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Effects of Unilateral Nephrectomy on Erythrocytosis and Arteriosclerosis Induced in Rats by Intrarenal Injection of Nickel Subsulfide*

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Summary. Administration of Ni_3S_2 to rats by unilateral inrarenal (ir) injection (5 mg/rat) caused erythrocytosis, arteriosclerosis, and abnormal plasma concentrations of asparagine, glycine, histidine, and lysine. Resection of the ipsilateral (Ni_3S_2-treated) kidney on the fourth day after the ir injection prevented erythrocytosis, amino acid disturbances, and severe arteriosclerotic lesions (fibrous intimal plaques and focal medial necrosis), but did not prevent early arteriosclerotic lesions (subintimal oedema with splitting of elastica). The early arteriosclerotic lesions appear to be initiated by vascular dissemination of Ni_3S_2 particles immediately post-injection, whereas the erythrocytosis, amino acid disturbances, and advanced arteriosclerotic lesions depend upon continued presence of the Ni_3S_2-injected kidney. Resection of the contralateral (non-injected) kidney has no effect upon Ni_3S_2-induced erythrocytosis, arteriosclerosis, or amino acid disturbances. Glomerulomegaly and mesangial hyperplasia developed in control rats following unilateral nephrectomy, owing to compensatory renal hypertrophy. Glomerulomegaly was more pronounced in Ni_3S_2-treated rats following contralateral nephrectomy than following ipsilateral nephrectomy, suggesting that erythrocytosis and compensatory renal hypertrophy act synergistically to enhance glomerulomegaly.

Key words: Nickel subsulfide – Nephrectomy – Erythrocytosis – Glomerulomegaly – Arteriosclerosis

Introduction

Administration of nickel subsulfide (Ni_3S_2) to rats by intrarenal (ir) injection (5–10 mg/rat) produces a remarkable assortment of pathological responses:

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(*a*) intense erythroid hyperplasia occurs in bone marrow and spleen from 1 week–6 months post-injection (Morse et al. 1977; Hopfer et al. 1978 and 1980; McCully et al. 1982); (*b*) renal cancers develop in Ni_3S_2-treated kidneys commencing at approximately eight months post-injection (Jasmin and Riopelle 1976; Sunderman et al. 1979); (*c*) hyperplasia of salivary glands occurs at one week post-injection and persists for at least four months (McCully et al. 1982); and (*d*) arteriosclerotic lesions appear in the aorta and in arteries and arterioles of the heart, lung, brain, kidney, and other organs as early as one week post-injection and become most prominent at 8–10 weeks (McCully et al. 1982). Histopathological findings in kidneys of Ni_3S_2-treated rats include inflammation and fibrosis along the needle track (Morse et al. 1977; Hopfer et al. 1980), phagocytosis of Ni_3S_2 particles by mononuclear cells and glomerular mesangial cells (McCully et al. 1982), renal hypertrophy, pronounced glomerulomegaly, and mesangial cell hyperplasia (McCully et al. 1982), and crystalline mitochondrial incluions in renal tubular cells (Jasmin 1978; Jasmin et al. 1979).

