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BIOCHEMICAL MECHANISMS AND PATHOBIOLOGY OF α_{2u}-GLOBULIN NEPHROPATHY

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INTRODUCTION

There is a growing list of chemicals that cause a low incidence of renal tumors in male rats. Upon acute exposure, some of these compounds cause a toxic syndrome in male rat kidneys that is referred to as α_{2u}-globulin (α_{2u}) nephropathy (α_{2u}–N) (Table 1). This syndrome is characterized by an excessive accumulation of protein droplets in lysosomes of proximal tubule epithelial cells, the presence of cellular casts at the junction of the proximal tubule and the thin loop of Henle, and regenerative tubules (1).

The proximal tubule is a segment of the kidney nephron that can be divided into three distinct morphological segments, P_1, P_2, and P_3 (Figure 1; 2), each with functional differences. The P_1 segment, and to a lesser extent the P_2, is involved with active transport of ions resulting in the concentration of substances in the tubular fluid. The P_2 segment in the rat has a well-developed lysosomal-phagosomal system, which contains relatively large dark-staining lysosomes, suggesting that this segment is important for the uptake and catabolism of proteins. The involvement of these large lysosomes in the

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Table 1 Chemicals that cause protein droplet nephropathy and renal tumors in male rats.

Chemical	Reference
Unleaded gasoline	65, 66, 67
1,4-Dichlorobenzene	5, 68
d-Limonene	8, 64
Isophorone	7, 69
Dimethyl methylphosphonate	6, 91
Perchloroethylene	10, 90
Pentachloroethane	9, 90
Hexachloroethane	93

catabolism of proteins taken up from the glomerular filtrate has been demonstrated by histochemical studies showing the uptake and breakdown of horseradish peroxidase (3). There are fewer lysosomes in the P_3 segment, which appears simpler in structure and function. The reabsorption of sodium chloride and other solutes, as well as the passive secretion of urea, continue in this segment.

The massive protein-droplet or crystalloid-body accumulation that occurs after exposure to selected chemicals is localized in the P_2 segment of the nephron (4). The accumulation of protein droplets in these proximal tubules represents an increase in the size and number of secondary lysosomes. Lysosomal overload results, which progresses to degeneration and necrosis of individual cells lining the P_2 segment. This cell necrosis subsequently results in the release of debris that accumulates at the junction between the P_3 segment of the nephron and the thin loop of Henle, forming granular casts. The tubular epithelial cell necrosis and exfoliation leads to restorative cell proliferation in the P_2 segment as a compensatory response to the increased cell loss from toxicity. Chronic exposure of male rats to these compounds produces an exacerbation of these lesions, mineralization of the renal medulla and ultimately, it is believed, the induction of renal neoplasia in up to 25% of the animals (5–10).

Several compounds that cause renal tumors in male rats lack genotoxicity in short-term tests and in the kidney DNA-repair assay (11–13). Evidence thus far suggests that the increased incidence of renal tumors in male rats associated with chronic exposure to these chemicals is linked to nephrotoxicity-induced increases in cell proliferation (14, 14a).

A number of studies have shown that the protein droplets that accumulate in the proximal tubule cells in male rat kidneys are composed of α_{2u}. This low molecular weight protein is synthesized in large amounts in the liver of male rats (15, 16). Evidence thus far indicates that the development of α_{2u}-N appears to be dependent on the presence of this protein, since mammals that

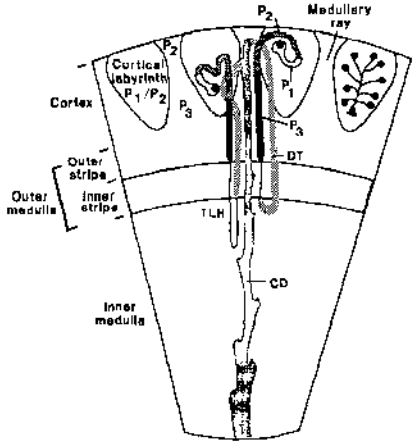

Figure 1 Schematic diagram of kidney illustrating proximal tubule segments (P_1, P_2, P_3) with respect to the various regions of the kidney. TLH, thin loop of Henle; DT, distal tubule; CD, collecting duct. (Reproduced from (4) with permission by US and Canadian Academy of Pathology, Inc.)

do not synthesize α_{2u}, such as female rats, guinea pigs, monkeys, dogs, and mice do not show symptoms of renal toxicity (17). There is no evidence that humans have α_{2u}, but it is clear that they do have related low molecular weight proteins. Because the syndrome appears to be dependent on the presence of α_{2u}, logically, humans would not be at equal risk of developing α_{2u}-N or renal cancer when exposed to these chemicals unless the related proteins respond in a similar manner. It is important that the biochemical mechanism for chemically induced α_{2u}-N be elucidated in order to determine human risk for developing α_{2u}-N and renal cancer when exposed to these agents.

