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Yeast Stimulation of Bone Marrow Mitosis in the Soft-Shelled Turtle, *Pelodiscus sinensis* Crother for Cytogenetic Investigations

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ABSTRACT

We report a simple method involving the injection of a active yeast to increase the number of mitotic cells in the bone marrow tissue of the soft-shelled turtle, *Pelodiscus sinensis* Crother. Intramuscular injections of a suspension of active yeast into the hind leg region elevated the bone marrow mitotic index by as much as 10-fold. The metaphases induced using this method showed excellent chromosome spreading when air-dried. This method should prove useful to investigators who wish to use bone marrow as a source of chromosomes for soft-shelled turtle cytogenetic studies.

Key words: bone marrow, chromosome, mitosis, soft-shelled turtle, yeast stimulation.

INTRODUCTION

The Trionychidae family inhabits the seas of central Asia and the soft-shelled turtle, *Pelodiscus* (=*Trionyx*) *sinensis* Crother, 2000 is indigenous to Southern China, Taiwan, Northern Vietnam, Far Eastern Russia, Korea, Japan, Bonin Islands, Indonesia (Timor), and Japan, and has been introduced into Thailand and Hawaii (Webb, 1962; Gaffney, 1975). The soft-shelled turtle is considered a nutritious food and is a commercially important aquaculture species that is in high demand in the Korea, China, and Japan. In the recent years, the techniques required to farm this species have been rapidly developed in southern Korea.

Recently considerable chromosome information on the soft-shelled turtle has been presented by Oguma (1936, 1937), Susaki (1950), Bickham et al. (1983) and Fujii (1983). However, these reports quote low mitotic indices, particularly during the winter and when animals are out of condition. Moreover, comparatively little information is available on chromosome analysis in the soft-shelled turtle. Furthermore, soft-shelled turtle chromosomes have in general high in number. New methods are required to in the soft-shelled turtle to obtain excellent quality slides showing high mitotic indices to allow precise chromosome analysis.

Thus, if the number of dividing cells in soft-shelled turtle tissue samples could be increased, this would greatly help chromosomal analysis and other related

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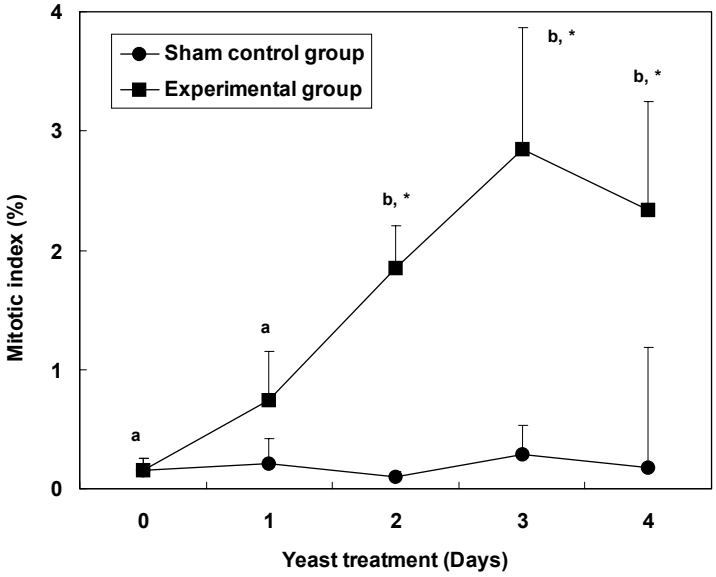

Figure 1. Mean mitotic indices of untreated animals (control group, n = 10, at initial time), of animals treated with dextrose solution only (sham control group, n = 7, 8, 6 and 8, at days 1, 2, 3, and 4, respectively), and animals treated with active yeast suspension (experimental group, n = 10, 9, 12 and 11, at 1, 2, 3, and 4 day, respectively). Bars indicate standard deviations. Same alphabetic letters are not significantly different (P < 0.05). Astrick indicate significant difference from the sham control and experimental group values (P < 0.05).

experimental studies. Cole and Leavens (1971) were the first to suggest the use of yeast to stimulate mitosis in the hemopoietic tissues of reptiles. Here, we describe a new method of increasing the rate of mitotic division in soft-shelled turtle hemopoietic tissue, involving the injection of active yeast.