The erythrocytosis induced by ir injection of Ni_3S_2 is apparently mediated by enhanced renal production of erythropoietin, since erythropoietin activity is increased in serum of Ni_3S_2-treated rats (Solymoss and Jasmin 1978; Hopfer et al. 1979), and since the erythrocytosis regresses following removal of the Ni_3S_2-injected kidney (Jasmin and Solymoss 1975). Stimulation of erythropoiesis in bone marrow and spleen leads to sustained elevations of blood haematocrit, haemoglobin concentration, and reticulocyte count; incorporation of ^{59}Fe into erythrocytes is increased, and blood volume is greatly expanded (Jasmin and Solymoss 1975 and 1978; Morse et al. 1977; Hopfer et al. 1978 and 1980; Oskarsson et al. 1981). Ni_3S_2-induced erythrocytosis is inhibited by simultaneous ir injection of manganese dust (Hopfer and Sunderman 1978), or by sustained sc infusion of a nickel-chelator, sodium diethyldithiocarbamate (Sunderman et al. 1982). Ni_3S_2 is the most potent of 17 nickel compounds that have been tested for erythropoietic stimulation by ir administration to rats (Sunderman and Hopfer 1982). Induction of erythrocytosis by ir injection appears to be specific for nickel compounds, since ir injections of Au, Cd, Co, CoS, Cr, Cu, Fe, Mn, and Pb dusts do not stimulate erythrocytosis in rats (Jasmin and Riopelle 1976; Sunderman and Hopfer 1982). Administration of Ni_3S_2 to rats by im, ip, iv, intrahepatic, or intrasplenic injections does not induce erythrocytosis; ir injection seems to be the sole route of Ni_3S_2 administration that stimulates erythropoiesis (Jasmin and Solymoss 1978; Hopfer et al. 1980; Sunderman and Hopfer 1982). The present paper describes the effects of unilateral nephrectomy upon the haematological, biochemical, and histopathological responses to ir injection of Ni_3S_2 in rats.

Materials and Methods

Nickel subsulfide (αNi_3S_2, median particle diameter $<2\,\mu m$) was provided by INCO Ltd., Toronto, Canada; its chemical and physical properties and criteria of purity have been described previously (Hopfer and Sunderman 1978). The experimental animals were 60 male

rats of the Fischer-344 strain (Charles River Breeding Laboratories, Inc., Kingston N.Y., USA), approximately 8 weeks old at the time of ir injection (mean body wt = 156 g, s.d. = 9 g). The rats were kept in stainless-steel cages and were fed Purina laboratory rat chow and water ad libitum. The rats were randomly divided into six groups of 10 rats (groups *A–E*). Each rat in the vehicle control groups (*A, B,* and *C*) was anesthetized with diethyl ether; the right kidney was exposed by a subcostal lumbar incision; 0.1 ml of injection vehicle (sterile NaCl solution, 0.14 mol/litre) was slowly injected into the cephalic pole of the kidney by use of a tuberculin syringe with a 25 gauge needle; the muscles and fascia were sutured with silk, and the skin incision was closed with surgical clips. Rats in the test groups (*D, E,* and *F*) were treated similarly, except that 5 mg of Ni_3S_2 was injected ir in 0.1 ml of NaCl vehicle. On the fourth day after the ir injection, rats in groups *B* and *E* were subjected to right nephrectomy, and rats in groups *C* and *F* were subjected to left nephrectomy. The surgical technique for nephrectomy was similar to that for ir injection; the ureter and renal vessels were ligated with silk sutures, and the kidney was excised with scissors.

At 2, 4, 6, and 8 weeks after ir injection, the tip of each rat's tail was incised with a scapel, and blood (80 µl) was collected into heparinized capillary tubes for measurement of haematocrit (Strumia et al. 1954). At 9 weeks after the ir injection, blood (3 ml) was obtained by cardiac puncture for measurement of plasma amino acids. The heparinized blood samples were centrifuged within 5 min; the plasma was immediately deproteinized with sulfosalicylic acid; the protein-free extracts were stored at −20° C until analysis. Amino acid concentrations were measured by ion-exchange chromatography, as described by Sunderman and Horak (1981), using a Beckman model 119CL amino acid analyzer.

Immediately after cardiac puncture, each rat was killed by inhalation of diethyl ether. Specimens of aorta, femoral bone marrow, brain, heart, kidneys, liver, lungs, and spleen were fixed in 10% neutral buffered formalin. Paraffin-embedded sections were sectioned at 4 µm, stained with hematoxylin and eosin (H & E), and examined by light microscopy. Maximum diameters of 40 glomeruli in each kidney were measured with a calibrated ocular micrometer, as previously described (McCully et al. 1982), and mean glomerular volumes were calculated, assuming spherical shape, by the equation $V = 4/3\ \pi r^3$. Arteriosclerotic changes were identified by systematic microscopic examination of the aorta (including arch, thoracic, and abdominal segments) and the arteries and arterioles of the heart, brain, lungs, and kidney. Arteriosclerotic lesions were scored according to stage and severity, in the following categories: (*a*) subintimal oedema and splitting of elastica, with or without hyperplasia of smooth muscle cells, (*b*) fibrous intimal plaques, and (*c*) focal medial necrosis adjacent to fibrous intimal plaques. Incidences of the specific arterial lesions were determined for each experimental group.

