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Distribution of Lectin Bindings in the Testes of the Greater Japanese Shrew Mole, *Urotrichus talpoides*

Takuo MIZUKAMI¹⁾, Sachi KUWAHARA²⁾, Masako OHMURA²⁾,
Yasuko IINUMA²⁾, June IZUMIKUBO²⁾, Mio HAGIWARA²⁾,
Masamichi KUROHMARU¹⁾, Yoshihiro HAYASHI¹⁾ and Takao NISHIDA²⁾

¹⁾Department of Veterinary Anatomy, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, and ²⁾Laboratory of Anatomy and Physiology, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 252-8510, Japan

Abstract. The binding patterns of 20 lectins in the testes of the greater Japanese shrew mole (*Urotrichus talpoides*) were investigated by light microscopy. Eleven lectins (WGA, sWGA, LEL, STL, Jacalin, PSA, LCA, BPA, Con-A, WGA and PHA-E) exhibited a positive reaction in the cytoplasm and acrosomal region of spermatids and in the cytoplasm of Sertoli cells. LEL, BSL-I, DSA, sWGA, BSL-I and PHA-L were intensely positive in the cytoplasm of Leydig cells. The binding patterns in the acrosomal region of spermatids were divided into three types. For LEL, ECL and STL, the reaction was detected in the acrosomal regions from Golgi to cap phase spermatids and disappeared in acrosome and maturation phase spermatids. For SBA, VVL, ECL and DSL, the reaction was detected in the acrosomal region from Golgi to acrosome phase spermatids. No reaction was recognized in the acrosome of maturation phase spermatids. In DBA and Jacalin, the cytoplasm of maturation phase spermatids was positive although no reaction was detected in the acrosomal region of all spermatids. sWGA, BPA and LEL exhibited a strong reaction in the plasma membrane of zygotene spermatocytes at stages X and XI. BSL-II revealed a strong reaction in the cytoplasm of spermatogonia.

Key words: Lectin, Histochemistry Testis, Spermatogenesis, Greater Japanese shrew mole

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It is well known that glycoconjugates are widely distributed in mammalian tissues, including those of the testis, liver [1], brain [2, 3] and the immune system [4], and play important roles in cell differentiation, maturation, cell-cell interactions, signal transduction [5], embryonic development [6] and transformation of cells [7-9]. Lectins have been used as histochemical reagents to investigate the localization of glycoconjugates in various tissues, due to their specific binding affinity for sugar residues of glycoconjugates. Some lectins play an

essential role during the ontogenetic processes of cell adhesion and cell recognition, and are also involved in signaling events (nuclear lectins) [5], such as autocrine or paracrine mechanism and innate defence [4]. In testes, the distribution and function of lectins have been studied in the human [10-12], some rodents [13-20], domesticated animals [21-24], wild mammals [25-27], and reptiles [28, 29]. However, in insectivores, only one report [30] is available at present. Although the lectin bindings in the testis of the musk shrew [30] were somewhat different from those of other mammals, it is not clear whether the peculiar lectin-

binding patterns in the musk shrew (*Suncus murinus*) testis are common among all insectivores or are unique to that animal. In order to solve this problem, the present study was conducted to study the lectin bindings in the testis of the greater Japanese shrew mole (*Urotrichus talpoides*) (insectivore), and to compare them with those of the musk shrew [30] and other mammalian species [10–29].

Materials and Methods

Animals

Greater Japanese shrew moles, weighing 14 to 20 g, were collected at the university forest in Chichibu, Saitama Prefecture. Most of them were captured with a Sherman's trap baited with flour paste or soybean.

Light microscopy

Five adult male greater Japanese shrew moles were anesthetized with pentobarbital (5 mg/100 g body weight, i.p.). Then, the testes were surgically excised and immersed in Bouin's solution overnight. The samples were cut into slabs, dehydrated in a graded series of ethanol, and embedded in paraffin wax. They were sectioned at 4 μ m, stained with hematoxylin and eosin and/or

periodic acid Schiff (PAS)-hematoxylin and observed using light microscopy.