α_{2u}-GLOBULIN

The liver is the major site of α_{2u} synthesis in the adult male rat (16, 18, 19). Neither female or immature male rats synthesize this protein in their livers (18). This protein is synthesized and secreted by the hepatic parenchymal cells (15, 16) at a rate of 90–180 μg/gram liver/hour (20). Because the protein is rapidly secreted, α_{2u} constitutes only 0.1% of the total liver protein (21). Nucleotide sequencing studies conducted by Unterman et al (22) show that mature hepatic α_{2u} contains 162 amino acids and has a molecular weight of 18,700 Da. Unprocessed α_{2u} contains a 19 amino acid leader sequence that is cleaved prior to secretion of the protein (23, 24). The unprocessed form of α_{2u} is reported to be glycosylated, but the sugar residues appear to be removed prior to secretion of the protein from the liver (23).

Studies on the action of various hormones on α_{2u} synthesis show that androgens, thyroid hormones, glucocorticoids, pituitary growth hormone, and insulin promote biosynthesis, whereas estrogen treatment completely suppresses its synthesis (25–30). It is concluded that the androgenic induction of α_{2u} is dependent on the synergistic effects of the growth and developmental hormones (26). Preliminary investigations show that the earliest appearance of α_{2u} detected in the male rat liver occurs at 40 days of age, which corresponds to the onset of circulating testosterone and puberty. α_{2u} first appears in the urine at about the same time (25). The normal daily urinary excretion of α_{2u} in the male reaches a maximum by 60 days of age and constitutes the principal urinary protein in the mature male rat (31, 32), accounting for approximately 30% of total protein excreted or 20 mg/day (21, 32).

α_{2u} is found in tissues other than the liver, including the kidney, submaxillary, lachrymal, preputial and mammary glands, and the anterior pituitary. Neither the kidney nor the pituitary contain mRNA for α_{2u}, and it is believed that their source of protein is derived from plasma (33). α_{2u} in female rat kidneys is believed to originate in the submaxillary, lachrymal, and mammary glands. There is approximately 120-fold less α_{2u} in female kidney than in male rat kidney (34). The hormonal and developmental regulation of α_{2u} synthesis in each of these tissues is unique, probably due to slightly different α_{2u} genes being transcribed in the various tissues (35).

Metabolic Fate of α_{2u}-Globulin

Because α_{2u} is a low molecular weight protein, it readily passes through the glomerular filter of the nephron. Approximately 50% of α_{2u} is reabsorbed and the remainder is excreted in the urine (36, 37). Like other low molecular weight proteins, α_{2u} is reabsorbed by the proximal tubule segment of the nephron. The normal pathway for reabsorption of proteins is to be reabsorbed initially by sites at the apical border of the proximal tubular cells. The proteins undergo endocytosis and are concentrated into vesicles called phagosomes. α_{2u} has been localized within the proximal tubule cell lysosomes of male rats by the use of immunohistochemical techniques (38). Those phagosomes that contain protein migrate to the cell interior where they fuse with lysosomes (39). Enzymatic cleavage of proteins occurs in the phagolysosomes by a mixture of proteases. These proteolytic enzymes cut proteins at various sites from either the ends (exolytic) or at points within the protein (endolytic). The products of hydrolysis (amino acids, peptides) can then permeate the endolysosome membrane, cross the contraluminal cell membrane and return to the circulation (39).

Lane & Neuhaus (40, 41) discovered multiple isoforms of α_{2u} in kidney and urine and found that the renal forms were more acidic than the urinary

forms. These investigators concluded that the multiple urinary and renal α_{2u} forms were the result of metabolism by the kidney of a single α_{2u} form secreted by the liver into the blood, but could not provide direct evidence. However, later studies using ^{125}I-labeled α_{2u}-globulin injected into male rats demonstrated that α_{2u} secreted by the liver is metabolized by the kidney to forms 1400 Da smaller (42). This work showed only one molecular weight form in liver, plasma, and urine, with two forms present in kidney. The lower molecular weight α_{2u} present in the kidney is believed to be a product of the higher molecular weight liver form.