Null hypotheses for differences between mean values for blood haematocrit, plasma amino acid concentrations, and glomerular volumes in the experimental groups were tested by Student's *t*-test; the significance of differences between incidences of arteriosIcerotic lesions was tested by Fisher's exact test (Siegel 1956).

Results

Blood haematocrits of the rats at 2, 4, 6, and 8 weeks after ir injection of vehicle or Ni_3S_2 are listed in Table 1. Haematocrit values for the three control groups were pooled, since there were no significant differences between the mean haematocrit values in rats that received an ir injection of vehicle without nephrectomy (group *A*), with right nephrectomy (group *B*), or with left nephrectomy (group *C*). In rats that received an ir injection of Ni_3S_2 without nephrectomy (group *D*), mean haematocrit values were significantly increased throughout the period of observation. Erythrocytosis was completely prevented by resection of the injected kidney on the fourth day after Ni_3S_2 administration (group *E*). Under the same conditions, resection of the contralateral kidney did not affect the development of erythrocytosis (group *F*).

Table 1. Blood haematocrit of rats at specified intervals after an injection of NaCl vehicle (0.14 mol/litre, 0.2 ml/rat) or Ni_3S_2 (5 mg/rat, in 0.2 ml of vehicle) into the right kidney. Unilateral nephrectomy was performed in certain groups on the fourth day after the ir injection

Group	Ni_3S_2 dose (mg/rat)	Resected Kidney	No. of rats	Blood haematocrit (%, mean ± s.d.)			
				2 weeks	4 weeks	6 weeks	8 weeks
A	0	None	10	49±2	52±2	51±2	53±2
B	0	Right	10	47±1	50±2	51±1	50±3
C	0	Left	10	48±2	50±2	51±1	50±1
All controls (*A*+*B*+*C*)			30	48±2	51±2	51±2	51±3
D	5	None	10	62±3*	70±3*	74±3*	75±3*
E	5	Right	10	47±1**	49±1**	52±2**	49±2**
F	5	Left	10	61±2*	70±5*	76±3*	76±3*

* $p<0.001$ versus group *A* and versus all controls (groups *A*+*B*+*C*)
** $p<0.001$ versus group *D* and versus group *F*

Concentrations of amino acids in rat plasma at 9 weeks after injections are listed in Table 2. Data for the three control groups were pooled, since the mean values did not differ significantly among these groups. In rats that received an ir injection of Ni_3S_2 without nephrectomy (group *D*), plasma asparagine concentration was significantly increased, and plasma glycine, histidine, and lysine concentrations were significantly diminished. These abnormalities were prevented by resection the Ni_3S_2-injected kidney (group *E*); the abnormalities were unaffected by resection of the contralateral kidney (group *F*).

Autopsies and histological examinations of the rats were performed at 9 weeks after the ir injection. No significant extra-renal lesions were observed in the control rats that received an ir injection of NaCl with or without unilateral nephrectomy (groups *A*, *B*, and *C*). The following pathological findings were noted in practically all rats of group *D*, which received an ir injection of Ni_3S_2 without nephrectomy, and in rats of group *F*, which were treated by ir injection of Ni_3S_2 and subsequent resection of the contralateral kidney: (*a*) pronounced erythroid hyperplasia in bone marrow; (*b*) extramedullary hematopoiesis in spleen, liver, and lung; (*c*) marked vascular congestion of spleen, liver, lung, and kidneys; and (*d*) subcapsular fibrosis and fibroblastic hyperplasia along the needle track in Ni_3S_2-treated kidneys. Particles of Ni_2S_2 were abundant along the needle track and in the parenchyma of the injected kidney; Ni_3S_2 particles were sparsely distributed at distant sites throughout the body, including especially the lungs, bone marrow, and contralateral kidney.

