^11\),\(^12\).

Most pathologists agree that the earliest CPN lesion, by light microscopy, is represented by small cross sections of basophilic tubules surrounded by a thickened basement membrane in the renal cortex. Only later does another one of the hallmarks of CPN, glomerulosclerosis, become
evident. By the end of 2-year carcinogenic studies, the advanced stage of CPN may result in end-stage kidney and death of the animal due to chronic renal failure. In fact, mortality associated with CPN is an issue with serious implications in the conduct of some carcinogenic studies. CPN may be modulated by a number of factors including diet, administration of hormones and many other experimental manipulations. Recently, the type of diet particularly with regard to the protein content and/or caloric restriction have been investigated as ways to reduce the severity or limit the progression of CPN, thereby, extending the life of the animal.

CPN has been described as a degenerative to atrophic disease with compensatory hypertrophy and hyperplasia. The proliferative rate of CPN has been demonstrated by cell labeling studies, which have shown cell proliferative rates within CPN affected tubules increased over that of normal tubules. In advanced stages of nephropathy, a number of proliferative tubule profiles may be recognized as having an increased number of lining epithelial cells. These tubule profiles are problematic for many pathologists as they may be mistaken for atypical tubule hyperplasia (ATH), the precursor lesion of renal adenoma. Hard and Seely recently published recommendations to assist in the differentiation of CPN tubules from actual ATH and early renal tubule adenomas. An association between the presence of ATH and renal tubule tumors (RTT) has been observed from several carcinogenic studies in which chemical administration exacerbated the severity of CPN. Retrospective studies of control and treated animals have consistently shown a relationship between the increased severity of CPN and the presence of ATH and RTT. The underlying factors associated with this relationship are not known but, most likely, are multi-factorial and complex.

The interpretation of a renal tumorigenic response related to CPN is important for risk assessment analysis since apparently there is no human renal disease which is similar to CPN. Therefore, it has recently been postulated that RTT linked to CPN has no relevance for extrapolation to human risk assessment because CPN is a rodent-specific disease. This review was written primarily for toxicologic pathologists, especially study pathologists, who would like a concise yet instructive review of CPN with emphasis on pathology and pathology-related issues. Furthermore, this review is not intended to be a comprehensive overview of all aspects of CPN. Current investigations of CPN associated tumorigenesis are also presented. These studies have been the interest of the authors for some time. This postulated carcinogenic “Mode of Action” is unique and undoubtedly will have to undergo further examination and review by both researchers and by federal agencies responsible for chemical and drug regulation.

Histological Spectrum of CPN Pathology

As noted previously, CPN is characterized by a spectrum of histological changes including regenerative tubules (basophilic tubules) lined by a thickened basement, the presence of eosinophilic hyaline proteinaceous tubule casts, glomerulosclerosis, interstitial fibrosis and infiltration of a variable mononuclear cell infiltrate. In recent studies, it has been reported that the earliest change by light microscopy of H&E stained sections is the presence of one or more cortical solitary basophilic tubule cross sections surrounded by a thickened basement membrane (Fig. 1). The thickened basement membrane is one of the key components of CPN throughout the course of the disease. The presence of this change is helpful in distinguishing early CPN and regeneration due to toxic insult. If one examines CPN-affected kidneys in detail, degenerative to apoptotic cells, as well as mitotic figures may be evident and responsible for the regenerative appearance. However, in most instances, these changes are not fully appreciated unless thorough examination under higher power is conducted. In some instances, affected tubules may appear as slightly dilated. Therefore, the tubule appearance is due to the presence of increased numbers of lining epithelial cells with basophilic cytoplasm and crowded, slightly enlarged and vesicular nuclei, all responsible for the overall basophilia noted on H&E staining. As the number of affected tubules increase, a minimal infiltration of mononuclear cells becomes evident within the interstitium. At this time, a few eosinophilic hyaline tubule casts may be noted in Henlé’s loops within the medulla. Hyaline casts are observed in the absence of basophilic tubules, early CPN is still usually suspected because affected CPN tubules in the cortex are most likely out of the plane of section. Shortly thereafter, changes within affected glomeruli may be noted. These changes reflect the early segmental and basement membrane thickening of the glomerular tuft and Bowman’s capsule. Occasionally, a lightly eosinophilic staining and amorphous material may be present in Bowman’s space. Variably-sized, eosinophilic protein droplets in sporadic tubules may also be observed. Proteinuria or the loss of urinary protein in the urine, associated with CPN, is best determined by measuring urinary albumin excretion. By electron microscopy, it has been reported that glomerular injury may be observed. However, there is no clear evidence that these changes precede the earliest tubule changes.