Proposed Physiological Functions of α_{2u}*-Globulin*

Urine of male rodents contains a factor which accelerates the onset of puberty in female rodents (43). This factor appears to act by stimulating the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into blood (43, 44). This puberty-accelerating pheromone is under androgenic control (45) and appears either to be protein-bound or to be a portion of a large protein molecule (46). One proposed function of α_{2u} is that it may act as a carrier for a pheromone since it has a high rate of excretion in urine, is under androgenic control, and is believed to be a transport protein (47).

Other evidence suggests that α_{2u} is necessary for spermatogenesis due to its ability to maintain normal plasma concentrations of follitropin and lutropin (48). In estrogen-treated rats, α_{2u} had an effect on both LH and FSH. In these studies, α_{2u} protected spermatogenesis (49) and stimulated body growth and growth of testes, seminal vesicle, prostate, and increased serum testosterone concentrations. In contrast, Ekstrom (42) did not observe an effect of α_{2u} on the weight of prostate, seminal vesicles, and testes in estrogen-treated male rats. He attributes these differences to the possibility that the α_{2u} preparations from the two laboratories were different (42). One may have contained a factor involved in the stimulation of LH and FSH. Because α_{2u} is proposed to be a carrier protein, the physiological ligand may very well be this factor involved in maintaining spermatogenesis. Despite various proposals concerning its possible function, e.g. transporting a pheromone (47) or being involved in the regulation of spermatogenesis (50, 51), the function of α_{2u} remains yet to be definitively demonstrated.

α_{2u} *Superfamily of Proteins*

α_{2u} is classified as part of a family of low molecular weight transport proteins based on their similar primary amino acid sequences, proposed functions, and similar subunit molecular weights (52–55). Some of these proteins are known to serve as carriers for small lipophilic molecules, although the function of others remains undetermined. Members of this family include human retinol binding protein (RBP), apolipoprotein D, α_1-acid glycoprotein (AGP), α_1-

microglobulin, bovine β-lactoglobulin (BL), rat odorant binding protein and mouse major urinary protein (MUP).

RBP and BL have a similar three-dimensional protein structure (56). Another similar feature is their ability to bind retinol (57, 58). The common structural feature of RBP and BL polypeptide chain is that it is folded in an eight-stranded β-barrel that encloses the bound retinol. Using the Chou & Fasman secondary-structure prediction procedure, AGP shows a similar fold (54). It is believed that all the proteins in this family contain this β-barrel in which the interior cavity is lined with largely hydrophobic side chains that can interact favorably to bind a lipophilic molecule (54). The composition of amino acids in this region may differ between each protein, thereby determining their ligand specificity. More information on the structure and function of these proteins is needed to determine whether human proteins in this family may also be involved in the development of a similar α_{2u}-N. Although MUP is in the same family as α_{2u}, male mice do not develop α_{2u}-N when exposed to the chemicals that cause this syndrome in male rats.

BIOCHEMISTRY OF PROTEIN DROPLET NEPHROPATHY

α_{2u}-Globulin nephropathy is a syndrome that occurs in male rats exposed to a diverse group of compounds. Chemicals causing this renal toxicity include decalin (59–64), unleaded gasoline (UG) (1, 4, 65–67), 1,4-diclorobenzene (1,4-DCB) (68), d-limonene (d-Lim) (64), isophorone (IPH), (69) as well as several pharmacological agents (70). UG, a chemical mixture, causes α_{2u}-N upon acute exposure and a low incidence of renal tumors after two years of exposure (65, 67).

Because UG is a complex mixture of hydrocarbons, a critical question is whether the nephropathy of this mixture is associated with any specific chemical or class of chemicals within the mixture. Results from studies evaluating the various component fractions of UG indicated that the nephrotoxic activity, as evidenced by the appearance of protein droplets, is associated with saturated aliphatic compounds (66). Acute toxicity studies found the most active nephrotoxins to be highly branched hydrocarbons. Included in this group was 2,2,4-trimethylpentane (TMP), which constitutes approximately 5% of the total mixture of UG. Much of the work on the biochemical mechanism of α_{2u} nephropathy has been performed using TMP as a model compound.

Initial studies using ^{14}C-TMP showed a species and sex difference in its disposition in rats and mice (71). Male rat kidneys contained a 10-fold greater amount of ^{14}C-TMP derived radiolabel than did female rats 72 hours after exposure. Male mice did not retain ^{14}C-TMP in any organ, including kidney.

