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Journal of Invertebrate Pathology 90 (2005) 32–38
Journal of INVERTEBRATE PATHOLOGY
www.elsevier.com/locate/yjipa

Mitotic arrest and toxicity in *Biomphalaria glabrata* (Mollusca: Pulmonata) exposed to colchicine

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Received 22 February 2005; accepted 19 April 2005
Available online 13 June 2005

Abstract

Continuous exposure of *Biomphalaria glabrata* snails to 0.1% colchicine resulted in a significant increase, relative to non-exposed snails, in the number of arrested mitotic figures in the amebocyte-producing organ (APO) as soon as 4 h, with peak numbers after 12 h of exposure. The number of circulating hemocytes was significantly elevated at 24 h. However, by 72 h both the number of mitotic figures in the APO and the concentration of circulating hemocytes in the hemolymph had returned to control levels. Hemocytes appeared to possess normal morphology throughout this exposure, including the formation of long filopodia with supporting rodlike structures that have been reported to contain microtubules. Snail survival decreased as a function of exposure time. Significantly fewer snails, relative to controls, survived a 48-h exposure, and only 1 out of 30 snails recovered from a 72-h exposure to 0.1% colchicine. Colchicine-exposed snails displayed intoxicated behavior, even upon removal from the colchicine solution, although no histopathology was evident in the CNS of snails exposed for 72 h.

Keywords: *Biomphalaria glabrata*; Amebocyte-producing organ; Hemocyte; Hematopoiesis; Mitosis; Colchicine; Toxicity; Molluscs

1. Introduction

Colchicine is a phytoalkaloid that binds to tubulin and prevents its polymerization into microtubules, thereby blocking formation of the mitotic spindle and arresting nuclear division at metaphase (Jordan and Wilson, 1999). Consequently, colchicine has long been used experimentally to visualize metaphase chromosomes in cytogenetic studies, and to induce polyploidy in plants (Eigsti and Dustin, 1955). Although colchicine has been employed as an anti-inflammatory drug, acting in part by disrupting both microtubule-dependent and microtubule-independent functions of neutrophils (Molad, 2002), at doses slightly higher than therapeutic it is quite toxic to humans, resulting in wide ranging effects, including bone marrow depression, pancytopenia, neuropathy, myopathy, and multi-organ failure (Harris et al., 2000).

Colchicine also is toxic to invertebrates. For example, dietary exposure of female *Drosophila melanogaster* to 100 ppm (0.01%) colchicine results in 100% mortality by 7 days, whereas lower concentrations induce reproductive abnormalities (Koch and Spitzer, 1983). Exposure of the snail *Indoplanorbis exustus* to 10 ppm (0.001%) colchicine for 7 days interferes with neurosecretion, oviposition, and egg viability, although survival of adult snails is not affected at this concentration (Hanumante et al., 1980). However, a 24-h exposure to 1000 ppm (0.1%) colchicine is non-toxic to the freshwater crustacean *Daphnia pulex* (Morrow et al., 2001).

Joky et al. (1985) used colchicine to study effects of infection with the digenetic trematode *Echinostoma caproni* on the hematopoietic amebocyte-producing organ (APO) (Lie et al., 1975) of the snail *Biomphalaria*

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0022-2011/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2005.04.005

glabrata. These authors exposed *B. glabrata* to miracidia of *E. caproni*, and at different times post-exposure, incubated snails in 0.1% (2.5 mM) or 0.5% (12.5 mM) colchicine for 18 h prior to fixation and histological examination of the APO. Mitotically arrested cells were observed in the APO, and a peak in APO hyperplasia occurred at 3–4 days post-exposure to miracidia.

