Effects of Sodium Intake and Nephrotoxin Dose on Acute Renal Failure in the Young Rat

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Summary

These experiments were undertaken to determine the differences between rats 3-4 and 7-8 wk of age with respect to the severity of acute renal failure (ARF) induced by two nephrotoxins, mercuric chloride (HgCl_2) and uranyl nitrate (UN). Two observations suggested that the younger rats might be more susceptible to ARF: ARF developed in response to a dose of HgCl_2 , which had no effect in the older rats, and mortality rates tended to be higher in the younger rats. Despite this possible age-related difference in susceptibility, increased sodium intake (giving 1% NaCl rather than tap water to drink) had comparable protective effects in both models of nephrotoxin-induced ARF in rats of both ages.

Speculation

Despite previously described age-related differences in the distal tubular handling of sodium, increased sodium intake resulted in qualitatively similar protection from nephrotoxic acute renal failure in both 3-4 and 7-8-wk-old rats. Thus, the protective mechanism due to increased sodium intake may be a consequence of increased proximal tubular fluid flow.

Despite intensive investigation, the pathogenesis of acute renal failure (ARF) remains controversial (5-11, 14-17, 23, 25-28, 34, 35, 37). A pathogenic role for the renin-angiotensin system has been postulated. This is based on the observations that, in certain experimental models of ARF, chronic sodium (Na) and potassium (K) loading suppress renal renin and reduce the severity of ARF whereas chronic Na deprivation augments renal renin and enhances the severity of ARF (14). Although we have confirmed that the severity of nephrotoxic ARF is inversely related to dietary Na intake and/or excretion, we have shown that this relationship is independent of changes in renal renin, at least in the mature rat (5). Very few data concerning ARF in the immature rat are available. Studies using the developing animal might provide some insights, inasmuch as there are age-related differences in the activity of the renin-angiotensin system and in the ability to handle volume expansion and dietary Na loads in many species, including the rat (1, 3-5, 6, 18-21, 24, 32, 33). Any of these factors might affect the susceptibility to and/or the severity of ARF.

In a previous study of 2-, 4- and 8-wk-old rats, no pattern was found between the severity of mercuric chloride-induced ARF and either basal renal renin, plasma renin, or the increase in plasma renin 6 h after injection. However, there were age-related differences: mortality was highest in the 2-wk olds, and those that survived exhibited a delayed recovery, similar to what has been found in mature rats subjected to Na deprivation (5).

In the present studies, younger (3-4-wk old) and older (7-8-wk old) rats were compared with respect to the effect of varying dietary Na on two nephrotoxic models of ARF: mercuric chloride (HgCl_2) and uranyl nitrate (UN). In addition, the dose-response to HgCl_2 of these two age groups were compared. It should be noted that since the weaning age of rats is 18-20 days, the younger rats of these studies were of necessity older than the youngest rats of the previous study (5).

MATERIALS AND METHODS

Sprague-Dawley rats, bred in our laboratory so that the exact postnatal ages were known, were studied at 3-4 wk of age (40-80 g body weight) and at 7-8 wk of age (150-250 g body weight). All were weaned at 18-20 days of age.

Effect of dietary Na. Each rat was put in a metabolic cage for 2-3 days before the experiments and given either a low Na diet (tap water plus "Na-free diet, Rat Modified," from Nutritional Biochemicals, Cleveland, OH), a normal Na diet (tap water plus Purina Rodent Chow, from the Purina Company, St. Louis, MO), or a high Na diet 1% NaCl rather than tap water, plus Purina Rodent Chow). Fluid intake and urine output during the 24 h immediately preceding the experiments were recorded. The urine was analyzed for Na and K. Then randomly selected animals on each of the three dietary regimes, from both age groups, were sacrificed for the determinations of mean baseline blood urea nitrogen (BUN) and serum creatinine (S_{Cr}). The remaining rats were injected subcutaneously with 1 ml/kg body weight of either 4.7 mg HgCl_2 /ml or 2.5 mg UN/ml. Tail vein blood samples (0.2 ml) were drawn on the second day after HgCl_2 and on the second and fifth days after UN. On the fifth day after HgCl_2 and on the seventh day after UN, animals were sacrificed and blood was obtained for the determinations of both BUN and S_{Cr} .

HgCl_2 dose-response. Other rats given the normal Na diet were handled as described above except that they were injected with 1.5 or 3.0, rather than with 4.7, mg HgCl_2 /kg body weight. In all cases, the volume was 1 ml/kg body weight.