Among pathologists, discussion regarding the earliest lesion, namely the basophilic tubules, often causes debate on the most correct terminology pathologists should use to describe this change. Some pathologists refer to all changes associated with CPN as “CPN or nephropathy” while others diagnose either “basophilic tubules” or “tubule regeneration”. Because “basophilic tubules” may result from a number of differing pathogenic mechanisms, the authors do not recommend the use of “basophilic tubules” as a descriptor for the earliest lesion. However, if “basophilic tubule” is used then it is suggested that this term be defined in the narrative portion of the pathology report with regard to its association with CPN. Furthermore, it is highly recommended not to separate and grade each component of CPN individually. This effort requires considerable
diagnostic consistency and time and often results in lengthy and confusing incidence tables which add little to the overall interpretation of a chemical effect. All of the components of CPN should be grouped together as CPN or alternately, as nephropathy.

As the animal ages, the extent within the kidney and the severity of CPN-related areas increase noticeably. Minimal changes usually result in multiple foci of CPN-affected areas randomly found throughout the cortex (Fig. 2). As the disease progresses, hyaline casts become more prominent and are seen extending down through the outer to inner medulla within Henle’s loop. With increasing severity, foci of CPN-affected areas begin to merge and become confluent with each other resulting in a more diffuse change. Continued progression of CPN results in striking tubule, glomerular and interstitial changes. Foci of basophilic tubules are remarkable due to increased amounts of thickened basement membrane material. An occasional cortical tubule becomes hypertrophic with increased amounts of pale eosinophilic staining cytoplasm. Glomeruli may either appear hypertrophic or atrophic due to glomerulosclerosis and the presence of adhesions between the glomerular tuft and Bowman’s capsule. The presence of interstitial fibrosis often results in tubule dilatation and/or cyst formation. This phenomenon appears to be the result of interactions between fibrogenic growth factors produced by tubule epithelial cells, macrophages and myofibroblasts. In “End-Stage” CPN hyaline casts may also be seen in collecting ducts. In advanced to end-stage CPN, little normal renal cortical tissue remains (Fig. 3). Grossly, these kidneys are enlarged, pale and with a pitted surface. Animals that have died from end-stage renal disease often have diffuse mineralization of tubule basement membranes. Secondary hyperparathyroidism and mineralization of other tissues may be noted.

Additionally, in more advanced cases of CPN small irregularly-shaped, edematous and hyperplastic outgrowths of epithelium are often noted on the surface of the renal papilla (Fig. 4). These projections seemingly have little pathological significance. Vascular or hemorrhagic lesions are not a prominent part of the overall histological spectrum of CPN. On rare occasion only, the authors have noted perivascular inflammatory cell infiltrates and even some vasculitis and thrombosis. A light golden-brown pigment is often noted in CPN tubules and interstitial tissue. This pigment seems to be predominantly iron-positive and presumably hemosiderin.

Grading the severity of CPN is mainly a subjective evaluation often based on the pathologist’s training and experience. There is little published guidance on how to grade CPN. Most CPN grading schemes grade CPN on a 3, 4 or 5 grade scale, representing severities from minimal to markedly-severe (End-Stage). Although all of these grading schemes are appropriate, if used consistently, grades 1–4 or 1–5 are more likely to help in the determination of an exacerbated chemical effect and are used more regularly on a global basis. Depending on the length of the study, study pathologists may also modify their grading scheme for more acute studies versus carcinogenicity studies. Furthermore, in any event of a potential chemical effect involving CPN, pathologists should provide a detailed description of the grading criteria for CPN in the narrative report. This provides information for the regulatory agencies to use in interpreting and comparing data from these studies and assists the peer review pathologist in determining the accuracy and consistency of the study pathologist when peer review is conducted.

