Urol Res (2000) 28:97–103

ORIGINAL PAPER

L. Cai · D. X. Deng · J. Jiang · S. Chen
R. Zhong · M. G. Cherian · S. Chakrabarti

Induction of metallothionein synthesis with preservation of testicular function in rats following long term renal transplantation

Received: 9 March 1999 / Accepted: 1 October 1999

Abstract Metallothionein (MT), as an acute phase or stress-response protein and free radical scavenger, is related to inflammation and cellular protection from oxidative damage. In order to evaluate long-term testicular damage and the role of MT following renal transplant, nine allogenic (Fisher 344 → Lewis) and seven isogenic (Lewis → Lewis) renal transplants were performed and the recipient rats were followed for 140 days when allografts develop chronic transplant rejection. Testicular weight, light microscopic morphology, and lactate dehydrogenase-X enzyme activity were assessed. Testicular MT was determined by Cd-heme assay, and was localized immunocytochemically using a polyclonal rabbit antibody. No differences in testis weight, morphology, or LDH-X enzyme activity were found between allograft and isograft recipients. Testicular MT level was significantly increased in the testis of allograft recipients. Testicular zinc (Zn) and copper (Cu) levels, but not iron (Fe) level, were significantly higher in testis with allograft kidney than that with isograft kidney. In addition, Cu/Zn ratio was also significantly high in the allograft group. However, the MT level did not show any significant correlation either with Cu and Zn alone or with Cu/Zn and Fe/Zn ratios. These data suggest that allogenic stimuli may induce MT synthesis in the recipient testis. The increased MT level in an allograft may offer a protective action from oxidative damage in the testis.

Key words Renal transplantation · Metallothionein · Testicular function · Testicular metals

L. Cai · D. X. Deng · S. Chen · S. Chakrabarti (✉)
Department of Pathology, Medical Science Building,
The University of Western Ontario,
London, N6A 5C1, Canada
e-mail: schakrab@julian.uwo.ca
Fax: +1 519 661 3370

J. Jiang
Department of Surgery,
The University of Western Ontario, London, Canada

R. Zhong
Departments of Pathology, and Surgery,
Microbiology and Immunology,
The University of Western Ontario,
Multi-Organ Transplant Program,
London Health Sciences Center,
London, Ontario, Canada

M. G. Cherian
Departments of Pathology and Pharmacology
and Toxicology, The University of Western Ontario,
London, Canada

Introduction

Transplantation of kidney, and more recently of other organs, has become a clinical reality for patients with end-stage organ failure [3, 15]. With the prolonged life span of transplant recipients, biological changes may occur in other organs. Hence proper assessment of testicular functional and structural changes in patients following transplantation is important [13, 24]. However, there are only a few studies on the evaluation of testicular changes after renal transplantation in animal models with long-term follow-up.

Metallothioneins (MTs) are a group of low-molecular weight (6000–7000 daltons), cysteine rich (30%) intracellular proteins with a high affinity for transitional metals such as the essential zinc (Zn), copper (Cu), and toxic cadmium (Cd) [7]. MT-I and MT-II are the major two of four isoforms found so far. MT is expressed in various species and tissues, including testis. A high basal level of MT has been demonstrated in the testicular tissues both by immunohistochemistry and by in situ hybridization [10, 11, 22]. However, MT in testis cannot be induced by injection of metals such as Zn and Cd which are major inducers of hepatic MT synthesis [11, 31].

Due to a high content of cysteine, MT is not only involved in the detoxification of heavy metals but also acts as a free radical scavenger both in vitro and in vivo, and its scavenging activity is more efficient against free radicals than others [21, 28]. Already we have demonstrated the protective effect of MT against radiation- and

transient metal (Cu or Fe)-induced oxidative damage [4–6]. MT has also been considered as an acute-phase or stress-response protein since it is induced readily by a variety of stress-related factors such as glucocorticoids, bacterial lipopolysaccharide (LPS), interferon, alkylating agents, and irradiation [14,19,25]. Many of these inducers can generate reactive oxygen radicals, and result in tissue or cell injury and inflammation, which elicit acute phase responses. Acute phase or stress responses are the local and systemic reactions of the organism to disturbances of its homeostasis due to tissue injury, infection, immunological disorder, or stress conditions. In allogenic organ transplantation, immune reaction and inflammatory responses may produce reactive oxygen species. All these responses and intermediates are able to induce MT protein synthesis. There is evidence of the elevation of MT synthesis either regionally or systematically after organ transplantation [9] and tumor implantation [16,27]. To test the hypothesis that testicular functional and structural changes following allo-transplantation may be related to overexpression of MT protein, the present study investigated testicular structure and function in recipients following long-term renal transplant along with MT protein synthesis and metal concentrations in the testis.

