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Nuclear Instruments and Methods in Physics Research B 231 (2005) 333–337

NIM B
Beam Interactions with Materials & Atoms

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Tin accumulation in spermatozoa of the rats exposed to tributyltin chloride by synchrotron radiation X-ray fluorescence (SR-XRF) analysis with microprobe

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Available online 23 March 2005

Abstract

Organotin compounds are widely used in industry and its environmental contamination by these compounds has recently become a concern. It is known that they act as endocrine disruptors but details of the dynamics of Sn in reproductive organs are still unknown. In the present study, we attempted to determine Sn distribution in the testis of rats exposed to tributyltin chloride (TBTC) by inductively coupled argon plasma-mass spectrometry (ICP-MS) for microdissectioned seminiferous tubules and cell-selective metal determination of synchrotron radiation X-ray florescence (SR-XRF) analysis.

TBTC was orally administered to rats at a dose of 45 μmol/kg per day for 3 days. One day later, Sn was detected in the microdissectioned seminiferous tubules at a level approximately equivalent to that in the testis. Significant stage-specificity of Sn accumulation was not observed in the experimental model. Sn was also detected in spermatozoa at the stage VIII seminiferous tubule, which are the final step of spermatogenesis in the testis. These data indicate that Sn accumulates in germ cells as well as in spermatozoa in a short period of TBTC exposure.

PACS: N87.83.a; 87.59.e

Keywords: X-ray fluorescence analysis; Organotin; Testis; Sperm

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0168-583X/$ - see front matter
doi:10.1016/j.nimb.2005.01.079

1. Introduction

Organotin compounds including tributyltin (TBT) are used in various technical applications such as wood preservatives, disinfectants, and antifouling paints. Environmental contamination has recently become a concern as they are potential as endocrine disruptors [1]. They exhibit reproductive toxicity in experimental animals [2–4] but the dynamics of Sn in reproductive organs is poorly understood.

Within the seminiferous epithelium of the testis, male germ cells progress through mitosis, meiosis, and cellular differentiation to become spermatozoa. This process is known as spermatogenesis. Seminiferous tubule of rat contains a few spermatogonia, the primitive germ cells, along the basement membrane, one or several layers of spermatocytes farther in, and groups of spermatids next to the lumen of the tubule (Fig. 1). These types of germ cells undergo a series of developments, which are further classified into 14 stages [5]. Germ cells at different stages of the seminiferous tubules respond differently to exogenous stimuli [6–8]. In the previous study, we demonstrated stage-specific profiles of trace elements, such as zinc, copper, manganese, and selenium, in the seminiferous tubules with a microdissection technique [9,10]. Cell-specificity of elemental distribution among the three types of germ cells in the seminiferous epithelium, however, still remains unclear.

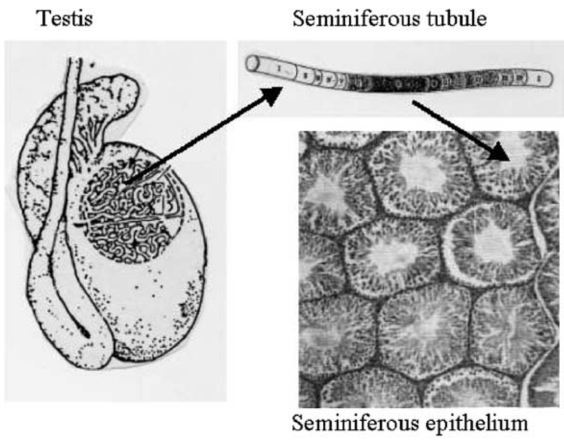

Fig. 1. Structure of the rat testis. Germ cells are arranged in the seminiferous epithelium in order of cell development. Each tubule has three types of germ cells; spermatogonia along the basement membrane, one or several layers of spermatocytes farther in, and groups of spermatids next to the lumen of the tubule.

Synchrotoron radiation X-ray fluorescence analysis (SR-XRF) with a microprobe is a simple and useful way to investigate the precise distribution of elements in tissues [11,12]. 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 with major element, such as calcium and potassium, unlike the Sn L-line. In addition to the microdissection technique, in the present study, we applied the analytical method for cell-selective determination of Sn in the seminiferous epithelium and the Sn dynamics in the testis was examined in Wistar male rats exposed to tributyltin chloride (TBTC).

2. Experimental

2.1. Chemicals

Chemicals were obtained as follows: optimal cutting temperature (OCT) compound from Sakura Finetechnical Co., Ltd. (Tokyo, Japan); tributyltin chloride (TBTC) from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan); Corn oil from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); custom multi-elements standard from SPEX Certi Prep (Netuchen, USA); nitric acid (ultrapure analytical reagent) from Tama Chemicals (Kawasaki, Japan).

2.2. Animals and treatments

Wistar male rats (7 weeks) were obtained from SLC Japan, Inc. (Shizuoka, Japan). Animals were allowed to acclimatize to an environment of controlled temperature, humidity, and light/dark cycles for 3 weeks before the study. TBTC was dissolved in corn oil and administered to rats (10 weeks, 305–320 g, $n = 4$) by oral injection at a dose of 45 μmol/kg per day for 3 days. As a control, rats (10 weeks, 325–336 g, $n = 3$) were given plain corn oil. The day following the last administration of