**CPN Associated Cell Proliferation and Proliferative Lesions**

As previously noted, CPN has been described as a degenerative to atrophic disease with compensatory hypertrophy and hyperplasia. The prominent regenerative tubules noted with CPN are part of the compensatory hyperplastic response. The term “simple tubule hyperplasia” was introduced to denote a tubule appearance consisting of a single cell layer of increased numbers of lining epithelial cells and to help differentiate CPN tubules from ATH or, alternately diagnosed as, “renal tubule hyperplasia”. Although pathologists differ in their diagnostic approach to the early changes associated with CPN, “tubule regeneration” or “basophilic tubules” are terms used more frequently. Other pathologists simply include this early change within the overall diagnosis of CPN when they are reasonably comfortable with their diagnosis. The term simple tubule hyperplasia has not been routinely used to diagnose the early tubule change. However, the crowded nature of the lining cells of CPN-affected tubules implies the presence of simple tubule hyperplasia.

Although it was easy for pathologists to recognize the increased number of lining epithelial cells within the confines of a single tubule by routine H&E light microscopy, it was not until sometime later when investigators began to apply specialized techniques such as tritiated thymidine autoradiography to demonstrate the cell labeling index (LI) of cells undergoing DNA synthesis within areas of CPN. These studies have reported increased LI of up to 10 times greater in CPN affected areas than normal areas. It appears that the proliferation rate of affected tubules is the same for male and female rats; however, the number of affected tubules is greater in males. Proliferating cell nuclear antigen (PCNA) may also be used to measure CPN cell proliferating activity.

In examining kidneys from rats with advanced (moderate to marked severity) to end-stage CPN, it is common to observe tubules containing proliferative changes which exceed the normal expectation for tubule regeneration or simple tubule hyperplasia. A number of these tubule phenotypes have been problematic for pathologists often leading to inappropriate diagnoses and interpretation. However, recent guidelines have been published which offer recommendations in the interpretation of these proliferative changes associated with CPN. In addition, detailed criteria
were published in these studies to assist in the differentiation between CPN tubules and ATH. Therefore, a companion study, to the original paper by Hard and Seely, was conducted which investigated these challenging tubule profiles in more detail using special stains and serial-sectioning to better understand the origin and fate of these tubules. The results from this investigation supported the proliferative nature of these tubules but none progressed outside the confines of a single tubule and they lacked the additionally reported features of ATH and, accordingly, were not considered as preneoplastic lesions. Furthermore, the guidelines recommended by Hard and Seely were prepared specifically for cases involving advanced CPN in preclinical rodent studies and were not meant to be used in studies outside the context of advanced CPN. It was suggested that these proliferative tubule profiles be regarded as part of advanced CPN and not diagnosed separately.

Aside from tangential sections or cell proliferation responses due to the presence of inflammatory cells, many of these CPN tubule profiles contained focal to segmental accumulation of tubule lining cells which exceeded more than a single cell layer (multicellular layering) within the confines of the tubule (Fig. 5) or in some cases a dilated tubule (Fig. 6). These tubules, in general, were surrounded by a thickened basement membrane and contained cells morphologically similar to adjacent CPN lining cells. Even though in a few cases, these tubules were solitary and larger than some of the other adjacent regenerative tubules, their irregular outline and thickened basement membrane helped distinguish them from preneoplastic tubule hyperplasia. Tangential sections through these larger CPN tubule profiles often had a “plaque-like” appearance which required careful examination to aid in discriminating this appearance from ATH (Fig. 7). These “plaque-like” structures lacked the
high proliferative rate demonstrated by PCNA in ATH\textsuperscript{36}.

The diagnosis of ATH within the context of marked to end-stage CPN should be made from the standpoint of several histological criteria. Guidelines for the diagnosis of ATH are presented in Table 1. Perhaps one of the most reliable criteria is the presence of fibroblasts which are seen encircling the ATH tubule suggesting early expansile growth of the lesion (Fig. 8). ATH may either be graded or not graded, however, in any grading scheme, the most severe lesion should be regarded as a borderline lesion with renal tubule adenoma.