Lectin histochemistry

The lectins (Vector laboratories, Burlingame, CA, USA) used in this study are shown in Table 1. The sections (4 μ m) obtained from the paraffin block described above were deparaffinized, rehydrated, and treated with 0.01 M phosphate-buffered saline (PBS; pH 7.4) and 1% bovine serum albumin (BSA)-PBS. The sections were incubated with biotinylated lectins (Vector, 25 μ g/ml) in 1% BSA-PBS for 60 min. After washing with PBS, they were treated with 1% BSA-PBS and incubated with avidin-biotin peroxidase complex (ABC; Vector) for 30 min. They were then washed again with PBS, immersed in diaminobenzidine (DAB, 0.2 mg/ml)-H₂O₂ (0.005 %) for 5 min, rinsed in distilled water, and stained with hematoxylin before observation using light microscopy. Negative controls without lectins were processed in parallel.

Results

Reaction in Sertoli cells and Leydig cells

Nine lectins (WGA, LEL, STL, Jacalin, LCA, PSA, ConA, PHA-E and BPA) exhibited a positive reaction in the cytoplasm of Sertoli cells (Fig. 1a).

Table 1. Lectins used in this experiment

Common name	Scientific name	Binding specificity
LCA	<i>Lens culinaris</i>	α -man
PSA	<i>Pisum sativum</i>	α -man
ConA	<i>Canavalia ensiformis</i>	α -man, α -glc
BSL-II	<i>Bandeiraea simplicifolia</i>	glcNAc
DSL	<i>Datura stramonium</i>	(glcNAc) ₂
WGA	<i>Triticum vulgaris</i>	(glcNAc) ₂ , NeuNAc, Sia
sWGA	<i>Triticum vulgaris</i>	(glcNAc) ₂ , NeuNAc
LEL	<i>Lycopersicon esculentum</i>	(glcNAc) ₃
STL	<i>Solanum tuberosum</i>	(glcNAc) ₃
SBA	<i>Glycine max</i>	galNAc
VVL	<i>Vicia villosa</i>	galNAc
DBA	<i>Dolichos biflorus</i>	α -galNAc
Jacalin	<i>Artocarpus integrifolia</i>	α -gal $\rightarrow$ OMe
BSL-I	<i>Bandeiraea simplicifolia</i>	α -gal, α -galNAc
SJA	<i>Sophora japonica</i>	β -galNAc
PNA	<i>Arachis hypogaea</i>	β -gal(1 $\rightarrow$ 3)galNAc
ECL	<i>Erythrina cristagalli</i>	β -gal(1 $\rightarrow$ 4)galNAc
BPA	<i>Bauhinia purpurea</i>	β -gal(1 $\rightarrow$ 4)galNAc
UEA-I	<i>Ulex europaeus</i>	α -L-fuc
PHA-E	<i>Phaseolus vulgaris</i>	oligosaccharide
PHA-L	<i>Phaseolus vulgaris</i>	oligosaccharide

Fig. 1. Lectin-bindings in the testis of the greater Japanese shrew mole. a) PHA-E exhibits a positive reaction in the cytoplasm of Sertoli cells. b) ECL exhibits a strong reaction (arrow) in the plasma membrane of zygote spermatocytes at stages X and XI. c,d) BSL-I is intensely positive in the cytoplasm of Leydig cells. a) $\times 200$, b) $\times 320$, c) $\times 200$, d) $\times 600$.

Table 2. Localization and intensity of lectin bindings in spermatids

		Spermatid				Lectin
		Golgi phase	cap phase	acrosome phase	maturation phase	
Type-I	Cytoplasm	-	-	-	++	LEL, ECL, STL
	Acrosome	+++	+++	-	-	
Type-II	Cytoplasm	-	-	++	+++	SBA, VVL, DSL
	Acrosome	+++	+++	-	-	
Type-III	Cytoplasm	-	-	+++	+++	DBA, Jacalin
	Acrosome	-	-	-	-	

LEL, BSL-I, PSA, sWGA and PHA-L were intensely positive in the cytoplasm of Leydig cells (Fig. 1c-f).