Further studies on the disposition of TMP using ^{3}H-TMP demonstrated that male rats selectively retained TMP-derived radiolabel in their kidneys and that 2,4,4-trimethyl-2-pentanol was the major metabolite present in male rat kidney, but absent in female kidneys (72). A significant increase in the renal concentration of α_{2u} was also observed 24 and 48 hours after dosing. When kidney cytosol prepared from ^{3}H-TMP treated male rats was subfractionated, 60% of the radiolabel was in the cytosol (73).

The accumulation of TMP-derived radiolabel, protein droplets, and α_{2u} in kidney proximal tubules suggested an association between chemical and protein. Gel fitration chromatography of the cytosol showed that approximately 20% of the radiolabel coeluted with the low molecular weight protein fraction. Using an ELISA, this protein fraction contained α_{2u}. The remaining radiolabel eluted in the molecular weight range of less than 1000 Da and was freely dialyzable, suggesting that it represented free metabolites.

Purification of α_{2u} from TMP-treated male rats demonstrated that TMP-derived radiolabel was specifically associated with α_{2u} (74). Noncovalent, reversible binding was found to exist between chemical and α_{2u} since the complex dissociated in the presence of SDS. Using gas chromatography-mass spectrometry, the radiolabel that coeluted with the protein fraction containing α_{2u} was determined to be 2,4,4-trimethyl-2-pentanol (TMPOH) (73).

Other chemicals that cause α_{2u}-N such as 1,4-DCB, IPH and d-lim are also shown to bind reversibly to α_{2u} after in vivo exposure. In the case of 1,4-DCB, both the parent compound and a metabolite, 2,5-dichlorophenol, are associated with the fraction containing α_{2u} (68). After d-lim exposure, the major metabolite, d-limonene-oxide, along with some parent compound was found to bind reversibly to α_{2u} (75), whereas IPH exposure resulted in only the parent compound binding to α_{2u} (69). Information on the ability of various chemicals to bind reversibly to α_{2u} along with the accumulation of chemical and α_{2u} in the kidney suggests that the formation of the chemical-α_{2u} complex somehow is responsible for the accumulation of the protein in the proximal tubule cells of male rats.

Hydrolysis of α_{2u}-Globulin

There are several possible explanations for the accumulation of protein droplets in the proximal tubule. One would be an increase in the synthesis of α_{2u} which would result in lysosomes overloaded with protein. Olson et al (76) have shown that increased synthesis does not occur, because mRNA for α_{2u} in liver is not increased after UG chemical exposure. Another plausible explanation would be an increase in the ability of α_{2u} to be reabsorbed into the proximal tubule cells. Although this has not been directly measured, Viau et al (77) measured β_2-microglobulin excretion as a functional test of the reabsorptive capacity of the kidney, and did not see any change after

isoparaffinic solvent exposure to male rats. Also, there was no difference in the urinary clearance of α_{2u} between control and treated rats.

Another way in which a chemical may disrupt the normal pathway of protein uptake and metabolism in the proximal tubule cells resulting in protein droplet accumulation is lysosomal dysfunction. Accumulation of protein within the lysosomes of the proximal tubules suggests that the intracellular processing of proteins by kidney cells might be disrupted by chemical exposure. This may be due to either an alteration in lysosomal enzyme activity or a change in the ability to hydrolyze the protein-chemical complex. Murty et al (78) have shown that lysosomal enzyme activity is not decreased after UG exposure. In our laboratory, in vitro studies that measure cathepsin B activity confirmed their results.

The most plausible mechanism of protein droplet accumulation is that the protein forms a complex with either the administered chemical or one of its metabolites, making the protein more resistent to hydrolysis (Figure 2). This hypothesis was tested in vitro using purified α_{2u} from untreated male rat kidneys ("free" α_{2u}) and purified protein from TMP-treated male rats ("bound" α_{2u}). Using proteinase K, the "bound" α_{2u} was more resistant to hydrolysis then was "free" α_{2u} (79). Rivera Torres et al (80) have demonstrated that d-limonene-oxide-bound α_{2u} is also more resistant to hydrolysis using normal lysosomal enzymes. The basis for reduced protein digestibility after the formation of the chemical-protein complex is currently being addressed. Some possible explanations may be that there is a change in the

