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Validation of Impaired Renal Function Chick Model with Uranyl Nitrate

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Uranium is a highly toxic element when soluble salts are administered parenterally, whereas the index of toxicity is very low when ingested (NAS Subcommittee 1980). Due to low gastrointestinal absorption, uranium is not considered to be a chemotoxic threat as an environmental contaminant (Voegtlin and Hodge 1949). Most absorbed uranium is excreted in the urine with tissue deposition primarily occurring in bone and kidney tissue (Passow et al. 1961). In vivo, uranium is present in tetravalent (U^{4+}) or hexavalent (U^{6+}) forms, with U^{6+} as the most stable form. In the salt form, uranium is one of the oldest substances used experimentally to induce mammalian renal failure. Leconte first used uranium as a nephrotoxic agent in 1854 (MacNider 1929). Renal damage occurs when uranium reacts chemically with the protein of columnar cells lining the tubular epithelium, leading to cellular injury and necrosis (Tannenbaum 1951; Hodge et al. 1973; Voegtlin and Hodge 1949). Uranyl nitrate (UN) is the most common uranium salt utilized for nephrotoxic modeling (Avasthie et al. 1980; Dickson 1909; Flamenbaum et al. 1974; Haley 1982; Mac Nider 1929; Pelayo et al. 1981; Stein et al. 1975).

The development of an impaired renal function (IRF) chick model (Harvey et al. 1984) required a suitable nephrotoxic compound, such as UN, for validation, yet toxicity data for chickens were notably absent in the literature. The objective of the present study was to validate the IRF model with UN, based upon preliminary nephrotoxic dosages developed in this laboratory (Kubena et al. 1983).

MATERIALS AND METHODS

Two hundred day-old Leghorn cockerals (Hy-Line, W-36) were wing banded and placed in electrically heated batteries (20 chicks/deck). The chicks received continuous light and were fed commercial starter ration and tap water, ad libitum. At 3 weeks of age, the surgical ligation of ureters to reduce renal mass by 80% producing IRF (Harvey et al. 1984) was performed on 80 chicks while sham surgery was performed on 50 chicks. Chicks were randomly selected from each of 3 groups (non-surgery, sham surgery, IRF), assigned to treatments of 20 each, and consisted of non-surgery untreated (1), sham untreated (2), IRF untreated (3), non-surgery treated (4), sham treated (5), and IRF

treated (6). Following a 5 day acclimatization period, chicks in treatments 1, 2, and 3 were injected with 1.0 ml of physiological saline, while chicks in treatments 4, 5, and 6 were injected with 250 mg UN/kg body weight. Uranyl nitrate, 6-hydrate (Fisher Scientific Products, Houston, TX) was dissolved in physiological saline and concentrations (wt/v) calculated on UN content less the hydrated portion of the compound. Dosages were administered subcutaneously at the base of the neck via a 22 ga needle and tuberculin syringe. Concentrations of UN solutions were adjusted so that volumes of injections did not exceed 1.2 ml/chick.

At 48 hrs post-injection, 15 randomly selected chicks from each treatment were bled for biochemical analyses via cardiac puncture, killed by cervical dislocation, and subjected to necropsy. An atomic-absorption spectrophotometer (Perkin-Elmer Model 403, Perkin Elmer, Norwalk, CT) was used to determine serum levels of calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) as outlined in the manufacturer's manual. Uric acid (UA) and creatinine (Creat) were determined on automated equipment (Technicon Auto Analyzer Model 1, Tarrytown, NY), according to the manufacturer's recommended procedures. Serum levels of albumin (Alb), blood urea nitrogen (BUN), acetyl cholinesterase (ACH), gamma glutamyl transferase (GGT), glucose (GLU), inorganic phosphorous (P), total protein (Tot Prot), cholesterol (Chol), alkaline phosphatase (Alk Phos), lactic dehydrogenase (LDH), and aspartate aminotransferase (AST) were determined on automated equipment (Gilford Model 3500, Gilford, Oberlin, OH) according to the manufacturer's recommended procedures. Two other serum components were determined with the same automated equipment; however, different procedures were used for these determinations, i.e., creatine kinase (CK) (CKNAC, BIO-DYNAMICS ICS/BMC Procedure, Indianapolis, IN) and triglycerides (Trig) (Dow Triglycerides Determination, Dow Diagnostics, Indianapolis, IN). Reagents for the determination of Alk Phos, BUN, Chol, GLU, Tot Prot, P, LDH, and AST were purchased from Worthington Diagnostics, Freehold, NJ. Reagents for the determination of UA (Technicon Reagent Methodology N13B) and Creat (Technicon Reagent Methodology N11B) were purchased from Technicon Corp., Tarrytown, NY. Triglyceride determinations were performed using the reagents purchased from Dow Chemical Co. Calcium, Mg, K, and Na standards were purchased from Varian Techtron Pty. Limited, Mulgrave, Australia. Reagents for ACH determinations were purchased from Boehringer-Mannheim Diagnostics, Inc., Houston, TX; whereas, GGT determinations were made using reagents purchased from Bio-Dynamics/bmc, Indianapolis, IN. Reagents for Alb determinations were purchased from Gilford Diagnostics, Cleveland, OH.

