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INFLUENCE OF *IN VIVO* COLCHICINE ADMINISTRATION ON MITOTIC INDEX IN CHINCHILLA BONE MARROW CELLS

Abstract

The aim of our research was to increase the number of mitoses in the bone marrow cells in chinchillas through the prior in vivo administration of colchicine. An experiment was carried out on 16 chinchillas variety Standard. The animals were divided into two groups: a control and an experimental group. The control group consisted of 8 animals, from which bone marrow were isolated and chromosome preparations were made according to the principles of the classical method of Tjio and Whang (1962) with ours modifications, while the study group consisted of animals to which colchicine had previously been administered in vivo. The preparations were stained in the classical technique using differential Giemsa stain. The results were analysed by computing and comparing the mitotic index (MI) in both groups. The analysis showed a higher proportion of dividing cells in the study group than in the control group. The differences in mitotic indices between the groups were statistically significant at $p<0,05$.

Key words: *chinchilla, bone marrow, colchicine, mitotic index, metaphase plates*

Introduction. A technique enabling chromosomes to be obtained directly from bone marrow was first described by Tjio and Whang (1962) and applied to small animals. The presence of a larger number of cells in directly obtained bone marrow than in the case of blood lymphocyte culture makes it possible to investigate chromosomes without the need for cell cultures (Olszewska 1981). The classical procedure for making chromosome preparations from bone marrow requires modification and specification for each species, mainly because of interspecies differences in the sensitivity of cells to the action of antimitotic substances (Stokłosowa et al. 2006). In order to obtain as many dividing cells as possible, it is necessary not only to make a proper selection of the antimitotic compound and its concentration, but also to establish the optimum duration of its action. In animal cytogenetics the compound of this type normally used is colchicine, a powerful toxin which paralyses the mitotic spindle, leading to an accumulation of cells chiefly in metaphase (Macgregor 1988). Bone marrow cells are obtained by puncturing the sternum or ilium in the case of large animals, and the femur in the case of small animals. A disadvantage of this technique is the difficulties involved in obtaining bone marrow *in vivo*, and it is therefore rarely used in cytogenetic experiments on large animals (Świtoński et al. 2006). It is used mainly in karyotype analysis of small animals such as rats (Tjio and Whang 1972, Moore et al. 1995) and mice (Ganasoundari et al. 1996).

It is also possible to use this method with *Chinchilla lanigera* Mol. (Rodentia, Chinchillidae). This is a breeding animal which is easy to keep and breed, although its

reproduction rate is low (Novacek 1992, Nes et al. 1989). Naturally it is the number of weaning young that determines the profitability of any animal breeding .

The diploid number of chromosomes in this species has been determined at 2n = 64. A total of 62 meta- or submetacentric autosomes have been identified. The largest in the complex is the metacentric X chromosome, while the meta- or submetacentric Y is among the smallest chromosomes (Nes 1963, Galton et al. 1965, Fredga 1966, Hsu and Benirschke 1967, Hong et al. 1982, Kuchta et al. 2010). It should be pointed out that in most publications to date concerning chinchilla karyotype the analysis has been performed on chromosomes obtained from lymphocyte cultures. A study was therefore undertaken with the aim of increasing the mitotic index (MI) in chinchilla bone marrow cells through the prior *in vivo* administration of colchicine.

Materials and Methods. The material consisted of bone marrow taken from the femur immediately after the animals were slaughtered for fur. The experiment was carried out on 16 breeding chinchillas of the Standard variety. All of the animals were at a similar age of 5–6 months, with average body mass 500 g, and came from the same breeding farm in southern Poland. The chromosomes were obtained directly from the bone marrow cells in accordance with the procedure developed by Tjio and Whang (1962), with ours modifications. The slides were stained in the classical technique with Giemsa stain (Bratkowska 1981).

In vivo injection of colchicine: In order to increase the incidence of cells in mitosis in the bone marrow cells, 3 hours prior to slaughter the animals in the experimental (8 chinchillas) received intraperitoneally a 0.1% aqueous solution of colchicine in a quantity of 0.1 ml per gram of body weight, i.e. 5 ml per animal. The control group (8 animals) received 5 ml of *Aqua pro injectione* solution (Polfa).