**Evidence of CPN Associated Renal Tumorigenesis**

Although no definitive study has been designed or conducted to test the hypothesis of direct CPN associated renal tumorigenesis in rodents, there is a body of scientific evidence that strongly suggests that there is a relationship of CPN to renal carcinogenesis. It is doubtful if a study could be specifically designed to unequivocally prove this hypothesis so we are left with the information we have to date and hope new investigations will lead to further support of this hypothesis.

Over the years, several investigators have remarked on observations from various studies that there appeared to be a relationship between CPN and the development or risk of tubule neoplasms\textsuperscript{37–39}. However, Dietrich and Swenberg noted that in some National Toxicology Program (NTP)
bioassay studies, which enhanced CPN, tumors were not increased\(^{38}\). This could have been due to CPN not being exacerbated to a severe enough stage. In one study conducted to specifically investigate this relationship, the NTP data base was reviewed for the severity of CPN and the presence of renal tumors in control tumor-bearing F344 animals within studies is difficult. For instance, some nongenotoxic chemicals exacerbate CPN and result in tumors\(^{41}\). However, several of these chemicals also demonstrate nephrotoxicity and, therefore, may be influencing tumorigenesis by other mechanisms\(^{35}\). Hydroquinone, one such chemical, was reported to exacerbate CPN and resulted in increased numbers of tubule adenomas\(^{35}\). During the investigation into a possible mode of action, a treatment-associated, statistically significant increased severity grade of CPN in animals with ATH or adenomas was observed. In addition, the ATH and adenomas occurred in areas of severe to end-stage CPN.

Subsequently, two additional investigational studies utilizing the NTP bioassay study results of ethyl benzene and quercetin attempted to determine a mode of action of a small number of renal tumors in animals with exacerbated CPN\(^{24,25}\). These investigations used an expanded scale of CPN severity grade to re-evaluate affected kidneys and determined the distribution of tumors in relationship to the severity of CPN. Both of these investigations supported the role of exacerbated CPN as a mode of action underlying the development of renal tumors. Recently, a proposed set of criteria for recognizing a possible role of CPN-associated tumorigenesis from preclinical studies has been published\(^1\). These criteria are abstracted in Table 2.

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<th>Table 2. Criteria of CPN Associated Tumorigenesis</th>
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<td>Slight but usually statistical increase in renal tubule tumors</td>
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<td>Exacerbation of CPN to advanced degrees of severity at doses associated with tumor increase</td>
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<td>Tumors are usually adenomas (typically basophilic) which are often of small size or borderline with ATH</td>
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<td>Absence of any cellular alterations indicative of chemical toxicity in parenchyma that is not involved in the CPN process</td>
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The pathogenesis of CPN-associated tumorigenesis is most likely multi-factorial and complex. Cellular proliferation plays a role in tissue regeneration and carcinogenesis\(^{42,43}\). Therefore, several of the proposed mechanisms suggested for CPN tumorigenesis include promotion by indirect secondary mechanisms facilitating the clonal expansion of altered cells, increasing the spontaneous error rate in DNA replication or decreasing the time for fixation of mutational events\(^{43}\).

The question of the importance of CPN tumorigenesis and human risk assessment has been examined by Hard and Kahn\(^1\). In their paper, they looked at several of the leading causes of chronic renal disease in humans (ie: hypertensive, diabetic and post-infection nephropathies) and compared these conditions to CPN of rodents. Additionally they compared the age progression, clinical pathology parameters such as proteinuria, and diet modifications of CPN with the same human renal diseases. From their extensive review they could not find any human disease counterpart which was similar to rodent CPN. Therefore, they concluded that renal tubule tumors linked to CPN by chemical exacerbation in a rodent preclinical study have no relevance for extrapolation in human risk assessment.

In rodent preclinical studies, a number of rodent specific mechanisms of toxicity/carcinogenesis have been shown to have little to no relevance for human risk assessment\(^{44}\). It would appear that the accumulating body of evidence seems to support the postulated “Mode of Action” of CPN tumorigenesis within the framework for human relevance analysis\(^{45}\).

### References

34. Hardisty JF. Personal communication. 2007.