Materials and methods

Animals and treatment

Two strains of rats, Lewis (LEW) and Fisher 344 (F344) rats, were obtained from Jackson Laboratories (Bar Harbor, Maine, USA) and were housed at the Animal Care Facility, University of Western Ontario, London, Ontario, in accordance with guidelines established by the Canadian Council on Animal Care (1984). Only male rats weighing 250–300 g were used for transplantation. The renal transplantation was used as the surgical model [8]. The end-to-side anastomosis in the left side was performed between donor renal vessels and recipient abdominal aorta and inferior vena cava. The urinary tract reconstruction was performed by anastomosis between donor ureter and recipient bladder. To avoid the effect of artifacts on left-side testicular vessels, only right-side testes of the recipient rats were used in this experiment. Two groups of rats were investigated: F344 to LEW combination represented allogenic renal transplantation (ALLO, $n = 9$), and the isogenic LEW to LEW combination (ISO, $n = 7$). All LEW recipients with F344 allografts were treated with a therapeutic dose of cyclosporine A (CsA) at 1.5 mg/kg per day for 10 days to prevent any initial acute rejection episode. One hundred and forty days after renal transplantation, the rats were sacrificed and the testis on the right side was removed and weighed. Three (ISO) and six (ALLO) testes were used to analyze MT protein level, LDH-X enzyme activity and metal levels. The remaining testes (four each for ISO and ALLO) were fixed in 4% paraformaldehyde in phosphate buffer.

Methods

For histological assessment, 5-μm-thick sections were cut consecutively from the tissue blocks previously fixed in formaldehyde and were mounted onto glass slides. They were stained using hematoxylin and eosin (H&E) to evaluate the testicular morphology. Testicular histology was evaluated using comprehensive parameters including rupture of tubules, degeneration of germ cells, germ cell disarray, loss of sperm/spermatids, edema, hemorrhage, and fibrosis/granuloma [2]. Each testis was scored on a scale of 0 to 4 for each parameter, with the highest score indicating the most widely distributed pathology for that parameter. For quantitative estimation of the overall histological changes in the testes, the total histological score was assigned by adding the scores of all parameters for each testis from all the rats in a given group [2].

MT was localized in cells by immunocytochemical staining as previously described [12]. First, sections were deparaffinized and dehydrated, and then immersed in 3% H_2O_2 with methanol for 30 min to remove the endogenous peroxidase activity. Sections were further incubated with 10% normal goat serum for 1 h, followed by incubation with polyclonal rabbit anti-MT serum (1: 1000) at 4 °C overnight. The sections were washed in Tris-HCl buffer (0.05 M, pH 7.6) and were sequentially incubated with (1) biotinylated goat anti rabbit IgG, and (2) avidin-biotin horseradish peroxidase complex following the manufacturer's instructions (ABC kit, Vector Laboratories, Burlington, Calif., USA). The color was developed by immersing slides in 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB) with 0.33% H_2O_2 and counterstained with hematoxylin. Normal rabbit serum, substituted for the primary antibody, was used as the negative control. MT staining intensity was evaluated in different stages of seminiferous tubules between ISO and ALLO groups.

The lactate dehydrogenase-X (LDH-X) enzyme activity in testis was measured by methods published previously [2]. Testes were homogenized in 0.25 M sucrose and then centrifuged at 10,000 rpm for 6 min to collect the supernatant. All the processes were performed at 4 °C or on ice. The reaction mixtures contained 100 μl of testicular supernatant, 0.15 mM α-ketovaleric acid, and 0.15 mM NADH in a final volume of 3.0 ml 0.05 M phosphate buffer solution (pH 7.4). LDH-X activity was determined by the changes in absorption of NADH per minute, at 340 nm with a spectrophotometer (Ultrospec II, LKB-Wallac, Gaithersburg, Md., USA). The reaction was initiated by the addition of the supernatant and followed with the decrease in absorption of NADH at 340 nm. One unit of LDH-X activity was defined as the amount of enzyme catalyzing the oxidation of 1 μmol of NADH in 1 min. The activity of LDH-X was expressed as U/g wet tissue.