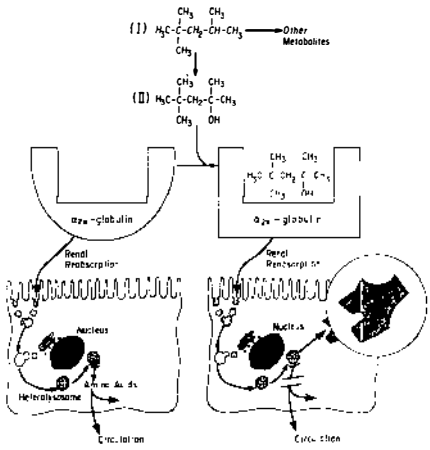

Figure 2 Proposed mechanism for α_{2u}-N in male rats. There is a reversible binding of chemical (or metabolite) to α_{2u}-globulin which changes the structure of the protein. This alteration makes the protein less digestible by lysosomal enzymes resulting in an accumulation of α_{2u} in the P_2 renal epithelial cells. (Reproduced from (92) with permission by Academic Press, Inc.)

tertiary structure of the protein when chemically bound or that the chemical is bound to a region on the protein that is the critical active site for protease digestion.

In Vitro Binding

In order to study characteristics of the chemical-protein complex, an in vitro method to evaluate chemical binding to α_{2u} was developed. These studies characterized the binding of 2,4,4-trimethyl-2-pentanol (TMPOH) to α_{2u} in vitro using ^{3}H-TMPOH and kidney cytosol as the source of α_{2u}. The binding affinity (K_d) of ^{3}H-TMPOH to α_{2u} was calculated to be on the order of 10^{-7}M (81). Compounds that cause α_{2u}-N in male rats were found to compete in vitro with ^{3}H-TMPOH for binding to α_{2u}. The relative affinity of each compound for α_{2u} was compared using their apparent inhibition constant values (K_i) determined from their IC_{50} value (concentration that inhibits 50% of the original binding) and the K_d for the ^{3}H-TMPOH-α_{2u} complex. It was demonstrated that chemicals known to bind to α_{2u} in vivo compete with ^{3}H-TMPOH for binding to α_{2u} to varying degrees. For example, when male rats were treated with d-lim, d-limonene-oxide and a small amount of parent compound is bound to α_{2u} isolated from their kidneys (75). This is consistent with the in vitro data on these compounds which showed that d-limonene-oxide has a stronger affinity ($K_i=10^{-7}$M) for α_{2u} than d-lim ($K_i=10^{-4}$M). Other compounds that bind to α_{2u} in vivo, such as IPH, 1,4-DCB and 2,5-DCP have K_i values that range from 10^{-4} to 10^{-6}M (81). From these data, it appears that factors other than binding affinity may determine the ability of chemicals to induce protein droplets.

Retinol was also tested for its ability to compete with ^{3}H-TMPOH for binding to α_{2u} in order to investigate whether α_{2u}, like RBP and BL, also binds retinol. In vitro, retinol competed well with ^{3}H-TMPOH for binding to α_{2u} (81), but when administered orally to male rats did not cause protein droplets. It is not clear whether retinol, or one of its metabolites, binds to α_{2u} in vivo. Although chemical binding to α_{2u} appears to be a prerequisite for development of α_{2u}-N, binding affinity may only be one factor to consider. It is possible that a conformational change in α_{2u} takes place when the chemical is bound. Although this was not detected by circular dichroism (S. J. Borghoff & J. A. Swenberg, unpublished observations), a minor change in the protein structure may take place which disrupts its normal digestion by lysosomal proteases.

Structure-Activity Relationships

Halder et al (66) tested the hydrocarbon compounds typically found in UG for α_{2u}-N inducing potential. The results from this study revealed a positive structure-activity response relating the degree of alkane branching to the potency of the nephrotoxicity. Since it is believed that the chemical binding to

α_{2u} is involved in the initiation of α_{2u}-N, it may prove useful to evaluate possible structure-activity relationships on the relatively diverse set of compounds that are known to bind to α_{2u}.

In their study, Halder et al (66) gavaged rats with various chemicals and evaluated α_{2u}-N. However, the active compounds, i.e. chemicals binding to α_{2u}, were not identified. Studies are currently underway to determine the structure-activity relationship among the chemicals known to bind, using the ability of selected compounds to compete in vitro with ^{3}H-TMPOH for binding to α_{2u} as a measure of activity. Molecular modeling programs are being used to build the chemical structures and determine features such as volume, dipole moments, charges on atoms, as well as partition coefficients that appear necessary for binding. Preliminary results reveal that when the volumes of the four compounds exhibiting the greatest relative affinity to α_{2u} were drawn, other compounds with a lower affinity usually had portions of the molecule that protruded beyond the boundaries of the higher affinity chemicals (82).