Analyses. Methods for measurement of urinary Na and K, BUN and S_{Cr} have been described in recent publications from this laboratory (5-8, 10).

Statistics. All results are expressed as means $\pm$ S.E. Analysis of variance (13) and Scheffe contrasts (36) were used to assess the statistical significance of differences between groups in multiple comparisons. Otherwise, the unpaired *t* test was used (13).

RESULTS

Within each of the six major groups of rats (e.g., animals of two age groups were given either high, normal, or low Na diets), there were several subgroups (e.g., those sacrificed for baseline BUN and S_{Cr} , those injected with UN or with one of three doses of HgCl_2). There were no statistically significant differences ($P > 0.05$) between these subgroups within any of the six groups, with respect to fluid intake, urine output, and Na and K excretion rates measured during the 24-h period preceding the experimental day. Therefore, results for the subgroups were combined and mean values are presented in Table 1. In general, fluid intake, urine volume, and Na excretion all increased with increasing dietary Na in both age groups. Food intake was not measured in these animals. Thus, it is impossible to draw firm conclusions concerning the % of dietary Na and K excreted in the urine. However, it is likely that food intake per unit body weight was higher, and therefore that Na and K intakes were higher, in the younger rats during this period of rapid weight gain (3, 4, 6). This might explain why all groups of younger rats excreted more K

than the corresponding groups of older rats and why the younger rats fed the normal Na diet excreted more than twice as much Na as the older rats.

BUN and S_{Cr} were simultaneously determined at the time of sacrifice (baseline, 5 days after $HgCl_2$, 7 days after UN) or at the time of death. There were no significant differences in either baseline BUN or baseline S_{Cr} among the three major groups of older rats (high, normal, and low Na diets) or among the corresponding three major groups of younger rats ($P > 0.05$). Therefore, all baseline values were combined, and the means for the two age groups are shown in Figure 1. Also shown are the individual BUN and S_{Cr} values measured either at sacrifice or death following UN injection. An excellent correlation was found between the individual values for both the older rats ($r^2 = 0.85$; $y = 28.3x + 13.7$; $P < 0.001$) and the younger rats ($r^2 = 0.88$; $y = 98.8x - 65.4$; $P < 0.001$). However, the slopes of the relationships were clearly different between the two age groups ($P < 0.001$).

Figure 2 shows the correlation between BUN and S_{Cr} at sacrifice or death following $HgCl_2$ injection. Because of the large numbers of overlapping individual values, only the means of the various groups and subgroups were plotted. The coefficients of determination shown in Figure 2 were based on group and subgroup means; those based on individual values were equally high and significant ($r^2 = 0.90$; $y = 52.1x - 27.2$; $P < 0.001$ for older rats and $r^2 = 0.86$; $y = 95.1x - 38.2$; $P < 0.001$ for younger rats). The

slopes for the two age groups were significantly different ($P < 0.001$, based on individual values).

Because of the excellent correlations in all cases between BUN and S_{Cr} , increases in either would be equally valid indices for reductions in glomerular filtration rate (GFR). However, for the purpose of assessing age-related differences in severity of ARF, it was decided that BUN was more meaningful. There was only a 20% difference between mature and immature rats with respect to baseline BUNs (14.6 ± 0.4 versus 18.2 ± 0.6 mg%, respectively) whereas there was a 35% difference between them with respect to baseline S_{Cr} (0.80 ± 0.02 versus 0.52 ± 0.02 mg%, respectively). Thus S_{Cr} would not discriminate between an older rat with a GFR 50% of normal and a younger rat with a GFR 33% of normal, whereas BUN might. BUN is not a completely satisfactory index due to possible age-related differences in diets. That this was not a major limitation is suggested by the lack of a significant difference between younger and older rats with respect to mean % increase in BUN/% increase in S_{Cr} following the injection of UN or the 4.7 mg/kg dose of $HgCl_2$ (1.40 ± 0.1 , $n=95$ younger rats versus 1.6 ± 0.1 , $n=92$ older rats; $P > 0.05$).