MT total protein content in the testis was determined by a Cd-heme assay as described elsewhere [12]. The dissected tissues were homogenized in 0.25 M sucrose and centrifuged at 20,000 rpm for 20 min. An aliquot of the resulting supernatant fraction, diluted with 30 mM Tris-HCl buffer (pH 8.0), was incubated with 10 ppm ^{109}Cd solution with known specific activity to saturate the metal-binding sites of MT. Excess Cd was removed by addition of rat hemolysate to the assay tubes followed by heat treatment in a boiling water bath, which caused precipitation of Cd-haemoglobin and other proteins, except MT which is heat stable. The denatured proteins were removed by centrifugation at 10,000 rpm for 2 min. Hemolysate treatment/heat denaturation/centrifugation steps were repeated three times. The Cd concentrations in the final supernatant were calculated from the radioactivity of the ^{109}Cd that were measured by a γ counter (1272 Clinigamma, LKB Wallac; Turku, Finland) and were converted to MT concentration on the basis of 7 g-atoms of Cd/MT [12]. The total testicular MT concentrations were expressed as μg/g wet tissue.

Testicular Zn, Cu and iron (Fe) concentrations were determined by an atomic absorption spectrophotometer (Varian Spectra-AA 30, Georgetown, Ontario) using an air-acetylene flame after tissue digestion by nitric acid as described previously [12]. Zn, Cu and Fe concentrations were expressed as μg/g wet tissue.

Results

A successful experimental renal transplant model was confirmed by more than 140 days of post-operative survival in most recipient rats [100% and 90% survival rate in isogenic (ISO) and allogenic (ALLO) renal transplantation groups, respectively]. At this point all

allografts developed histologically confirmed chronic transplant rejection (Data not shown). High serum creatinine (4.42 ± 2.70 vs 2.03 ± 0.63 in ALLO group, $P < 0.05$) and low urine creatinine (44.09 ± 24.3 vs 100.19 ± 46.44 in ALLO group, $P < 0.05$) were also seen in the allograft recipients in spite of similar urine volume. The testicular weights of testis in the ISO and ALLO group were 1.15 ± 0.11 and 1.26 ± 0.06 g per testis, respectively. They were significantly (Student *t*-test, $P < 0.01$) lower than that of age-matched normal rats (1.58 ± 0.03 g per testis, observation in our laboratory). Allogenic renal transplantation did not change the ratio of testis weight to bodyweight as compared to ALLO groups (Fig. 1, Student *t*-test, $P > 0.05$). Morphologically, most testes, either from the ISO group or ALLO group, showed nearly normal spermatogenesis. The seminiferous tubules showed spermatogonia, spermatocytes, spermatids, and mature sperms with normal microscopic morphology (Fig. 2A, B). No statistically significant difference (Fig. 1) was found in histological assessment of the testes from ISO and ALLO group (Student *t*-test, $P > 0.05$).

LDH-X is an isozyme of the LDH enzyme that is present only in primary spermatocytes and spermatids. The activity of LDH-X in testicular extract is reduced when the spermatids and primary spermatocytes were lost due to testicular damage [2]. No statistical difference (Fig. 1) was found in the testicular LDH-X activity between ISO and ALLO groups (Student *t*-test, $P > 0.05$).

The weight of epididymis in ISO group was 0.51 ± 0.02 g per epididymis, about 91–97% of value for normal epididymis (0.54 ± 0.01 g per epididymis, observation in our laboratory). There was no difference (Student *t*-test, $P > 0.05$) in epididymis weight or morphology between ISO and ALLO group (Fig. 1). A large amount of sperms with normal morphology were found in the sections of the epididymis (Fig. 2E, F), suggesting almost normal spermatogenesis in testis of ISO group.

