Cell-selective Determination of Sn in Spermatozoa of Rats Exposed to Tributyltin Chloride by Synchrotron Radiation X-ray Fluorescence Analysis (SR-XRF) using Microprobe

Marine pollution due to organotin compounds added to ship bottom paints or fishnet antifoulants and the contaminated food have been a recent concerns as the pollutants are potential endocrine disruptors. Organotin compounds exhibit reproductive toxicity in experimental animals but the dynamics of Sn in reproductive organs is poorly understood.

Seminiferous tubules of rat testis contains a few spermatogonia, which are primitive germ cells, along the basement membrane, one or several layers of spermatocytes farther inside, and groups of spermatids next to the lumen of the tubule (Fig. 1). These types of germ cell undergo a series of developmental processes, which are further classified into 14 stages, and become spermatozoa. Germ cells at different stages of seminiferous tubules respond differently to exogenous stimuli. A novel technique, therefore, is expected to reveal cell-specific profiles of trace elements in the testis.

Sensitive analysis by analytical methods in common use is difficult for trace Sn. Synchrotron radiation X-ray fluorescence analysis (SR-XRF) using a microprobe is a simple and useful method of investigating the precise distribution of elements in tissues. The use of high-energy incident X-rays is suitable for detecting small amounts of Sn in biological samples because it excites the XRF of the Sn K-line, which is not interfered by major elements, such as calcium and potassium, unlike the Sn L-line. In the present study, we applied the analytical method for the cell-selective determination of Sn in the testis of Wistar male rats exposed to tributyltin chloride (TBTC) [1].

SR-XRF measurements were performed at beamline **BL37XU**, using an energy dispersive SR-XRF system with monochromatic X-rays [2]. Spermatozoa in a seminiferous tubule stage VIII, which is the final stage of spermatogenesis in the

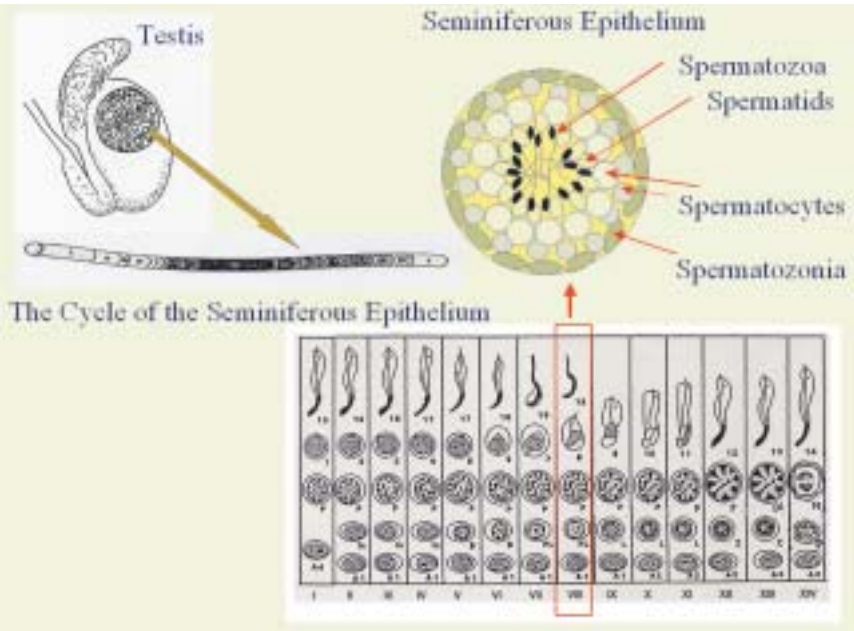

Fig. 1. Structure of rat testis. The illustration on the lower part is a arrangement of germ cells in the seminiferous epithelium. Rat seminiferous tubules are classified into 14 stages in the order of development. The specific associations in the vertical columns represent specific stages (Roman numerals) of the epithelium cycle. The illustration on the upper part is a cross section of a stage VIII tubule.

testis, were subjected to cell-selective Sn determination (Fig. 2). X-rays (37.5 keV; beam size, 3 × 3 μm^2) were used to irradiate spermatozoa of the testicular specimen. The analytical position was confirmed by comparison with the Zn imaging of the stage VIII seminiferous tubules because Zn concentration is higher in spermatozoa than in other types of germ cells at this stage as reported previously [3].