Making of microscopic preparations directly from chinchilla bone marrow cells: The procedure involved several stages. First the bone marrow was flushed through each end of the excised and cleaned femur isolated from the carcass, using a solution of PBS with 0.1% colchicine (37°C). The material was centrifuged for 5 minutes at 1500 rpm. The supernatant was poured off, the cells suspension was well mixed in a shaking apparatus (Vortex) and a 0.05 M KCl hypotonic solution was added (37°C), after which the cells were incubated at 37°C for 40 minutes [in the methodology of Tjio and Whang (1962) the incubation time was 25 minutes]. After incubation the material was centrifuged for 5 minutes (1500 rpm). The cell suspension was then fixed with Carnoy's solution, and again centrifuged for 5 minutes. In view of the large volume of cell precipitate, fixing was repeated not 3 times (as given by Tjio and Whang) but 5 times, with the addition each time of 10 ml of Carnoy's solution, which had been refrigerated for 2 hours prior to use. Precleaned microscope slides previously placed in a freezer were allowed to collect condensation.

The microscopic slides were made by dropped the same quantity of cell suspension. Five drops of cells suspension were dropped on each basic slide, by use of Pasteur pipet. Prior to microscopic analysis the air-dried slides were stained with a 10% aqueous solution of Giemsa stain. According to Bratkowska (1981) the time interval of staining usually ranges from 20 to 30 minutes, and must be suitably chosen for each species individually. After a series of trials it was found that the optimum staining time for chinchilla bone marrow cells was 25 minutes.

The effect of colchicine administered *in vivo* on the number of mitotic divisions in the analysed preparations was determined based on the mitotic index, which was calculated using the following formula:MI = M /N * 100%; where MI - denotes the mitotic index, M - the number of dividing cells, and N - the total number of cells.

Microscopic analysis was carried out using a NIKON biomedical microscope (Eclipse E600) coupled with a NIKON digital camera (DS-Fi1-U2). Analysis was made of 5 slides from each animal, a total of 80 preparations. For determination of the number of cells, 10 fields were selected at random in each of the slides under 250 magnification. The cells were counted using the MultiScan Base program. To determine the significance of the differences in MI between the study group and control group, the results were analysed using the t-Student test for matched pairs (Sokal and Rohlf 1981). The computations were performed using the Statistica 9 package.

Results. For each animal, between ~3600 and ~9600 bone marrow cells were analysed under the microscope, with the percentage of dividing cells being calculated. The dispersion of the breeding material in most of the preparations in the experimental and the control groups was comparable. However differences were observed in the quantity of dividing cells, particularly in metaphase – there were more such cells in the experimental group (Figure 1). Moreover it was found much more frequently in the preparations from the experimental group that the metaphase plates were complete and the chromosomes better distributed. Significantly, no damaged bone marrow cells were detected in the analysed preparations, in spite of the use of two doses of colchicine: one *in vivo* and one during the rinsing out of the marrow from the bone.

The value of the mitotic index (MI) in the study group was higher (1.76–4.22) than in the control group (0.77–1.95) (Table 1). The differences between the analysed groups were statistically significant (t-Student test, $p<0,05$). This implies that giving the animals colchicine *in vivo* three hours before the sample of biological material is taken increases the value of the mitotic index in a suspension of bone marrow cells.

Table 1.

Mitotic index (MI) for animals in the control group and study group.