Figure 3 shows the development of ARF following UN injection. Mean baseline BUNs for each of the six major groups, as well as mean BUNs at 2, 5, and 7 days are shown. BUNs (baseline and 5 days) were compared using one-way analysis of variance and Scheffe contrasts. Mean control BUNs were not significantly

Table 1. Baseline weight, fluid intake, and urinary excretion

Diet	Age group	Number	Wt (g)	Fluid intake ² (ml)	Urine ² (ml)	$U_{Na}V^2$ (μ Eq)	U_KV^2 (μ Eq)
High Na	7-8 wk	34	195 $\pm$ 6	24.6 $\pm$ 1.6	13.2 $\pm$ 0.8	3470 $\pm$ 239	952 $\pm$ 61
	<i>P</i> value ³			<0.01	NS	<0.02	<0.001
	3-4 wk	48	54 $\pm$ 1	29.8 $\pm$ 1.2	11.6 $\pm$ 0.6	2827 $\pm$ 147	1675 $\pm$ 97
Normal NA	7-8 wk	80	195 $\pm$ 3	13.2 $\pm$ 0.6	5.5 $\pm$ 0.3	267 $\pm$ 14	391 $\pm$ 25
	<i>P</i> value			<0.001	NS ⁴	<0.001	<0.001
	3-4 wk	104	56 $\pm$ 1	19.0 $\pm$ 0.7	5.1 $\pm$ 0.2	711 $\pm$ 29	952 $\pm$ 41
Low Na	7-8 wk	46	189 $\pm$ 5	13.4 $\pm$ 0.7	4.9 $\pm$ 0.4	56 $\pm$ 5	495 $\pm$ 30
	<i>P</i> value			<0.005	NS	NS	<0.001
	3-4 wk	48	60 $\pm$ 1	15.8 $\pm$ 0.4	4.2 $\pm$ 0.3	49 $\pm$ 8	962 $\pm$ 72

¹ Mean $\pm$ S.E.

² Fluid intake, urine volume, and Na and K excretion rates ($U_{Na}V$ and U_KV , respectively) were measured during the 24 h preceding the experiments; all are expressed per 24 h per 100 g body weight.

³ *P* values were calculated using the unpaired *t* test.

⁴ NS, $P > 0.05$.

Fig. 1. Correlation of blood urea nitrogen (BUN) with serum creatinine in older (7-8 wk) and younger (3-4 wk) rats given low, normal, and high Na diets. The chemical determinations were performed on samples collected at death or at sacrifice (on the seventh day) following the injection of uranyl nitrate (2.5 mg/kg body wt). The mean value for control rats (non-injected) is also shown.

Fig. 2. Correlation of blood urea nitrogen (BUN) with serum creatinine in older and younger rats (7-8- and 3-4-wk-old, respectively) given low, normal, and high Na diets. The chemical determinations were performed on samples collected at death or at sacrifice (on the fifth day) following the injection of mercuric chloride (at the dosages indicated). Mean values for non-injected (controls) and injected rats are shown; number of rats in each group is indicated.

Fig. 3. The development of acute renal failure in older and younger rats (7-8- and 3-4-wk-old, respectively) given low, normal, and high Na diets as assessed by increases in blood urea nitrogen (BUN) following the injection of uranyl nitrate (2.5 mg/kg body wt). Control values were measured in non-injected rats. Means $\pm$ S.E. are shown; number of rats in each group is indicated.

different among the six groups of rats shown in Figure 3 ($P > 0.05$). In all six groups, mean BUNs on the fifth day were significantly higher than control ($P \leq 0.05$ at most). As assessed by BUN on the fifth day, high Na diet had a protective effect in rats of both ages; BUN was significantly lower in rats fed the high Na diet than in rats fed the control diet ($P < 0.0001$ and $P <$

0.001 for younger and older rats, respectively). However, the low Na diet did not enhance the severity of ARF in a clear cut manner. On the one hand, the difference between mean BUNs of 7-8-wk-old rats fed the control and the low Na diets was of borderline significance ($P = 0.05$) whereas the difference in mean BUNs of the 3-4-wk-old rats fed the same two diets was not significant ($P > 0.05$). On the other hand, mortality rates were higher in 3-4 and 7-8 wk old rats fed the low Na diet (9/15 and 2/17, respectively) than in the corresponding groups fed the control diet (3/15 and 0/16, respectively). Comparison of these mortality rates, 3-4 versus 7-8-wk-old rats, suggests that the younger rats fed normal or low Na diets were more susceptible to, or had a more severe form of, ARF than the older rats. Indeed, BUNs on the fifth day were higher in the younger groups fed either normal or low Na diets than in the corresponding older groups ($P < 0.0001$). In contrast, there were no deaths in either younger or older rats fed the high Na diet and mean BUNs of both age groups on the fifth day were not significantly different ($P > 0.05$).

