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Development of metamorphosis assay using *Silurana tropicalis* for the detection of thyroid system-disrupting chemicals

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Abstract

The West African clawed frog (*Silurana tropicalis*), which resembles the South African clawed frog (*Xenopus laevis*), but is somewhat smaller, has a diploid genome and a shorter generation time. Therefore, *S. tropicalis* has the potential for use as a new model in ecotoxicology. We demonstrated a *S. tropicalis* metamorphosis assay based on *Xenopus* Metamorphosis Assay (XEMA) using 1 μg/L thyroxine (T4) and 75 mg/L propylthiouracil (PTU). Tadpoles at developmental stages 48–50 were exposed to chemicals for 28 days and total body length, developmental stage, and hind limb length were recorded every 7 days. Significant differences in developmental stage and total body length were found for both T4 and PTU after 7-day exposure, which were similar to the results of the XEMA ring-test using the same chemicals. Moreover, in the present study, we measured hind limb length as a new endpoint of thyroid axis. Significant differences in the hind limb length were encountered in both T4 and PTU treatments after 7 days of exposure. These results suggest that *S. tropicalis* can be used in a XEMA-like protocol to detect agonist and antagonist effects of chemicals on the thyroid system. Hind limb length is also a suitable endpoint in such protocols. A new test protocol detecting both thyroid disruption and reproductive effects of chemicals using *S. tropicalis* should be established in the near future.

Keywords: *Silurana tropicalis*; Amphibian; Metamorphosis assay; Endocrine disruptor; Hind limb; Thyroxine; Propylthiouracil

1. Introduction

For the detection and characterization of environmental chemicals with potential to disrupt function of the thyroid system, the Amphibian Metamorphosis Assay was selected by the OECD Task Force on Endocrine Disrupters Testing and Assessment (EDTA) as an in vivo assay (OECD, 2003). Thyroid hormone regulates a wide range of biological processes associated with development, somatic growth, metabolism, energy provision and reproduction in vertebrates. Exogenous chemicals that can interfere with thyroid hormone axis could pose a significant hazard to human and wildlife health (Colborn, 2002; Zoeller, 2003). The need for the development and validation of an in vivo assay for detection of thyroid system-disrupting chemicals arises from concern that a considerable number of compounds have the potential to interact with different aspects of

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thyroid function and thyroid hormone action (for review, Brucker-Davis, 1998; Kloas, 2002; Zoeller, 2003). Overall, amphibians represent a suitable model for monitoring reproductive performance (Fort et al., 2000, 2004; Pickford and Morris, 2003), advanced development including metamorphosis (Carr et al., 2003; Goleman et al., 2002a; Iwamuro et al., 2003), and sexual maturation (Goleman et al., 2002b; Hayes et al., 2002; Levy et al., 2004; Tavera-Mendoza et al., 2002a, b).

The South African clawed toad *Xenopus laevis* was selected as a test animal for the metamorphosis assay for detecting chemicals with agonistic and/or antagonistic thyroid hormone activities, since the metamorphosis induced by thyroid hormones (triiodothyronine (T3) and thyroxine (T4)) has been well characterized in this species. However, *Silurana tropicalis*, frequently called *Xenopus tropicalis*, has been recognized as a potential model animal recently, because of its advantages for genetics and its shorter life cycle than *X. laevis*. They are very similar morphologically, but there are some differences in phylogeny between these two species. Frequently, it is discussed which species name is appropriate for the tropical clawed toad used in the present study—whether it should be *X. tropicalis* or *S. tropicalis*. Phylogenetic analysis of albumins (Bisbee et al., 1977), together with studies on DNA content (Thiebaud and Fischberg, 1977) and chromosome numbers (Tymowska and Fischberg, 1982), has led to the hypothesis that the genus *Xenopus* consists of two distinct species groups: (1) *X. tropicalis* and its sister species *X. epitropicalis* in the *X. tropicalis* group and (2) all remaining *Xenopus* species in the *X. laevis* group. Cannatella and Trueb (1988), however, concluded a detailed phylogenetic and morphological characteristic study of the entire family Pipidae and presented a phylogenetic hypothesis of intergeneric relationships. These authors concluded that the *X. tropicalis* group is more closely related to the genera *Hymenochirus*, *Pipa*, and *Pseudhymenochirus* than it is to the *X. laevis* group. Consequently, they resurrected the genus name *Silurana* to replace *Xenopus* for the above group. Hence instead of *X. tropicalis* we will use the term *S. tropicalis*.

As found in differences between two species described above, *X. laevis* has several primary disadvantages as a model animal: (1) *X. laevis* requires 1–2 yr to reach sexual maturity, reducing its practicality for life-cycle experiments (Hirsch et al., 2002), and (2) *X. laevis* is tetraploid, containing duplicated gene copies of which many are nonfunctional. On the other hand, *S. tropicalis* features a smaller diploid genome and a much shorter life cycle (6–8 months) (Amaya et al., 1998; Hirsch et al., 2002). Little study of *S. tropicalis* as a toxicological tool has been performed to date, but some evidence has shown reasonable concordance between the two species (Fort et al., 2000, 2004; Song et al., 2003). There are no reports concerning the effect of thyroid hormones (T3 and T4) and anti-thyroid chemicals on the development of *S. tropicalis*.

Therefore, the development and validation of a metamorphosis assay using *S. tropicalis* will be valuable to demonstrate the usefulness of this species as a model animal. However, this species has not been well investigated with respect to the developmental biology and endocrinology of the thyroid system as compared with that of *X. laevis*. In the present study, we developed a *S. tropicalis* metamorphosis assay based on the *Xenopus* Metamorphosis Assay (XEMA).

2. Materials and methods

2.1. Test chemicals

Thyroxine (T4; CAS 51-48-9) and propylthiouracil (PTU; 6-*n*-propyl-2thiouracil; CAS 51-52-5) were purchased from Sigma (St. Louis, MO, USA) at the highest purity available. Stock solutions of T4 and PTU were prepared in 0.1 M NaOH and 0.7 M NaOH, respectively.

2.2. Animals

Silurana tropicalis was used for the assay. Adult male and female *S. tropicalis* were kindly supplied from the Institute for Amphibian Biology, Hiroshima University. Adult *S. tropicalis* were fed No.2 diet (Oriental Yeast Co, Ltd., Tokyo, Japan) and maintained in a static system at a temperature of $25 \pm 1\,^{\circ}C$ in dechlorinated tap water.

2.3. Rearing conditions

Spawning of adult *S. tropicalis* was induced by an injection of 500 U human chorionic gonadotropin (hCG; Gonatropin, Teikoku Hormone Mfg. Co., Ltd., Tokyo, Japan) per 100 g body weight into the dorsal lymph sac. Spawned eggs and developing tadpoles were initially reared in large holding tanks (e.g., 50-L tanks) filled with 40 L of rearing medium at $25 \pm 1\,^{\circ}C$ and pH 7.0 ± 0.5 during all phases of development. The synthetic rearing medium was formulated by adding 2.5 g of the commercial salt mixture Tropic Marine Meersalz (Tagis, Dreieich, Germany) to 10 L of deionized distilled water. All tanks were continuously aerated by airstones. During all phases of development, tadpoles were fed daily a commercial tadpole food, Sera Micron (Sera GmbH, Heinsberg, Germany). Feeding of tadpoles started at day 5 postfertilization. During the pre-exposure phase, the total daily food ration was increased along with tadpole growth. The amount of food was adjusted as needed so that the water became clear within