All data for biochemical analyses were compared statistically according to the general linear model procedure for analysis of variance and were ranked according to the Duncan's multiple range test (SAS Institute, Inc., 1982).

RESULTS AND DISCUSSION

The outstanding post-mortem feature of UN treated chicks included visceral gout; thickened peritoneal and air sac membranes; swollen mottled kidneys; and a dark swollen, congested liver. Histologic examination confirmed tubular nephrosis and hepatic necrosis. A characteristic putrid odor was noted in uricacidemic chicks upon opening of the abdominal cavity. Tissues at UN injection sites were edematous with yellow staining of the subcutaneous fat, connective tissue and musculature.

The validation of the IRF model dosed with UN was based primarily upon changes in serum biochemical parameters and not mortality, since this model's usefulness should be directly related to its increased sensitivity to nephrotoxic compounds. Mortality in treatments was low (0, 0, and 20% in 4, 5, and 6, respectively) and was attributed to the close proximity of bleeding times (48 hr) to dosing. Serum biochemical measurements (Tables 1,2,3) demonstrated that differences existed for treatments 4, 5, and 6 as compared to 1, 2, and 3. Observed changes included elevated levels of UA, BUN,

Table 1. Serum enzymatic activities of chickens 48 hrs. post-injection with 250 mg UN/kg body weight.

	Parameter[1]					
Treatment[2]	GGT[3]	Alk Phos	ACH	CK	AST	LDH
1 (NS UT)	14.90^{a}	1450^{a}	1127^{a}	1111^{a}	140^{b}	344^{b}
2 (Sham UT)	14.89^{a}	1492^{a}	1013^{a}	1109^{a}	143^{b}	370^{ab}
3 (Surgery UT)	11.90^{b}	1110^{abc}	1091^{a}	1181^{a}	145^{b}	497^{ab}
4 (NS T)	12.70^{ab}	1214^{ab}	678^{b}	1584^{a}	237^{a}	1051^{a}
5 (Sham T)	12.90^{ab}	883^{bc}	674^{b}	1610^{a}	226^{a}	560^{ab}
6 (Surgery T)	12.10^{ab}	666^{c}	614^{b}	1420^{a}	210^{ab}	926^{ab}

[1] Means of 15 chicks per treatment.
[2] NS = non surgery; UT = untreated (saline injection); T = treated (uranyl nitrate injection).
[3] GGT = gamma glutamyl transferase, Alk Phos = alkaline phosphatase, ACH = acetyl cholinesterase, CK = creatine kinase, AST = aspartate aminotransferase, LDH = lactic dehydrogenase.
a,b,c In the same vertical column, means followed by different letters are significantly different ($P<.05$) according to Duncan's multiple range test.

Table 2. Serum metabolite values of chickens 48 hrs post-injection with 250 mg UN/kg body weight

Treatment[2]	Parameter[1] UA[3]	BUN	GLU	Creat	Chol	Trig	Tot Prot	Alb
	mg/dl						gm/dl	
1 (NS UT)	6.53^{c}	1.31^{b}	308^{a}	.37^{d}	186^{a}	108^{a}	2.82^{ab}	1.32^{a}
2 (Sham UT)	6.07^{c}	1.26^{b}	292^{a}	.47^{bc}	157^{b}	92^{a}	2.62^{bc}	1.17^{b}
3 (Surgery UT)	10.95^{bc}	1.96^{a}	285^{a}	.43^{c}	208^{a}	80^{a}	3.17^{a}	1.05^{b}
4 (NS T)	8.01^{c}	1.77^{a}	307^{a}	.50^{b}	157^{b}	89^{a}	1.94^{d}	.74^{d}
5 (Sham T)	13.44^{b}	1.89^{a}	305^{a}	.48^{bc}	154^{b}	69^{a}	2.36^{c}	.87^{c}
6 (Surgery T)	22.24^{a}	2.03^{a}	251^{b}	.59^{a}	124^{c}	82^{a}	1.81^{d}	.56^{e}