Testicular MT protein was markedly increased in ALLO group as compared to ISO group (Fig. 3). Immunohistochemically, weak MT staining was present in the seminiferous epithelium in ISO group (Fig. 2C)

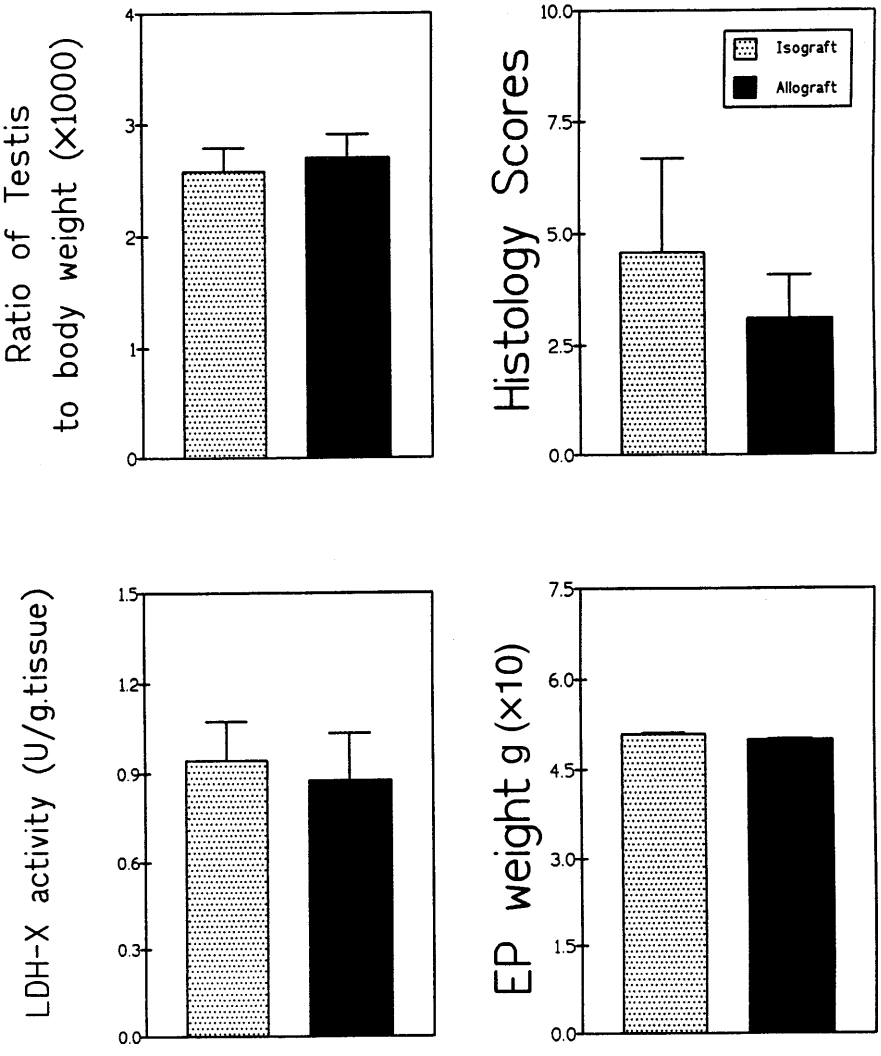

Fig. 1 General assessment of testis morphology and function in rats after renal transplantation. Ratio of testis to body weight (×1000), histological score, LDH-X activity and epididymes (*EP*) weight (×10 g) were compared between isograft and allograft groups