Histopathological examinations of rats in group *E*, which were treated by ir injection of Ni_3S_2 and subsequent resection of the ipsilateral kidney, revealed the following findings: the bone marrow was not hyperplastic, the myeloid/erythroid ratio of bone marrow cells was normal, extramedullary haematopoiesis was minimal, and the spleen, liver, lungs, and kidneys were not congested.

Table 2. Plasma amino acid concentrations in rats at 9 weeks after injection of vehicle or Ni_3S_2 into the right kidney (as specified in the caption to Table 1) with unilateral nephrectomy in certain groups four days after the ir injection

Amino Acids[a]	Plasma amino acid concentrations (μmol/litre, mean ± s.d.)			
	All controls[b] $N=29$	Group *D* $N=10$ Ni_3S_2 (5 mg) without nephrectomy	Group *E* $N=10$ Ni_3S_2 (5 mg) with right nephrectomy	Group *F* $N=10$ Ni_3S_2 (5 mg) with left nephrectomy
Ala	386 ± 48	358 ± 37	405 ± 62	383 ± 46
Arg	147 ± 22	151 ± 12	136 ± 16	160 ± 19
Asn	86 ± 14	98 ± 14*	83 ± 14	106 ± 15**
Asp	18 ± 5	25 ± 7	14 ± 16	21 ± 9
Cys	91 ± 19	96 ± 20	86 ± 16	82 ± 20
Cyt	3.7 ± 1.5	3.1 ± 0.8	4.9 ± 1.8	3.5 ± 0.5
Glu	63 ± 19	62 ± 19	66 ± 10	62 ± 18
Gln	950 ± 245	878 ± 233	1,027 ± 220	1,192 ± 236
Gly	216 ± 23	160 ± 28***	225 ± 24	189 ± 23**
His	69 ± 7	52 ± 6***	66 ± 5	55 ± 7***
Hyp	51 ± 9	49 ± 10	45 ± 9	60 ± 7
Ile	100 ± 12	105 ± 10	97 ± 13	101 ± 12
Leu	171 ± 24	166 ± 17	161 ± 22	168 ± 19
Lys	413 ± 42	353 ± 39***	410 ± 36	384 ± 37*
Met	49 ± 7	53 ± 8	48 ± 7	50 ± 5
Orn	52 ± 8	55 ± 8	59 ± 11	51 ± 5
Phe	62 ± 7	53 ± 8	61 ± 5	61 ± 6
Pro	145 ± 20	154 ± 18	141 ± 22	162 ± 25
Ser	175 ± 16	169 ± 14	168 ± 10	168 ± 14
Tau	190 ± 61	187 ± 27	182 ± 59	246 ± 98
Trp	66 ± 12	74 ± 14	63 ± 11	57 ± 13
Tyr	86 ± 12	94 ± 12	86 ± 9	94 ± 12
Val	208 ± 23	227 ± 28	194 ± 32	212 ± 23

[a] Abbreviations specified by the Commission on Biochemical Nomenclature (1975). Non-standard abbreviations are Cyt and Tau, which designate cystathionine and taurine, respectively

[b] Includes group *A* (without nephrectomy, $N=10$), group *B* (right nephrectomy, $N=9$), group *C* (left nephrectomy, $N=10$)

* $p<0.05$ versus all controls
** $p<0.01$ versus all controls
*** $p<0.001$ versus all controls