PATHIOBIOLOGY OF α_{2u}-GLOBULIN NEPHROPATHY

Normally, young male rats have a high rate of proteinuria which is attributed, in part, to the large amount of α_{2u} filtered and reabsorbed. As α_{2u} synthesis decreases with age, albumin excretion steadily increases over time. This increase in albumin excretion reflects the spontaneous glomerulonephrosis seen in aging rats of strains commonly used in safety assessment. In untreated male rats, the renal P_2 epithelial cells contain small protein droplets that parallel the proteinuria in these animals. These droplets stain positively for α_{2u} and are not noted in female rat kidneys (38). The higher rate of proteinuria that occurs in old male, but not female, rats may be due not only to the presence of α_{2u} and albumin, but also to the fact that renal cathepsin B activities (lysosomal endopeptidases) are significantly higher in females than in males (83).

After exposure to TMP or other chemicals listed in Table 1, there is an increase in the number and size of the protein droplets in male rat kidneys (Figures 3 and 4), with a concomitant increase in the concentration of α_{2u}. In severe α_{2u}-N, the accumulation of α_{2u} in the lysosome results in large polyangular lysosomes that are electron-dense and crystalloid when examined by electron microscopy (4, 61). These crystalloid protein droplets are associated with cytotoxicity. The injured cell is released from the basement membrane and sloughs into the lumen where it collects in granular casts in the thin loop of Henle or is excreted in the urine. As a consequence of cell death, there is regeneration of neighboring cells. Short et al (4) have observed that both protein droplet accumulation and increased cell proliferation are specifically localized in the P_2 segment of the proximal tubules of male rats exposed to

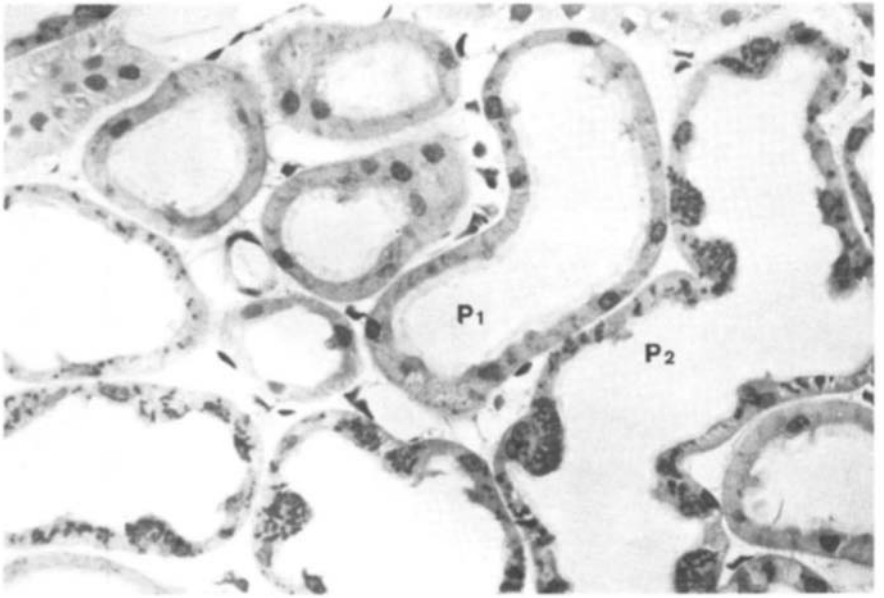

Figure 3 Immunohistochemical staining of α_{2u}-globulin in individual lysosomes in P_2 renal epithelial cells of a control male rat. (Reproduced from (92) with permission by Academic Press, Inc.)

UG or TMP. The proliferative response in the P_2 segment of the male rat nephron closely parallels the extent and severity of immunohistochemical staining of α_{2u} in the same region. None of these effects was observed in female rats. Figure 5 demonstrates in male rats the relationship between protein droplet accumulation and increased thymidine labeling of P_2 cells after TMP exposure.

Because of the site-specific localization and correlation between protein droplet accumulation, identification of α_{2u} within the droplet, and increases in cell proliferation, it was proposed that the excessive protein droplet accumulation results in continual compensatory cell division. One explanation for tumor formation is that this increase in cell proliferation enhances the likelihood of spontaneous mutational events and also encourages clonal expansion of initiated cells. These events augment the development of neoplasia above the incidence normally seen in control animals. This would explain the low incidence of renal tumors formed after 2 years of exposure to the compounds that cause α_{2u}-N (Table 1).