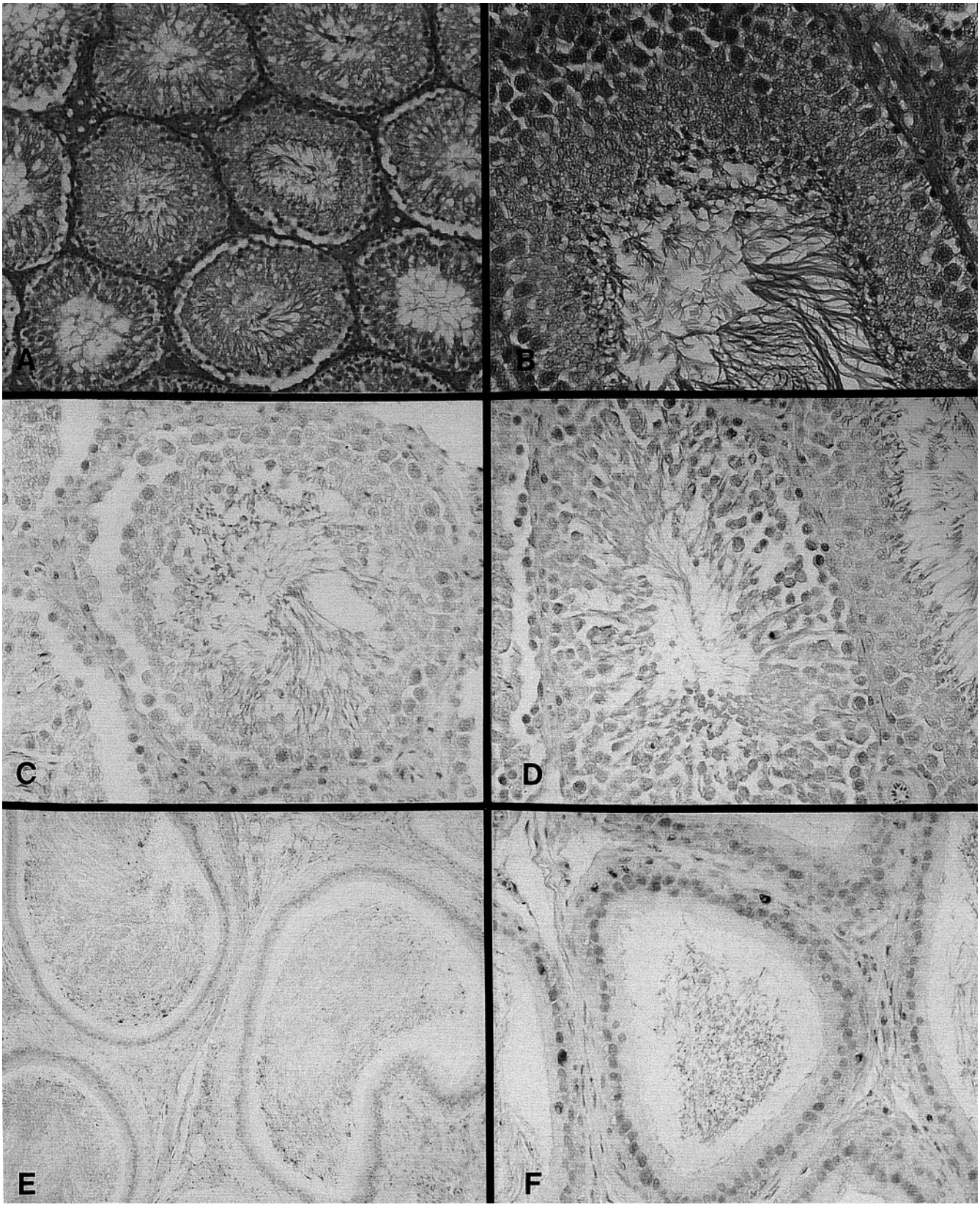

Fig. 2A–F Morphology and MT immunocytochemical staining pattern in rat testis after renal transplantation. **A, B** Seminiferous tubules with normal spermatogenesis from allografted rats (**A** ×100; **B** ×400); **C** weak immunocytochemical staining for MT in seminiferous tubules in ISO group; **D** Intense immunocytochemical staining for MT, in particular for spermatids and sperm, in seminiferous tubules in ALLO group; **E, F** immunocytochemical staining for MT in epididymis in ALLO group. There is intense MT staining in sperms inside of epididymis (**E** ×250) and a few epithelium cells with intense MT staining (**F** ×400)

whereas the strong MT staining was shown in ALLO group (Fig. 2D). With respect to the distribution of MT, late stage germ cells such as sperm and elongated and round spermatids showed generally more intense staining than early stage germ cells (spermatogonia and primary spermatocytes) (Fig. 2D). Stages VII–XIV of seminiferous showed a generally high intensity of MT staining than stages of I–VI of seminiferous (Data not shown). There is no difference for the patterns such as MT staining in the different type of cells and stages of seminiferous between ISO and ALLO groups. In the epididymis, immunoreactive MT was mainly localized in the interstitial tissues, the sperms and some epithelial cells (Fig. 2E, F). No inflammatory cell infiltrate was observed either in ALLO or ISO group.

Zn and Cu levels, but not the Fe level, are significantly higher in the ALLO group than in the ISO group (Table 1). No correlation between MT concentration and metal levels (either Zn or Cu) was found when the individual MT and metal levels were subjected to linear regression analysis. In ALLO group, the ratio of Cu to Zn was significantly increased. Furthermore, when individual MT concentrations were plotted against the Cu/Zn ratio, there was found to be a statistically insignificant correlation with Cu/Zn ratio.