Glomerulomegaly, hyperplasia of mesangial cells, and increased deposition of mesangial matrix were observed in the residual kidneys of control rats in groups *B* and *C*, which underwent unilateral nephrectomy four days after ir injection of NaCl vehicle. The same findings were consistently observed in rats of groups *D*, *E*, and *F*, which received an ir injection of Ni_3S_2 with or without unilateral nephrectomy. Measurements of glomerular volume in the six experimental groups are listed in Table 3. In rats of group *E*, which were treated by ir injection of Ni_3S_2 and subsequent resection of the ipsilateral kidney, the mean glomerular volume of the residual kidney

Table 3. Renal glomerular volumes of rats killed 9 weeks after injection of vehicle or Ni_3S_2 into the right kidney (as specified in the caption to Table 1) with unilateral nephrectomy in certain groups four days after the ir injection. Maximum diameters of 40 glomeruli in each kidney were measured by ocular micrometry; glomerular volumes were calculated by the equation $V = 4/3\ \pi\ r^3$

Group	Ni_3S_2 dose (mg/rat)	Resected kidney	No. of rats	Glomerular volume ($\mu m^3 \times 10^6$)[a]	
				Right kidney	Left kidney
A	0	None	10	0.71 ± 0.12	0.76 ± 0.13
B	0	Right	10	resected	1.13 ± 0.23*
C	0	Left	10	1.21 ± 0.20*	resected
D	5	None	10	1.28 ± 0.13*	1.16 ± 0.19*
E	5	Right	10	resected	1.25 ± 0.29*
F	5	Left	10	1.49 ± 0.25*, **	resected

[a] Mean ± s.d.

* $p < 0.001$ versus corresponding value for group *A*
** $p < 0.001$ versus corresponding values for groups *B*, *C*, *D*, and *E*

Table 4. Incidence of arteriosclerotic lesions in rats killed 9 weeks after injection of vehicle or Ni_3S_2 into the right kidney (as specified in the caption to Table 1) with unilateral nephrectomy in certain groups four days after the ir injection. The incidences of arteriosclerotic lesions are based on systematic microscopic examination of the aorta (arch, thoracic, and abdominal segments) and arteries and arterioles in heart, kidneys, brain, and lungs

Group	Ni_3S_2 dose (mg/rat)	Resected kidney	Incidence of arteriosclerotic lesions		
			Subintimal oedema with splitting of elastica	Fibrous intimal plaques plaque	Focal medial necrosis next to fibrous
A	0	None	5/10	2/10	0/10
B	0	Right	4/10	3/10	0/10
C	0	Left	6/10	2/10	0/10
All controls ($A+B+C$)			15/30	7/30	0/30
D	5	None	10/10*	10/10*	7/10*
E	5	Right	10/10*	5/10**	2/10**
F	5	Left	10/10*	9/10*	8/10*, [a]

* $p < 0.01$ versus all controls (groups $A+B+C$)
** $p < 0.05$ versus groups *D* and *F*

[a] Two rats had mural thrombi adjacent to foci of medial necrosis and intimal plaques

did not differ significantly from that of nephrectomized controls (groups *B* and *C*). On the other hand, when the contralateral (non-injected) kidney of Ni_3S_2-treated rats was resected (group *F*), the mean glomerular volume of the residual kidney was significantly greater than that of groups *B*, *C*, *D*, and *E*. Mesangial cell hyperplasia and deposits of mesangial matrix were observed in residual kidneys of Ni_3S_2-treated rats after ipsilateral or contralateral nephrectomy. When the non-injected kidney was resected, glo-

merular capillaries of the residual kidney were markedly congested, reflecting the presence of erythrocytosis.

Arteriosclerotic lesions in rats killed 9 weeks after ir injection of vehicle or Ni_3S_2 are summarized in Table 4. In control rats that received ir injection of NaCl vehicle, unilateral nephrectomy had no significant effect upon the incidence of arteriosclerotic lesions; therefore, data for the three control groups were pooled. In group *D*, which received ir injection of Ni_3S_2 without subsequent nephrectomy, and in group *F*, which received ir injection of Ni_3S_2 with resection of the contralateral kidney, the incidences of all categories of arterio-sclerotic lesions were significantly greater than in controls. In group *E*, which underwent ipsilateral nephrectomy following ir injection of Ni_3S_2, the incidence of subintimal oedema with splitting of elastica was significantly greater than controls and did not differ from groups *D* and *F*; however, the incidences of fibrous intimal plaques and focal medial necrosis in group *F* were significantly less than in groups *D* and *F*.