Control group	MI [%]	Experimental group	MI [%]
H9	70/6986 1.00	H1	346/9660 3.58
H10	154/7909 1.95	H2	356/8429 4.22
H11	92/7762 1.18	H3	166/7499 2.21
H12	68/6798 1,00	H4	189/7952 2.38
H13	44/3604 1.22	H5	149/7780 1.91
H14	48/6178 0.77	H6	135/7666 1.76
H15	62/6506 0.95	H7	130/5988 2.17
H16	54/3995 1.35	H8	139/4166 3.34
Mean	**74/6217**	**Mean**	**201/7392**

1.17	**2.69**

(H1 – H16) – tested animals

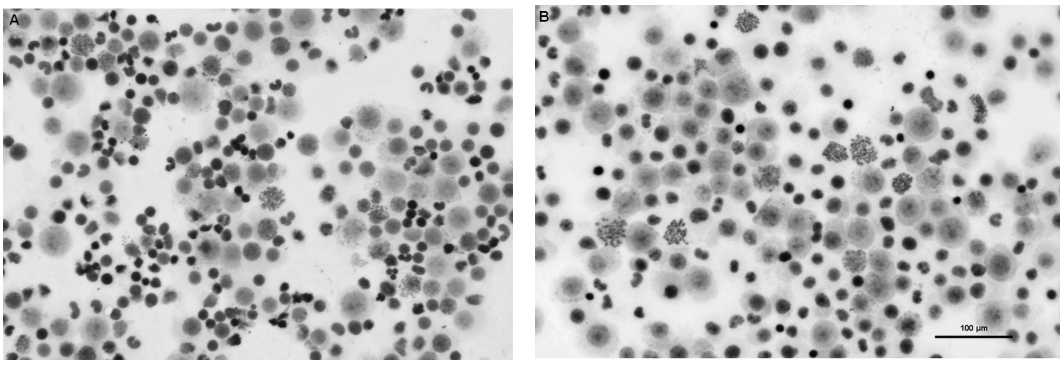

Figure 1. Bone marrow cells of *Chinchilla lanigera*: control group (A) and anexperimental group (B).

Discussion

Colchicine is an alkaloid of plant origin (obtained from *Colchicum autumnale L.*) with summary formula $C_{22}H_{25}O_6N$. It is a powerful mitotic toxin, whose presence leads to blocking of the mitotic spindle, which consequently enables the accumulation in the experimental material of cells in metaphase. For this reason, among others, the substance is widely used in cytogenetic research (Enikeeva and Goloshchapova 1998). There also exist several other plant-derived alkaloids with similar action. Among these are vincristine, vinblastine, coumarin, oleandrin, deacetyl oleandrin, and a preparation known as lutenurine (Gigant et al. 2009, Lettre-Heidelberg 1996). A substance sometimes used as a substitute for colchicine is 2-Methoxyestradiol, a narcotic with antimitotic action (Attalla et al. 1998). In spite of the similar effects of these substances, most cytogenetic research nonetheless uses colchicine. The present authors selected this alkaloid due to its easy market availability and the effectiveness of its action in previous experiments using other rodents (Meighan and Stich 1958, Bottura and Ferrari 1960, Rothfels and Siminovitch 1961, Tjio and Whang 1972, Kuchta et al. 2008).

Świtoński et al. (2006) found that, in order to increase the number of mitoses in the bone marrow, it is necessary to administer intraperitoneally 3 hours before slaughter a 0.1% solution of colchicine in a quantity of 0.1 ml per gram of body weight or to add it directly to the test tube containing the bone marrow sample. Our results suggest that, in order to obtain an increase in the mitotic index in chinchillas, colchicine should be administered twice: *in vivo* 3 hours prior to slaughter, and directly to the bone marrow in the test tube.

As a result of the use of colchicine, the chromosomes within the metaphase plates accumulated in the preparation are usually well divided, which makes later cytogenetic analysis significantly easier. Depending on the action time of colchicine, chromosomes are subject to shortening and thickening to different degrees (Olszewska 1981, Macgregor 1988).

In the present study, a single value was used for the alkaloid's action time. The metaphase chromosomes, well distributed as a rule over the whole area of the cell, were nonetheless quite often broken into chromatids or else showed too high a degree of contraction.

Summary

In further research, tests should be performed using different action times of colchicine on chinchilla chromosomes. The presented method of using colchicine to increase mitotic index, and optimization of the action time of that alkaloid, will lead to improvement in the quality of the chromosome preparations made from chinchilla bone marrow.

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