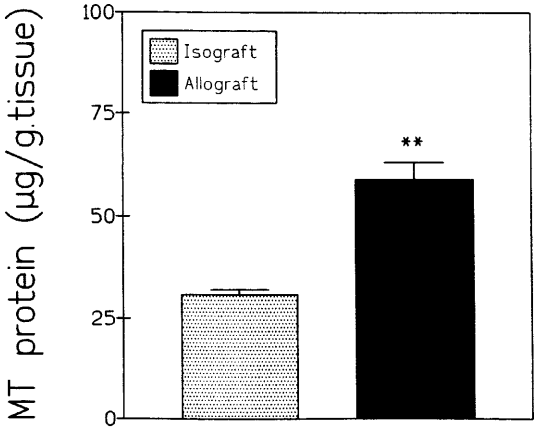

Fig. 3 MT protein level in testis after renal transplantation. Total MT protein in testis was measured by Cd-heme assay from rats with isografts ($n = 3$) and allografts ($n = 7$). $^{**}P < 0.01$ vs isograft (unpaired two-tailed Student t-test)

Discussion

Most of the studies on testicular functions after kidney [13,24] or liver [18] transplantation were assessed by measuring gonadal hormone levels such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and estradiol. Initally, low levels of these hormones and the suppression of testicular function were consistently found for a short period of time. These recovered to almost normal levels over the long term after renal or liver transplantation [13,18]. In the present study, the testis weight, morphology and LDH-X activity were used to assess testicular function. For both the ISO and ALLO groups we found no difference in these testicular functional and structural parameters. We also have found that MT level was increased in ALLO testes as compared to ISO-grafts which may be due to an immunological disturbance in testis of the rats that received allografted kidneys. As the investigations were carried out 140 days after the transplant, the increased MT level does not represent an acute reaction due to surgery. This notion is further supported by the fact that no MT level increase was seen in the ISO testis (Fig. 2C).

It has been well documented that MT synthesis can be induced by metals directly in almost all major organs in rats and mice except in testis [31]. In the present study, we found that increased MT synthesis was independent of metal alteration in the ALLO testes. This suggests that induction of MT synthesis in ALLO testis may not be due to metal accumulation. This result is consistent with previous reports where MT synthesis in testes was not induced after animals were injected with Cd, Zn or LPS [11,31]. However, it has been shown that freshly isolated or purified rat Leydig cells contain high basal levels of MT, and that they responded to exogenous Cd for MT induction [1,32].

Increased MT levels in ALLO testis as compared to ISO testis suggest that the induction of MT in the testis might be due to the allogenic stimuli which may cause inflammation and a stress response that in turn produces high levels of reactive oxygen species [9,14,19,25]. Oxidative stress triggers the release of a variety of mediators such as glucocorticoids and cytokines. In addition to metals, glucocorticoids can also induce MT synthesis [7,25]. Moreover, cytokines released as a result of allo-

Table 1 Metal levels in testis and its correlation with MT concentration after renal transplantation in rats. [a]Correlation of individual MT concentration with corresponding individual Zn, Cu or Fe statistically analyzed (two-tailed test). $^{*}P < 0.05$, $^{**}P < 0.01$ vs corresponding data in isograft group (unpaired two-tailed t-test)

Metal	Groups	(No. of rats)	Metal level (μg/g tissue)		Ratio of Cu or Fe to Zn	
			Mean ± SE	Correlation with MT[a]	Mean ± SE	Correlation with MT
Zn	ISO	(3)	25.00 ± 1.74	No ($P = 0.11$)		
	ALLO	(6)	30.14 ± 0.55*	No ($P = 0.73$)		
Cu	ISO	(3)	2.33 ± 0.13	No ($P = 0.57$)	0.095 ± 0.011	No ($P = 0.36$)
	ALLO	(6)	3.51 ± 0.23*	No ($P = 0.20$)	0.118 ± 0.007**	Marginal ($P = 0.087$)
Fe	ISO	(3)	41.80 ± 7.90	No ($P = 0.66$)	1.77 ± 0.40	No ($P = 0.55$)
	ALLO	(6)	46.00 ± 2.50	No ($P = 0.22$)	1.55 ± 0.12	No ($P = 0.12$)

genic renal transplantation may be another factor for the induction of MT synthesis in the testis. Cytokines such as IL-1, IL-6, TNF and interferon can induce MT synthesis in vivo, and cells in vitro [14,19,25]. Pretreatment with dexamethasone, a strong inhibitor of cytokine production, prevented the increase in the MT level induced by inflammatory agents [20]. As no evidence of local inflammation was detected, the enhanced testicular MT synthesis in the present study is probably due to the systemic immunological response either by the formation of reactive of oxygen species or by a cytokine mediated mechanism. Some authors have demonstrated that sex hormones can stimulate MT synthesis in prostate and coagulating gland of rats [29]. Although we did not measure hormone levels in the present study, it is unlikely that the short period of a reduced hormonal level seen in early post-transplantation period [13,18] will have a significant effect on the MT level in the testis at long-term follow-up. It is of further interest to note that no significant correlation of testicular MT with LDH-X activity, a hormone dependent testicular functional marker [30], was seen in this study.