Figure 4 shows the effect on HgCl_2 -induced ARF of variations in dietary Na in both age groups. As noted above, there was no effect of age or of dietary Na on baseline BUN; grand averages of BUN were 14.6 ± 0.4 and 18.2 ± 0.6 mg% for older and younger rats, respectively. Baseline BUN and BUN 2 days after HgCl_2 injection were compared using one-way analysis of variance and Scheffe contrasts. By the second day after the 4.7 mg/kg dose, BUN was increased above baseline in younger rats fed high ($P < 0.03$), normal ($P < 0.001$) or low ($P < 0.0001$) Na diets and in older rats fed normal ($P < 0.0001$) and low Na diets ($P < 0.0001$). The increase in BUN of older animals fed the high Na diet was of borderline significance ($P = 0.05$). An inverse relationship between dietary Na and the severity of ARF is apparent; BUN was significantly lower in rats fed the high Na diet than in rats fed the control diet ($P < 0.0001$ and $P < 0.001$ for 3-4- and 7-8-wk-old rats, respectively), and in turn, BUN was lower in rats fed the control diet than in those fed the low Na diet ($P < 0.05$ and $P < 0.0001$ for 3-4- and 7-8-wk-old rats, respectively). Also, mortality rates were inversely related to dietary Na: 3/25, 13/43, and 15/25 for the younger rats fed high, normal, and low Na diets, and 0/12, 4/28 and 7/13 for the older rats fed these respective diets and given the 4.7 mg/kg dose.

Figure 4 also shows the HgCl_2 dose-response in rats of both ages. The 1.5 mg/kg dose did not result in a significant increase in BUN on the second day in either 3-4- or 7-8-wk-old rats ($P > 0.05$); also there were no deaths resulting from this dose in either age group. The 3.0 mg/kg dose did not elevate BUN significantly in the older rats ($P > 0.05$), but it did in the younger rats ($P < 0.01$). At this dose, 3/21 younger rats and no older rats died. In both age groups, the 4.7 mg/kg dose increased BUN more than the 3.0 mg/kg dose ($P < 0.001$ and $P < 0.0001$ for 3-4 and 7-8 wk old rats, respectively).

Fig. 4. The development of acute renal failure in older and younger rats (7-8- and 3-4-wk-old, respectively) given low, normal, and high Na diets as assessed by increases in blood urea nitrogen (BUN) 2 and 5 days following the injection of various doses of mercuric chloride. Means $\pm$ SE are shown.

As was the case with the UN model, mean BUNs of younger and older rats fed the high Na diet were not significantly different ($P > 0.05$). However, BUNs were not significantly different between younger and older rats fed the control diet and given the 4.7 mg/kg dose ($P > 0.05$); moreover, BUN of the younger rats fed the low Na diet was actually lower than that of the older rats ($P < 0.0001$). This latter difference is somewhat misleading, however, although overall mortality rates of younger and older rats fed the low Na diet were similar (15/25 and 7/13, respectively), 5 of the 15 deaths of younger animals occurred during the first day, and in these, BUN at death averaged 136 ± 9 mg%. Thus, mean BUN in such a circumstance reflects values of the least severely affected.

Comparison of Figures 3 and 4 suggests that ARF developed more rapidly in the HgCl_2 model. In the UN model, BUN peaked around the fifth day whereas in the HgCl_2 model, it peaked around the second day and by the fifth day, recovery had either begun (BUN had begun to decrease) or the rat had died.

DISCUSSION

The precise mechanisms by which nephrotoxins such as HgCl_2 and UN induce ARF in experimental animals remain to be elucidated (10, 14, 16, 23, 34, 35). The critical target sites for these nephrotoxins as well as their modes of delivery to these sites remain conjectural (23, 35, 37). Moreover, the relationships of the various enzymatic, biochemical, structural and functional derangements that occur after nephrotoxin administration are still unclear (23, 25, 26, 35, 37). Decreased renal blood flow, tubular obstruction, and backleak of filtrate have all been reported to play major roles (9, 11, 14-17, 27, 34, 35, 37). Regardless of the mechanisms involved, in the mature animal, the level of Na intake and/or excretion has been demonstrated to have a profound effect on the severity of ARF in several experimental models (5, 7, 8, 14, 28). Indeed, excellent inverse correlations have been found between Na excretion in the 24-h period preceding nephrotoxin administration and the severity of ensuing ARF (5, 8, 14).