Discussion

The present study shows that early resection of the Ni_3S_2-injected kidney prevents Ni_3S_2-induced erythrocytosis in rats, whereas resection of the contralateral non-injected kidney has no such effect. These observations confirm and extend an earlier report that removal of the Ni_3S_2-injected kidney, following the development of erythrocytosis, causes regression of the plethoric condition (Jasmin and Solymoss 1975). The mesangial hyperplasia and glomerulomegaly that develop in both kidneys of rats after unilateral ir injection of Ni_3S_2 reflect polycythemia with expanded blood volume and generalized vascular congestion. Recent demonstration that cultured rat mesangial cells synthesize erythropoietin (Kurtz et al. 1982) confirms our suggestion (McCully et al. 1982) that mesangial cells are a site of erythropoietin production. However, the present finding that mesangial cell hyperplasia occurs without erythrocytosis in Ni_3S_2-treated rats with ipsilateral nephrectomy shows that mesangial hyperplasia is not invariably associated with increased production of erythropoietin. An interesting secondary result of this investigation is the demonstration that unilateral nephrectomy produces mesangial hyperplasia and glomerulomegaly in control rats. These responses are manifestations of the compensatory renal hypertrophy that occurs following unilateral nephrectomy (Marshall 1963; Malt 1969). Glomerular enlargement is more pronounced in Ni_3S_2-treated rats following contralateral nephrectomy than following ipsilateral nephrectomy, suggesting that erythrocytosis and compensatory real hypertrophy act synergistically to enhance glomerulomegaly.

This investigation demonstrates that ir administration of Ni_3S_2 to rats causes alterations of amino acid metabolism; these abnormalities are prevented by resection of the Ni_3S_2-treated kidney, but are unaffected by resection of the contralateral kidney. Diminished plasma concentrations of several amino acids, including glycine, histidine, and lysine, were previously reported in rats with acute renal damage from administration of nickel com-

pounds (Gitlitz et al. 1975; Horak and Sunderman 1980; Sunderman and Horak 1981). The diminutions of plasma amino acid concentrations were associated with aminoaciduria and proteinuria, as manifestations of nickel nephropathy. Increased plasma concentrations of asparagine have not been observed in rats with nickel nephropathy (Gitlitz et al. 1975; Horak and Sunderman 1980). In the present study the occurrence of hyperasparaginemia and diminished plasma concentrations of glycine, histidine, and lysine in Ni_3S_2-treated rats presumably reflects mild impairment of renal and hepatic function.

Previous studies showed that ir administration of Ni_3S_2 to rats produces vascular dissemination of Ni_3S_2 particles. At 1 h after an ir injection, Ni_3S_2 particles are predominantly located in the injected kidney, but are also scattered throughout the body, including the opposite kidney, lungs, bone marrow, lymph nodes, spleen, liver, and adrenals (Hopfer et al. 1980; McCully et al. 1982). Based upon the results of the present study, the authors suspect that embolization of Ni_3S_2 particles initiates the widespread endothelial lesions in arteries and arterioles. Progression from subintimal oedema and splitting of elastica to fibrous intimal plaques and focal medial necrosis is potentiated by the continued presence of Ni_3S_2-injected kidney. The progression from mild to severe arteriosclerotic lesions may involve (*a*) sustained release of Ni-complexes from the Ni_3S_2-injected kidney, (*b*) a direct effect of erythropoietin (or other metabolites, such as prostaglandins) released from the Ni_3S_2-injected kidney, or (*c*) indirect effects of Ni_3S_2-treatment, such as hyperviscosity and expanded blood volume.

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