Both Zn and Cu are structural components of Cu/Zn superoxide dismutase (Cu/Zn-SOD), and therefore, the increased testicular Cu and Zn in this study may indicate an increased Cu/Zn-SOD activity. Testicular SOD is inducible by sublethal injury and has been shown to offer protection in testicular injury [2,17,33]. However, it has been documented that a 50-fold increase in MT level is associated with only a three-fold increase in Cu/Zn-SOD in the cells treated with Cu [26]. A marked increase in MT with no change in Cu/Zn-SOD activity in the cells treated with Zn was also documented [23]. Therefore, the possible increased SOD activity in the present study may be not major contribution for protection from testicular damage as compared to increased testicular MT. However, the major underling protective mechanisms are unclear and may require further investigation.

In summary, we have demonstrated that testicular structure and function, morphology, and LDH-X enzyme activity are not affected by allogenic stimuli resulting from allografted kidney transplantation. The results show that MT synthesis is induced in the recipient testis with renal allografts but not isografts. The increased MT level may offer protection against testicular damage in allogenic renal transplant recipients.

Acknowledgments This study was supported in part by a research grant (M.G.C) from Medical Research Council of Canada and a research grant (S.C) from Multi-Organ Transplantation Program at the London Health Science Center, Canada. The authors thank Mr. K. Mukherjee for his excellent technical assistance.

References

1. Abel J, de Ruiter N, Kuhn-Velten WN (1991) Comparative study on metallothionein induction in whole testicular tissue and isolated Leydig cells. Arch Toxicol 65: 228–234
2. Agarwal A, Ikemoto I, Loughlin KR (1997) Prevention of testicular damage by free-radical scavengers. Urology 50: 759–763
3. Arend SM, Mallat MJK, Weslendorp RJW, Van der Woude FJ, Adams DH (1997) Patients survival after renal transplantation more than 25 years follow-up. Nephrol Dial Transpl 12: 1672–1679
4. Cai L, Koropatnick J, Cherian MG (1995) Metallothionein protects DNA from copper-induced, but not iron-induced cleavage in vitro. Chem-Biol Interact 96: 143–155
5. Cai L, Tsiapalis G, Cherian MG (1998) Protective role of zinc-metallothionein on DNA damage in vitro by ferric nitrilotriacetate (Fe-NTA) and ferric salts. Chem Biol Interact 115: 141–151
6. Cai L, Satoh M, Tohyama C, Cherian MG (1999) Metallothionein in radiation exposure: its induction and protective role. Toxicology 132: 85–98
7. Cherian MG (1995) Metallothionein and its interaction with metals. In: Goyer RA, Cherian MG (eds) Toxicology of metals – biochemical aspects. Springer, Berlin Heidelberg New York, p 121
8. Chin J, Zhong R, Duff J, Stiller C (1989) Microsurgical renal transplant models in rats: a comparison of four anastomotic techniques. Transplant Proc 21: 3351–3352
9. Courtade M, Carrera G, Paternain JL, Martel S, Carre PC, Folch J (1998) Metallothionein expression in human lung and its varying levels after lung transplantation. Chest 113: 371–378
10. Danielson KG, Ohi S, Huang PC (1982) Immunochemical detection of metallothionein in specific epithelial cells of rat organs. Proc Natl Acad Sci USA 79: 2301–2304
11. De SK, Enders GC, Andrews GK (1991) High levels of metallothionein messenger RNAs in male germ cells of the adult mouse. Mol Endocrinol 5: 628–636
12. Deng DX, Ono S, Koropatnick J, Cherian MG (1998) Metallothionein and apoptosis in the Toxic Milk mutant mouse. Lab Invest 78: 175–183
13. Handelsman DJ, Ralec Vl, Tiller DJ, Horvath JS, Turtle JR (1981) Testicular function after renal transplantation. Clinical Endocrinol 14: 527–538
14. Hidalgo J, Campmany L, Borras M, Garvey JS, Armario A (1988) Metallothionein response to stress in rats: role in free radical scavenging. Am J Physiol 255 (Endocrinol Metab 18): E518–E524
15. Katznelson S, Cecka JM (1997) The great success of Asian kidney transplant recipients. Transplantation 64: 1850–1852
16. Kloth DM, Chin JL, Cherian MG (1995) Induction of hepatic metallothionein I in tumor-bearing mice. Br J Cancer 71: 712–716
17. Kobayashi T, Miyazaki T, Natori M, Nozawa S (1991) Protective role of superoxide dismutase in human sperm motility: superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Hum Reprod 6: 987–991
18. Madersbacher S, Grunberger T, Maier U (1994) Andrological status before and after liver transplantation. J Urol 151: 1251–1254
19. Maret W (1995) Metallothionein and the acute phase response. J Lab Clin Med 126: 106–107
20. Min KS, Mukai S, Ohta M, Onosaka S, Tanaka K (1992) Glucocorticoid inhibition of inflammation-induced metallothionein sysntehsis in mouse liver. Toxicol Appl Pharmacol 113: 293–298
21. Miura T, Muraoka S, Ogiso T (1997) Antioxidant activity of metallothionein compared with reduced glutathione. Life Sci 60: 301–309
22. Nishimura H, Nishimura N, Tohyma C (1990) Localization of metallothionein in the genital organs of the male rat, J Histochem Cytochem 38: 927–933
23. Mulder TP, van der Sluys Veer A, Verspaget HW, Griffioen G, Pena AS, Janssens AR, Lamers CB (1994) Effect of oral zinc supplementation on metallothionein and superoxide dismutase concentrations in patients with inflammatory bowel disease. J Gastroenterol Hepatol 9: 472–477