The results obtained from the older rats of the present studies are consistent with an inverse relationship between severity of ARF (as assessed by either BUN or mortality following induction) and dietary Na intake and/or excretion. An inverse relationship

in the younger groups of rats was not as clear cut. On the one hand, high Na diet decreased and low Na diet increased the mortality resulting from injections of either UN or HgCl_2 in the younger rats. On the other hand, although increased Na intake and/or excretion was associated with a reduced BUN following injection of either UN or HgCl_2 , decreased Na intake and/or excretion was not associated with a significantly increased BUN in either model. At any rate, it can be concluded that increased Na intake and/or excretion has a protective effect in the younger as well as the older rat.

Certain findings are suggestive of increased susceptibility to, or increased severity of, ARF in the younger rats. First, except for similar mortality rates in younger and older rats fed the low Na diet and given the 4.7 mg/kg dose of HgCl_2 , mortality rates were consistently higher in the groups of younger rats, whether or not they were fed normal or low Na diets, and whether or not they were injected with UN or with the 3.0 or the 4.7 mg/kg dose of HgCl_2 . Second, the intermediate dose of HgCl_2 had no apparent effect on older rats (no deaths, no significant increase in BUN above baseline) whereas this dose, when given to the younger rats, resulted in three deaths and more than doubled the BUN of the survivors. It should be recognized that these comparisons are only suggestive. Differences in mortality rate might have an alternate explanation, namely, that because of age-related differences in food intake, reducing renal function by a given amount in younger rats produces larger distortions in electrolyte balances (plasma pH and potassium and phosphate concentrations), and with more drastic consequences, than in older rats. Moreover, direct comparisons of either BUN or SCr , between younger and older animals is not completely satisfactory because of differences in basal values and/or age-related differences in food intake per unit body weight. However, the similar values of % increase in BUN/% increase in SCr in rats of both age groups suggest that, short of measuring changes in glomerular filtration rate directly, the use of BUN as an index is not inappropriate. It is of interest that, whether mortality or BUN is used as an index of susceptibility or severity, the differences between the 3-4- and the 7-8-wk-old rats of the present study were less dramatic than those observed previously between 2- and 8-wk-old rats (6). Following the 4.7 mg/kg dose of HgCl_2 , BUN continued to increase between the second and fifth days in 2-wk-old rats, in contrast to the results presented in Figure 4 for rats 3-4-wk-old. The reason for this difference is not known. Slower elimination of nephrotoxin due to less fully developed glomerular and tubular functions in the 2-wk-old rats (2, 12, 19-20, 22, 30, 31) might have played a role.

In conclusion, although there are age-related differences in the renal handling of Na (3, 4, 24, 29, 32, 33) and there may be age-related differences in the susceptibility to and the severity of ARF, younger rats can be protected against nephrotoxin-induced ARF by increasing their Na intake and/or excretion. The mechanism is not known. Both long-term and short-term changes in Na intake and/or excretion are effective in mature rats, and the protective effects are independent of changes in the activity of the renin-angiotensin system (5, 7, 8, 10). It has been suggested that a state of sustained solute diuresis may increase the rate of nephrotoxin elimination and minimize prolonged tubular injury and obstruction (5, 7, 11, 23, 26, 27, 35). Whatever the mechanism, that the protective effect can be achieved in the young may have important clinical implications.

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ANNOUNCEMENT

The International Center for Interdisciplinary Studies of Immunology at Georgetown University will be holding an international workshop on "ACUTE DIARRHEA: ITS NUTRITIONAL CONSEQUENCES IN CHILDREN" at the Pan American Development Foundation in Washington, D.C. on May 7-10, 1982. The conference, which is being co-sponsored by the Nestle Corporation and the Pan American Development Foundation, will bring together a group of internationally renowned authorities in the fields of immunology and nutrition and will include the most recent advances in acute diarrhea in infancy including epidemiology, pathogenesis, therapy and prevention. A plenary session summarizing the contents of the workshop will be held on May 10 to which all interested individuals are cordially invited to attend. For further information, contact: Ms. Barbara Claudy, International Center for Interdisciplinary Studies of Immunology, Georgetown University, School of Medicine, Washington, D.C. at (202) 625-7437.