MATERIALS AND METHODS

Eighty-one soft-shelled turtles with a mean body weight of 685.9 ± 41.7(SD) g, and mean shell length and width of 17.5 ± 0.5(SD) cm and 14.4 ± 0.2(SD) cm, respectively, were used for this experiment. Yeast suspensions were prepared by thoroughly mixing 4 g of fresh active dry yeast and 6 g of dextrose in 25 ml of warm distilled water. This mixture was incubated as described by Lee and Elder (1980) at 40°C for 30 min until the yeast became active, as indicated by vigorous foaming. After anaesthetizing animals with 1000 ppm Lidocaine-HCl/ 1000 ppm $NaHCO_3$ at 25°C (Park et al., 2004a), they were injected intramuscularly (i.m.) into the hind leg region with 0.5 ml of the yeast suspension per 25 g of body weight.

The control group (initial time) comprised 10 individuals matched for weight and dimensions with the experimental group. The controls were not administered yeast or dextrose solution. The sham control group comprised 29 age/size matched individuals, and were administered dextrose solution only at 1, 2, 3 and 4 days. The experimental group comprised 42 individuals, which were administered yeast i.m., as described above also at 1, 2, 3 and 4 days. A mitotic arresting agent is administered intraperitoneally about 24 hrs following the final yeast injection. The direct preparations of chromosomes from bone marrow after injection with 0.05% colchicine (Sigma, USA) for 4 hrs. Following treatment with 0.075 M KCl for 30 min, and Carnoy fixation, slides were air-dried, Giemsa (Gurr's R-66, BDH, England) stained and examined under an optical microscope at 400X (Park et al., 1999, 2003, 2004b; Im et al., 2001; Kim et al., 2001). The mitotic rate per 3,000 nucleuses per slide without counting erythrocytes was determined. Mitotic rates were determined daily from the day after injection for three days.

Data were analyzed with the SPSS (Statistical Package for Social Sciences) statistical package. The results were analyzed using a one-way ANOVA. Means were separated by Duncan's multiple range test and were considered significantly different if $P<0.05$.

RESULTS AND DISCUSSION

Figure 1 shows mitotic rates in the four experimental groups, i.e., examined on days 1, 2, 3, and 4 post-injection. Our results indicated no demonstrable difference between the sham and control groups, and that the mitotic rates were elevated in active yeast treated animals. In particular, day 3 samples showed the highest mitotic rate (Fig. 1).

The control and sham groups had mitotic indices in

Figure 2. Microscopic photograph showing a portion of a bone marrow cell slide showing the highest mitotic rate at 3 days after active yeast injection. Arrows indicate mitotic figures. Bar indicates 100 μm.

the range 0.10~0.29%. However, the experimental groups on days 1, 2, 3, and 4 post-injection showed mitotic indices of 0.74% (3.5 times the sham control), 1.85% (18.5 times the sham level), 2.85% (9.8 times the sham control), and 2.34% (13 times the sham control), respectively.

Figure 2 shows that results obtained from bone marrow three days after active yeast treatment. Figure 2 shows a typical low power field and highly visible metaphases (18 arrows). We found that this i.m. yeast method consistently produced an elevated mitotic rate, and perhaps of greater importance, metaphases obtained by this method show excellent chromosome spreading when air-dried. As mentioned earlier by Lee and Elder (1980) in small mammals, we feel that this active yeast stimulation method offers a number of advantages in several areas of cytogenetical research on the soft-shelled turtle: (1) Consistently good chromosomal preparations can be made in the field with readily available relatively cheap equipment and raw materials; (2) useful cytogenetic data can be obtained from animals held in captivity for prolonged periods; and (3) a preliminary analysis showed no increase in aneuploidy or chromosomal aberration frequencies in cells subject to active yeast stimulation.

The reason for this increased mitotic activity and cell membrane fragility by active yeast stimulation in the soft-shelled turtle is not understood (Lee and Elder, 1980). These workers suggested that some component of the yeast suspension acts as a mitogen or that yeast cells directly stimulate B cell division as an immune response. The observed increases in hematopoietic cell numbers in the soft-shelled turtle may be caused by immunologic stimulation. A definitive conclusion on this issue will require further study.

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