[1] Means of 15 chicks per treatment.
[2] NS = non-surgery; UT = untreated (saline injection); T = treated (uranyl nitrate injection).
[3] UA = uric acid, BUN = blood urea nitrogen, GLU = glucose, Creat = creatinine, Chol = cholesterol, Trig = triglycerides, Tot Prot = total protein; Alb = albumin.
a,b,c,d,e In the same vertical column, means followed by different letters are significantly different ($P<.05$) according to Duncan's multiple range test.

Table 3. Serum mineral values of chickens 48 hrs post-injection with 250 mg UN/kg body weight

Treatment[2]	Parameter[1] Ca[3]	K	Mg	Na	P
	mg/dl				
1 (NS UT)	12.98^{abc}	24.52^{a}	2.76^{b}	385^{b}	7.05^{c}
2 (Sham UT)	11.88^{c}	23.57^{a}	2.76^{b}	371^{b}	6.87^{c}
3 (Surgery UT)	14.03^{a}	24.62^{a}	3.32^{a}	414^{a}	6.67^{c}
4 (NS T)	12.86^{abc}	17.88^{b}	2.46^{bc}	377^{b}	7.18^{c}
5 (Sham T)	12.40^{bc}	15.79^{b}	2.22^{c}	368^{b}	8.21^{b}
6 (Surgery T)	13.83^{ab}	19.35^{b}	2.73^{b}	377^{b}	9.35^{a}

[1] Means of 15 chicks per treatment.
[2] NS = non-surgery; UT = untreated (saline injection); T = treated (uranyl nitrate injection).
[3] Ca = calcium, K = potassium, Mg = magnesium, Na = sodium, P = phosphorus.
a,b,c In the same vertical column, means followed by different letters are significantly different ($P<.05$) according to Duncan's multiple range test.

Creat, AST, and P; while levels of GLU, Chol, Alk Phos, ACH, K, Tot Pro, and Alb declined.

Uric Acid levels of UN treated chicks (treatments 4, 5, and 6) were 1.2, 2.1 and 3.4 times, respectively, of treatment 1 (Table 2). Uric acid levels of treatment 3 were 1.7 times those of 1, while levels of treatment 6 were 2.0 times those of 3. Although not as sensitive an indicator of avian renal function as UA, Creat measurements suggested that the nephrotoxic effects of UN were more pronounced in treatment 6 than others. Hyperphosphatemia (Table 3), a common sequel of renal insult in mammalian systems, was noted in treatment 6 and was attributed to acute renal failure (Simeson 1980).

The reasons for declines in Chol, Alk Phos and ACH, treatment 6, are unknown. However, Chol and ACH are synthesized in the liver, and hepatocellular necrosis, described above, probably contributed to lowered circulating levels. The major portion of serum Alk Phos levels in young growing animals originate from osteoblasts (Kramer 1980). Uranium salts are known to replace Ca in bone (Passow et al. 1961) and perhaps a disruption of osteoblastic function also reduced circulating Alk Phos levels. Glucose, K, Tot Prot, and Alb levels were significantly decreased in treatment 6. This would suggest tubular damage had occurred since reabsorption of these molecules takes place in the proximal convoluted tubules (PCT) (Hook 1981, Siller 1981, Sturkie 1965). Potassium and UA metabolism are interrelated in that K is required for UA tubular transport. Therefore, high secretion rates of UA may cause an excessive loss of K ions (Austic 1983). Of the Tot Prot loss, the greatest percentage was probably Alb as it is routinely filtered by the glomerulus, then reabsorbed by the PCT. UN is toxic to PCT cells in mammals; it also binds with Alb to impair tubular protein transport (Hook 1981).

Reduced renal function, particularly that of the PCT, is the most predominate sign of UN toxicity in the chicken. Due to reabsorption and secretion deficiencies of tubules, excessive losses of GLU, Alb, and K occur. Identifying effects of UN allowed the authors to validate the sensitivity of an IRF chick model to a known nephrotoxic agent. Utilization of UN and the IRF model will be helpful to further investigations of renal responses to nephrotoxic compounds.

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