Reaction in spermatogenic cells

The spermatids of the greater Japanese shrew mole were easily subdivided into 4 phases (Golgi, cap, acrosome and maturation phases). Golgi, cap, acrosome and maturation-phase spermatids were equivalent to steps 1-3, steps 4-8, steps 9-12 and steps 13-15, respectively [31]. The binding patterns of lectins in the acrosomal region of spermatids

were divided into three types (Table 2). For LEL, ECL and STL (Fig. 2a, b), the reaction was detected in the acrosomal regions from Golgi to cap phase spermatids and disappeared in the acrosome and maturation phases. At the moment of spermiation, the residual body, probably derived from step 15 spermatids, showed a strong reaction. For SBA, VVL, ECL and DSL (Fig. 2c, d), the reaction was detected in the acrosomal regions from Golgi to acrosome phase spermatids. No reaction was detected in the acrosome of maturation phase

Fig. 2. Lectin-bindings in the acrosomal region of spermatids of the greater Japanese shrew mole. a and b) For STL and LEL, the reaction is detected in the acrosomal regions from Golgi to cap phase spermatids and disappears in the acrosome phase. c and d) For VVL, the reaction is detected in the acrosomal region from Golgi to acrosomal phase spermatids. e and f) For DBA, although no reaction is detected in the acrosomal region of all spermatids, the residual body derived from step 15 spermatids is positive. a) $\times 320$, b) $\times 600$, c) $\times 200$, d) $\times 320$, e) $\times 320$, f) $\times 600$.

spermatids. For DBA and Jacalin (Fig. 2e, f), although no reaction was detected in the acrosomal region of all spermatids, the cytoplasm of maturation phase spermatids was positive. swGA, BPA, LEL and ECL exhibited a strong reaction in the plasma membrane of zygotene spermatocytes at stages X and XI (Fig. 1b). BSL-II revealed a strong reaction in the cytoplasm of spermatogonia at stages VIII and IX (Fig. 3).

Discussion

The lectin binding patterns in the testis of the greater Japanese shrew mole were compared with those in other mammalian species, especially with those in the musk shrew. Similar to the case of the musk shrew [30], BPA was detected in the cytoplasm of Sertoli cells in the greater Japanese shrew mole. In other mammalian species

Fig. 3. BSL-II is detected in the cytoplasm of spermatogonia at stages VIII and IX. $\times 600$

previously studied, BPA did not reveal a positive reaction in Sertoli cells. BPA is thought to recognize the specific β -gal (1 $\rightarrow$ 3) galNAC sugar. Thus, the β -gal (1 $\rightarrow$ 3) galNAC sugar is present in the cytoplasm of Sertoli cells in insectivores. ConA and WGA showed an intense reaction in the acrosomal region of early round spermatids in the musk shrew [30], whereas these lectins revealed no reaction in that of the greater Japanese shrew mole. RCA-I, PNA, BPA and SBA bound to the acrosomal regions from Golgi to acrosomal phase spermatids and abruptly decreased in intensity in maturation phase spermatids of the musk shrew [30]. Nonetheless, in the greater Japanese shrew mole, reaction in the acrosomal region was detected with

LEL, ECL, STL, SBA, VVL, DSL, DBA, and Jacalin. Thus, in the acrosomal region of spermatids, SBA showed the same reaction. Previous reports have shown that SBA (indicating the distribution of galNac sugar) shows a positive reaction in the acrosome of spermatids in many mammalian species, such as human [10–12], rat [13–15], mouse [16], nutria [16], guinea pig [18], gerbil [18], tree shrew [25, 26], goat [21], boar [24], horse [23] and bull [22]. In the greater Japanese shrew mole, VVL (indicating the presence of galNAC sugar) was also observed in the acrosomal region of spermatids. BSL-II (indicating the distribution of glcNAC sugar) was detected in the acrosomal region of acrosome phase spermatids in the musk shrew [25]. Thus, glcNAC is present in the acrosomal region of spermatids in many mammalian species.