Short et al (14a) have tested UG and TMP for promoting effects in an initiation-promotion study of the kidney. Rats were initiated with N-ethyl-n-

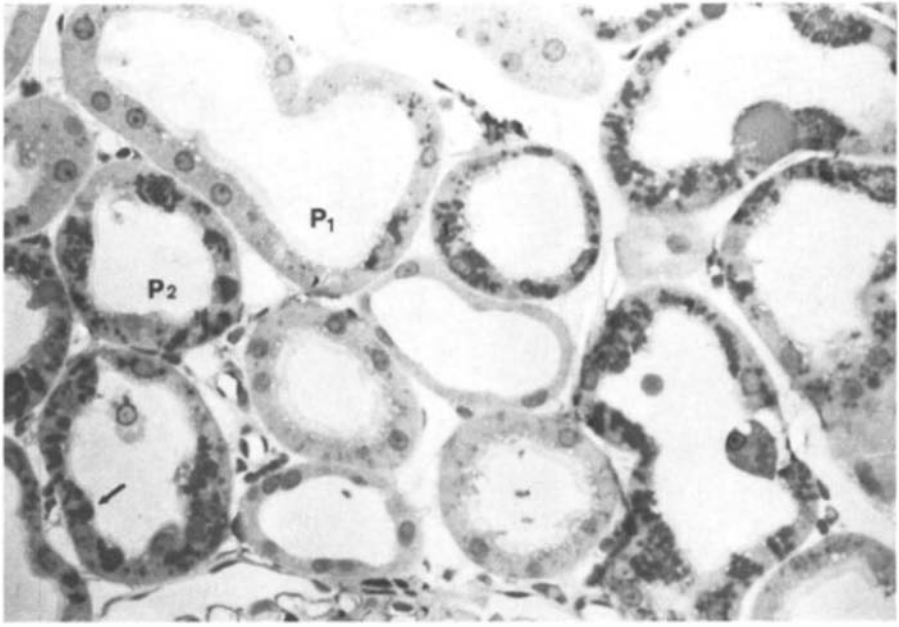

Figure 4 Immunohistochemical of α_{2u}-globulin in P_2 renal epithelial cells containing excessive numbers of large lysosomes from a male rat exposed to 50 ppm TMP for 3 weeks. (Reproduced from (92) with permission by Academic Press, Inc.)

hydroxylethylnitrosamine (EHEN) for 2 weeks and then exposed to various concentrations of either UG or TMP for up to 61 weeks. There were increases in the incidence of atypical cell foci (ACF), which are classified as a pre-neoplastic renal lesion, in male rats promoted with the high dose of UG and TMP. Also observed was a significant linear trend in the incidence of renal cell tumors (RCT) in male rats promoted with UG. The promoting effect of UG on ACF and RCT was demonstrated to be sex-specific, only occurring in male rats. These results were consistent with the hypothesis that protein-droplet inducing chemicals are promoters of renal neoplasia in a manner that is closely related to their ability to cause α_{2u}-N.

HUMAN RISK ASSESSMENT

The induction of renal neoplasia in male rats exposed to the chemicals that cause α_{2u}-N is of concern because of the widespread human exposure to these compounds. Although UG causes renal tumors in male rats (67), there is no statistically significant evidence from epidemiology studies to suggest a link

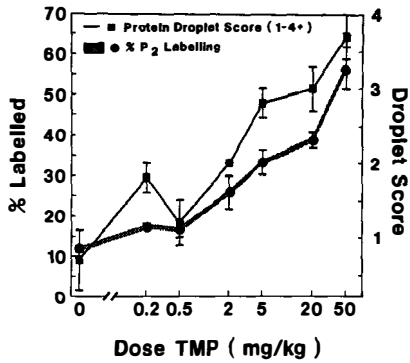

Figure 5 Dose-response relationship between the accumulation of protein droplets and increase in cell proliferation in the P_2 segment in TMP-treated male rats. (Reproduced from (4) with permission by US and Canadian Academy of Pathology, Inc.)

between occupational exposure to petroleum products and human kidney cancer (84–86). Epidemiological studies are not available for any of the other compounds that cause α_{2u}-N.