Four days after the first injection of TBTC (45 μmol/kg per day for 3 days, *p.o.*), Sn was detected in spermatozoa in the innermost area of the tubule. It indicates that an investigation of toxic effects after TBTC exposure on not only spermatogenesis but also spermiogenesis should be performed. High-energy SR-XRF analysis using a microprobe can be a powerful technique for investigating the elemental dynamics of Cd, Mo, U, and Cs as well as Sn in tissue with complex structures.

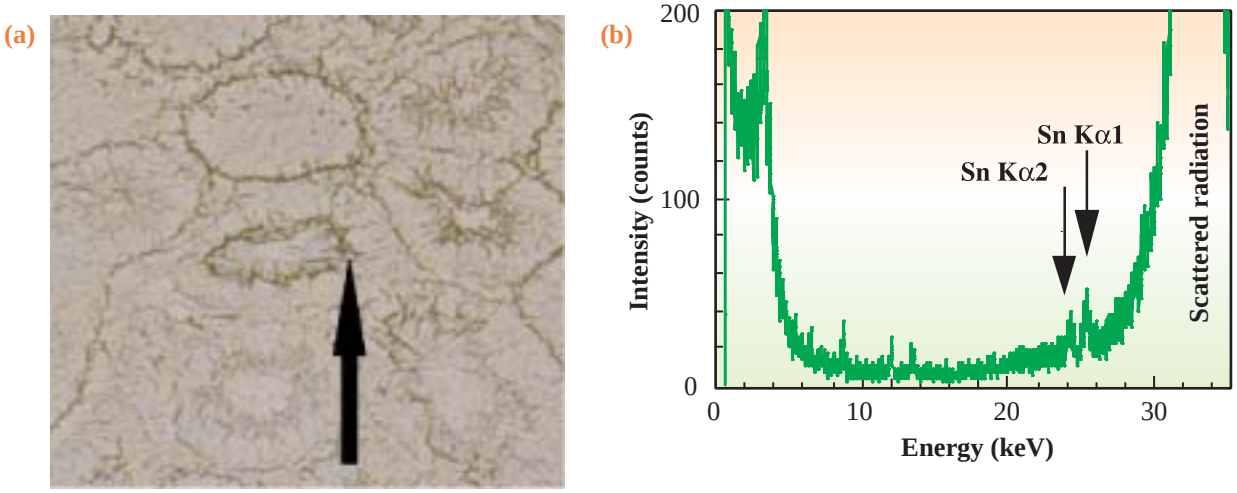

Fig. 2. Light microscopy of the testicular specimen **(a)** and X-ray fluorescence spectrum of the spermatozoa in the stage VIII seminiferous tubule **(b)**. A cryosection (30 μm) was obtained from the testis 1 day after TBTC administration (45 μmol/kg per day for 3 days, *p.o.*). The stage VIII seminiferous tubule has spermatozoa in its innermost. The arrow indicates the position of SR-XRF spot analysis. The Sn concentration of the testis was 0.5 μg/g wet weight.

S. Homma-Takeda

Environmental Radiation Protection Research Group, National Institute of Radiological Sciences

E-mail: shino_ht@nirs.go.jp

References

[1] *S. Homma-Takeda, Y. Nishimura, Y. Terada, S. Ueno, Y. Watanabe and M. Yukawa: Nucl. Instrum. Meth. Phys. Res. B* ***231*** *(2005) 333.*
[2] Y. Terada *et al.*: AIP Conference Proc. **705** (2004) 376.
[3] S. Homma-Takeda *et al.*: J. Radioanal. Nucl. Chem. **259** (2004) 521.