From the result of this and previous reports, it is possible to postulate that BPA (indicating the presence of β -gal(1 $\rightarrow$ 3)galNAC sugar) is specifically conserved in the cytoplasm of Sertoli cells in insectivores and that SBA (indicating the presence of galNac sugar) is conserved in the acrosomal region of spermatids across many mammalian species. Some peculiar lectin binding patterns were observed in the greater Japanese shrew mole as well as in the musk shrew, whereas slight differences were also recognized between the two species. In order to clarify the common lectin-bindings in insectivore testes, further studies using other kinds of insectivores, such as Talpidae, Soricidae and Crocidura should be undertaken.

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References

1. Ashwell G, Harford J. Carbohydrate-specific receptors of the liver. *Annu Rev Biochem* 1982; 51: 531–554.
2. Zenetta JP. Structure and functions of lectins in the central and peripheral nervous system. *Acta Anat* 1998; 161: 180–195.
3. Plendl J, Sinowatz F. Glycobiology of the olfactory system. *Acta Anat* 1998; 161: 234–253.
4. Kawasaki T. Structure and biology of mannan-binding protein, MBP, an important component of

- innate immunity. *Biochim Biophys Acta* 1996; 1473: 186–195.
5. Villalobo A, Gabius H-J. Signaling pathways for transduction of the initial message of glycode into cellular responses. *Acta Anat* 1998; 161: 110–129.
  6. Mann PL, Waterman RE. Glycocoding as an information management system in embryonic development. *Acta Anat* 1998; 161: 153–161.
  7. Muramatsu T. Alternation of cell-surface carbohydrates during differentiation and development. *Biochimie* 1988; 70: 1587–1596.
  8. Denis JW, Laferte S, Waghorne C, Breitman ML, Kerbel RS. Beta 1-6 branching of Asn-linked oligosaccharides is directly associated with metastasis. *Science* 1987; 236: 582–585.
  9. Rademacher TW, Parekh RB, Dwek RA. Glycobiology. *Annu Rev Biochem* 1988; 57: 785–838.
  10. Malmi R, Kallajoki M, Suominen J. Distribution of glycoconjugates in human testis. A histochemical study using fluorescein- and rhodamine-conjugated lectins. *Andrologia* 1987; 19: 322–332.
  11. Arenas MI, Madrid JF, Bethencourt FR, Fraile B, Paniagua R. Lectin histochemistry of the human testis. *Int J Androl* 1998; 21: 332–342.
  12. Wollina U, Schreiber G, Zollmann C, Hipler C, Günther E. Lectin-binding sites in normal human testis. *Andrologia* 1989; 21: 127–130.
  13. Arya M, Vanha-Perttula T. Lectin staining of rat testis and epididymis after ligation of excurrent ducts at different levels. *Biol Reprod* 1985; 33: 477–485.
  14. Vanha-Perttula T, Arya M. Lectin staining of rat testis and epididymis: effect of cyproterone acetate and testosterone. *Andrologia* 1985; 17: 301–310.
  15. Arya M, Vanha-Perttula T. Distribution of lectin binding in rat testis and epididymis. *Andrologia* 1984; 16: 495–508.
  16. Lee MC, Damjanov I. Anatomic distribution of lectin-binding sites in mouse testis and epididymis. *Differentiation* 1984; 27: 74–81.