24. Peces R, de la Torre M, Urra JM (1994) Pituitary-testicular function in cyclosporine-treated renal transplant patients, Nephrol Dial Transp l 9: 1453–1455
25. Sato M, Sasaki M, Hojo H (1993) Induction of metallothionein synthesis by oxidative stress and possible role in acute phase response. In: Suzuki KT, Imura N, Kimura M (eds) Metallothionein III: biological roles and medical Implications. Birkhauser, Basel, p 125
26. Steinkuhler C, Carri MT, Micheli G, Knoepfel L, Weser U, Rotilio G (1994) Copper-dependent metabolism of Cu, Zn-superoxide dismutase in human K562 cells. Lack of specific transcriptional activation and accumulation of a partially inactivated enzyme. Biochem J 302 (Pt 3): 687–694
27. Takeda A, Tamano H, Sato T, Goto K, Okada S (1995) Characteristic induction of hepatic metallothionein in mice by tumour transplantation. Biochem Biophys Acta 1243: 325–328
28. Thornalley PJ, Vasak M (1985) possible role for metallothionein in protection against radiation induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. Biochem Biophys Acta 827: 36–44
29. Tohyama C, Suzuki JS, Homma S, Karasawa M, Kuroki T, Nishimura H, Nishimura N (1996) Testosterone-dependent induction of metallothionein in genital organs of male rats. Biochem J 317: 97–102
30. Vaishnav MY, Moudgal NR (1991) Effect of specific FSH or LH deprivation on testicular function of the adult rat. Indian J Biochem Biophys 28: 513–520
31. Wahba ZZ, Miller MS, Waalkes MP (1994) Absence of changes in metallothionein RNA in the rat testes made refractory to cadmium toxicity by zinc pretreatment. Human Exp Toxicol 13: 65–67
32. Wang SH, Chen JH, Lin LY (1994) Functional integrity of metallothionein genes in testicular cell lines. J Cell Biochem 55: 486–495
33. Zhang H, Zheng RL, Wei ZQ, Li WJ, Gao QX, Chen WQ, Wang ZH, He J, Liang JP, Han GW, Huang T, Li Q, Xie HM, Zhang SM, Cai XC (1998) Effects of pre-exposure of mouse testis with low-dose $^{(16)}O^{8+}$ ions or ^{60}Co gamma-rays on sperm shape abnormalities, lipid peroxidation and superoxide dismuatse (SOD) activity induced by subsequent high-dose irradiation. Int J Radiat Biol 73: 163–167