In order to determine whether humans are at risk to develop cancer when exposed to the compounds that induce α_{2u}-N in male rats, the biochemical differences between male rats and humans as it relates to α_{2u}-N must be understood. The development of α_{2u}-N is dependent on the presence of α_{2u}, since strains of rats and other species that do not synthesize this protein do not develop this disease (17, 87). The question arises whether humans have α_{2u} or a similar protein which could possibly cause them be at risk to the syndrome when exposed to these chemicals. To date, there is no evidence that humans have α_{2u}. However, as mentioned previously, they do have several proteins within the same family as α_{2u} that are classified as transport proteins. This may not be of major importance because mice have a protein that is approximately 90% homologous to α_{2u} and do not develop α_{2u}-N when exposed to the chemicals that cause it in male rats.

In studying α_{2u}-N, there appears to be a number of critical factors necessary for the syndrome to occur. First, there is the synthesis of a high amount of a low molecular weight (LMW) protein that is freely filtered by the glomerulus and normally difficult to hydrolyze. After chemical exposure the binding of a chemical to this protein makes it more resistant to hydrolysis which leads to the accumulation of the protein in the proximal tubule cell lysosomes. To evaluate human risk for developing α_{2u}-N, human proteins similar to α_{2u} must be investigated to determine if they fulfill the requirements believed necessary to develop α_{2u}-N. One critical factor is not only how much protein is synthesized, but how much is actually filtered and reabsorbed by the

kidney. This value for proteins other than α_{2u} was difficult to find. The serum and urinary excretion levels were found for several human proteins and compared to α_{2u}. However, without knowing the amount of protein filtered, the level of protein that the kidney tubular epithelium is exposed to cannot be calculated.

Other information can be obtained on the characteristics of these proteins in relationship to the critical factors necessary for α_{2u}-N to develop. One such factor is the protein's ability to be catabolized. These experiments were performed in our laboratory where purified proteins such as AGP, BL, and RBP were hydrolyzed in vitro by PK and compared to α_{2u}. It was demonstrated that α_{2u} and AGP were the most resistant, while RBP and BL were 1,000–100,000-fold more susceptible to PK digestion (Table 2). AGP is a highly glycosylated protein (88); glycosylation is known to make a protein more stable and resistant to hydrolysis (89). The same may be true for α_{2u} and needs to be investigated. What is still not known is whether chemicals that bind to α_{2u} also bind to other members in the same protein family. If they do, it is important to determine how tightly they bind and whether their hydrolysis is affected when bound. Although α_{2u} is one of many proteins in the same family, whether any of these proteins participate in the sequence of events that leads to α_{2u}-N is currently being investigated. When more information on these human proteins is known, it will then be possible to evaluate the risk to humans exposed to the chemicals that cause α_{2u}-N in male rats.

SUMMARY

α_{2u}-N is a syndrome that has been characterized in male rats exposed to a number of environmental chemicals and pharmacological agents. The chemicals or their metabolites bind to α_{2u}, which is believed to lead to a less digestible chemical-protein complex. Because of the decreased hydrolysis of the chemical-protein complex in the lysosome, α_{2u} accumulates in the form

Table 2 Selected proteins from the superfamily of ligand-binding proteins

Protein	Molecular weight[b]	Species, tissue[b]	Ligand[b]	PK_{50}[a] (U/ml)
α_{2u}-globulin	18,700	rat, liver	unknown	9.8 ± 10
α_1-acid glycoprotein	18,944	human, urine	drugs, steroids	4.0 ± 5.2
Retinol binding protein	22,868	human, liver	retinol	$6.9 \times 10^{-6} \pm 2.1 \times 10^{-6}$
β-lactoglobulin	18,281	bovine, whey	retinol butane	$6.8 \times 10^{-8} \pm 2.5 \times 10^{-8}$

[a] PK_{50} represents the concentration of Proteinase K that hydrolyzed 50% of the protein. This value represents the mean ± SD of 3–4 separate observations.

[b] See Reference 53

of protein droplets. In extensive nephropathy, the accumulation of α_{2u} in the lysosome results in polyangular crystalloid droplets that lead to lysosomal overload and eventually cell death. This cell death stimulates restorative cell replication which promotes renal carcinogenesis in male rats.

As such, it is imperative that extrapolation of risk to humans of chemicals causing this syndrome be performed. Because the nongenotoxic mechanism for carcinogenesis in the male rat involves a unique protein, such extrapolations can only be done incorporating species differences in the critical factors that result in α_{2u}-N in rats. Presently, these data suggest a markedly reduced risk for humans compared to male rats.

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