  17. Söderström KO, Malmi R, Karjalainen K. Binding of fluorescein isothiocyanate conjugated lectins to rat spermatogenic cells in tissue sections. Enhancement of lectin fluorescence obtained by fixation in Bouin's fluid. *Histochemistry* 1984; 80: 575–579.
  18. Arya M, Vanha-Perttula T. Comparison of lectin-staining pattern in testis and epididymis of gerbil, guinea pig, mouse, and nutria. *Am J Anat* 1986; 175: 449–469.
  19. Malmi R, Söderström KO. Lectin binding to rat spermatogenic cells: effects of different fixation methods and proteolytic enzyme treatment. *Histochem J* 1988; 20: 276–282.
  20. Malmi R, Fröjdman K, Söderström KO. Differentiation-related changes in the distribution of glycoconjugates in rat testis. *Histochemistry* 1990; 94: 387–395.
  21. Kurohmaru M, Kanai Y, Hayashi Y. Lectin-binding patterns in the spermatogenic cells of the shiba goat testis. *J Vet Med Sci* 1991; 53: 893–897.
  22. Arya M, Vanha-Perttula T. Lectin binding pattern of bull testis. *J Androl* 1985; 6: 230–242.
  23. Verini-Supplizi A, Stradaoli G, Fagioli O, Parillo F. Localization of the lectin reactive sites in adult and prepubertal horse testes. *Res Vet Sci* 2000; 69: 113–118.
  24. Calvo A, Pastor LM, Bonet S, Pinart E, Ventura M. Characterization of the glycoconjugates of boar testis and epididymis. *J Reprod Fertil* 2000; 120: 325–335.
  25. Kurohmaru M, Maeda S, Suda A, Hondo E, Ogawa K, Endo H, Kimura J, Yamada J, Rerkamnuaychoke W, Chungsamarnyart N, Hayashi Y, Nishida T. An ultrastructural and lectin-histochemical study on the seminiferous epithelium of the common tree shrew (*Tupaia glis*). *J Anat* 1996; 189: 87–95.
  26. Kurohmaru M, Mizukami T, Kanai Y, Hondo E, Endo H, Kimura J, Rerkamnuaychoke W, Agungpriyono S, Nishida T, Yamada J, Hayashi Y. A lectin-histochemical study on the seminiferous epithelium of the northern smooth-tailed tree shrew (*Dendrogale murina*) and the Java tree shrew (*Tupaia javanica*). *Okajimas Folia Anat Jpn* 2000; 77: 63–68.
  27. Morigaki T, Kurohmaru M, Kanai Y, Mukohyama M, Hondo E, Yamada J, Agungpriyono S, Hayashi Y. Lectin-binding patterns in the testes of Java fruit bat (*Pteropus vampyrus*) and the Japanese lesser horseshoe bat (*Rhinolophus cornutus*). *J Reprod Dev* 2000; 46: 309–314.
  28. Labate M, Desantis S. Histochemical analysis of lizard testicular glycoconjugates during the annual spermatogenic cycle. *Eur J Histochem* 1995; 39: 201–212.
  29. Kurohmaru M, Hattori S, Hayashi Y. Lectin-binding patterns in the testes of the habu, *Trimeresurus flavoviridis*. *The Snake* 1998; 28: 1–4.
  30. Kurohmaru M, Kobayashi H, Kanai Y, Hattori S, Nishida T, Hayashi Y. Distribution of lectin binding in the testes of the musk shrew, *Suncus murinus*. *J Anat* 1995; 187: 323–329.
  31. Mizukami T, Kuwahara S, Ohmura M, Iinuma Y, Izumikubo J, Hagiwara M, Kurohmaru M, Hayashi Y, Nishida T. The cycle of the seminiferous epithelium in the greater Japanese shrew mole, *Urotrichus talpoides*. *J Vet Med Sci* 2001; 63: 31–35.
  32. Ishii N. Size and distribution of home ranges of the Japanese shrew-mole *Urotrichus talpoides*. *J Mamm Soc Jpn* 1993; 18: 87–98.
  33. Ishii N. Reproductive activity of the Japanese shrew mole, *Urotrichus talpoides* TEMMINCK. *J Mamm Soc Jpn* 1982; 9: 25–36.