Sullivan et al. (2004) reported increased mitotic activity in the APO of snails that had been injected with extracts of larval *Schistosoma mansoni*. To further study effects of larval schistosomes on the APO, we were interested in employing the method of Joky et al. (1985) both to facilitate identification of dividing cells in histological sections and to amplify mitotic responses. However, the optimal exposure conditions and possible toxic effects of colchicine for *B. glabrata* are not known, and the rationale for the 18-h exposure employed by Joky et al. (1985) is unclear. Therefore, we exposed *B. glabrata* to 0.1% colchicine, representing the lowest effective dose reported by these authors, for periods ranging from 1 to 72 h, in an attempt to obtain subthreshold (relative to effects on mitosis), effective, and toxic doses. Toxicity was assessed by measuring effects on (1) snail survival, (2) hemocyte concentrations, inasmuch as leukopenia occurs in humans following colchicine-induced bone marrow depression (Harris et al., 2000), and (3) histology of the central nervous system, since colchicine causes apoptosis in hippocampal neurons of rats both in vivo and in vitro (Kristensen et al., 2003).

2. Materials and methods

2.1. Snails

The wild-type Salvador strain of *Biomphalaria glabrata*, originally from Salvador, Brazil (Paraense and Correa, 1963) was used in this study. Snails were raised in heated (27 °C) 10-gal aquaria equipped with under-gravel filters and containing artificial pond water (APW), i.e., deionized water supplemented with Nolan–Carriker salt solution (Malek and Cheng, 1974), and were fed Romaine lettuce. Shell diameters measured 10–12 mm.

2.2. Colchicine exposure

Groups of 10 snails were immersed individually in 5 ml of 0.1% colchicine (Sigma Chemical, St. Louis, MO, or ICN Biochemicals, Aurora, OH) in APW in a covered 35-mm diameter plastic petri dish at 27 °C. Although snails sometimes attached to the lid of the dish by their headfoot, the solution clung to them, and as a result exposure to the colchicine was deemed to be continuous. Upon removal from the colchicine solution, the snails were briefly rinsed with deionized water. Separate sets of snails were exposed for each of the four studies (APO histology, hemocyte counts, survival, and brain histology) described below.

2.3. APO histology

Snails were exposed to colchicine for 1, 2, 4, 6, 12, 24, or 72 h, and subsequently dissected from their shells. The entire pericardial sac then was excised and fixed in Carnoy's fluid. Following paraffin embedding, the tissue was serially sectioned at 5 μm, and the sections were mounted on slides and stained with Delafield's hematoxylin. Sections were examined microscopically with the use of a 100× oil immersion objective, and total numbers of mitotic figures in the APO, which is the anterior wall of the pericardial sac, were counted. At each exposure period, pericardial sacs from 10 snails were examined. Pericardial sacs from 10 unexposed snails served as controls.

2.4. Hemocyte counts

Snails were bled after 1, 2, 4, 6, 12, 24, or 72 h exposure to colchicine. To obtain hemolymph, several millimeters of the outer whorl of shell were first removed with fine forceps, moisture was blotted from the exposed headfoot, and then the snail was induced to retract by forcefully prodding the headfoot with a capillary tube. As the snail retracted, hemolymph was expelled through the hemal pore (Sakharov and Rozsa, 1989) and was collected into the capillary tube. A second tube was employed if the first became clogged with mucus. Hemolymph was expelled from the capillary tube onto a piece of Parafilm (American Can Company, Greenwich, CT), and debris allowed to settle for 15 s. Then 25 μl of hemolymph were transferred to a phase Improved Neubauer hemacytometer (American Optical, Buffalo, NY), which was incubated in a humidity chamber for approximately 3 min. All adherent hemocytes, both granulocytes and hyalinocytes (Cheng, 1975), were counted in five 1-mm^2 areas of the hemacytometer using a phase contrast microscope, and the concentration of hemocytes/ml of hemolymph was calculated. At each exposure period, 10 snails were sampled. Hemocyte counts from 10 unexposed snails served as controls.

2.5. Survival

Following individual colchicine exposure for 24, 48, or 72 h, groups of 10 snails were transferred to covered plastic "shoebox" containers holding approximately 1 L of APW at 23 °C, and were periodically observed for abnormal behavior and mortality during the following 4 weeks, until they died. Snails undergoing decomposition or that lacked heartbeat were scored as dead and were removed from the container. A total of 3 groups of 10 snails were exposed for each time period. Controls consisted of 30 snails individually exposed to 5 ml APW for 72 h, followed by observation for 4 weeks.