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CELL PROLIFERATION OF THE MOUSE SEBOCYTES IN SKIN AND PREPUTIAL GLAND

Claudio Gustavo BARBEITO[1,2]; Vicente Alberto CATALANO[1]; Norma Viviana GONZALEZ[3]
[1]Cátedra de Histología y Embriología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Calles 60 y 118, La Plata, Argentina.
[2]Instituto de Patología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Calles 60 y 118, La Plata, Argentina.
[3]Cátedra de Histología y Embriología Animal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Calles 60 y 122, La Plata, Argentina.

Correspondence to: Barbeito, Claudio Gustavo.
Cátedra de Histología y Embriología. Facultad de Ciencias Veterinarias. UNLP. 60 y 118. La Plata (1900). Buenos Aires. Argentina.
E-mail: barbeito@fcv.unlp.edu.ar

Abstract. Preputial glands are specialized sebaceous gland of some rodent as mouse and rat. This gland is regulated by androgens and other hormones. In this work we analysed the mitotic activity of the alveolar and ductal cells of the mice preputial gland throughout a circadian period. The results were compared to data obtained from the skin sebaceous gland. The mitotic activity in the preputial sebocytes displayed a bimodal curve with the highest mitotic indices at 12:00 and 00:00 h while the lower values were reached at 20:00 and 08:00h. In the ductal cells, the mitotic activity showed a single mitotic peak at 12:00h and a trough at 20:00 h. In the sebocytes from skin glands, the acrophase was detected at 04:00 h. The average cell proliferation daily value was significantly higher in the preputial sebocytes when compared to cutaneous sebocytes. These results demonstrated the existence of quantitative and qualitative differences in the mitotic activity of the preputial gland sebocytes in relation to the skin sebocytes.
Keywords: cell proliferation, circadian rhythms, preputial glands, sebocytes.

INTRODUCTION

Preputial glands are alveolar complex holocrine glands; they are accessory to the male reproductive tract of some rodents, as the rat and the mouse. Due to its pheromones content, their products have been assigned functions related to territorial and sexual behavioral activities (11, 32). These glands' size and activities are larger in domineering aggressive males and their secretion is attractive to females (10, 11, 31). In addition, Marchlewska-Koj *et al.* (1990) found that the presence of these glands' extract induces estrous in virgin female mice.

Their main cellular type is the alveolar cell, mostly referred as sebocyte. Electron microscopy shows that the rat preputial gland resembles the human sebaceous gland, not only in terms of containing a sebocyte-like population of cells in an acinar arrangement at different maturational stages, but also in the morphology of its organelles (17). In these sense, preputial glands resemble the sebaceous skin glands and can be considered a specialization of these last. Accordingly, the preputial glands have been employed as an experimental model to study sebocytes growth and differentiation (27, 30).

Several hormones are known to influence these glands' activities (9). Insulin-like growth factor 1(IGF-I) and insulin, but not growth hormone (GH), stimulate *in vitro* cell proliferation of the glands. GH enhances preputial sebocytes differentiation (6). On the other hand, androgens have been found to stimulate preputial gland sebocytes as well as skin sebocytes (7, 13, 21, 24, 30).

Some hormones related to the preputial glands proliferation in the mouse exhibit daily variations as reported for androgens and insulin plasma levels (15, 23, 29). In addition, many cell

populations in the adult mouse exhibit a daily proliferation rhythm (2, 8, 25).

For a better understanding of the preputial cell proliferation, the following study was carried out to: 1) characterize its temporal structure in adult mice, and 2) to compare preputial and sebaceous skin glands cell proliferation.

MATERIALS AND METHODS

Forty-two male C3H / Avy strain mice, five month-old were used. Animals were housed under standardized conditions for a 24 h periodicity analysis in an ad hoc room with a 22 ± 2ºC temperature and lighting regimen of 12 h light - 12 h darkness (lights on 06.00-18.00 h). Pelleted food and water were provided ad libitum. Mice were divided into 6 lots (n: 6-8) for killing by decapitation and exsanguination at the following times of day: 00:00, 04:00, 08:00, 12:00, 16:00, and 20:00 h. Each mouse received an intraperitoneal colchicine dose of 2 µg / g body weight 4 h before sacrifice.

Preputial glands and a sample of auricular skin from each animal were fixed in 10% buffered formalin. The embedding was performed in paraffin. Five µm-thick sections were stained with H&E.

The observation was carried out under immersion oil objective (1000x). In the preputial glands, two cell-populations were monitored: alveolar cells (preputial sebocytes, PS) from the basal and parabasal layers and ductal cells (DC). No less than 3000 PS and 1500 DC per individual were monitored scoring mitosis along with the total number of cell nuclei every tenth field. In the cutaneous glands, the same procedure was employed for 3000 alveolar cells (cutaneous sebocytes, CS).

The mitotic index (colchicine metaphase / 1000 nuclei) (MI) for each animal and cell-type was calculated from data collected. Using these individual data, the arithmetic mean ± standard error from each lot and group was assessed. The significance of the differences between organs, lots and groups was analyzed by Anova and Student's t- test.

RESULTS

Table 1 and Figure 1 summarize the results on mitotic activity in PS, DC and CS. The mitotic activity in the PS presented important variations in the different time-points, displaying a bimodal curve (Figure 1) with the highest mitotic indices at 12:00 and 00:00 h while the lower values are reached at 20:00 and 08:00h (Table 1). In DC, the mitotic activity showed a single mitotic peak at 12:00 h and a trough at 20:00 h (Figure 1 and Table 1). For CS the acrophase was detected at 04:00 h (Figure 1 and Table 1).

The average cell proliferation daily value was significantly higher in PS (7.93±0.68) when compared to CS (5.6±0.3) (Table 1).

DISCUSSION

Both cell-populations from the mouse preputial glands under study presented mitotic daily variations. Our results demonstrated clear-cut qualitative and quantitative differences in the proliferation of PS and DC.

DC showed a higher mitotic activity than PS. This finding is in agreement with higher proliferation indices reported for ductal cells in other exocrine glands as the submaxillary gland (1). Moreover, whereas DC mitotic activity presented a single peak curve, PS had a different rhythmicity for the mitotic daily curve showed a bimodal characteristic. Similar circadian bimodal rhythms have been found in other mouse cell-populations (2, 25). For instance, the pars intermedia in female mice displays a comparable temporal structure at 28 d of age for two mitotic peaks are found at midnight and noon, whereas in adult females the mitotic daily curve is still bimodal but peaks are found at 04:00 and 16.00 h (2).

The mitotic index values were significantly higher in PS than in CS. Furthermore, the daily

mitotic curve's structure for PS revealed a bimodal pattern whereas CS curve displayed a single peak.

Socially dominant male mice have larger preputial glands than do subordinate males (4). A similar situation has been observed in rats for dominant individuals that had heavier preputial glands compared to subdominant and subordinate rats (20). Accordingly to their sexually-related role, PS are stimulated to proliferate and differentiate by androgen (13, 14, 18) as in other sebaceous glands, namely CS. As both PS and CS posses receptors for androgen and other hormones as the melanocortin 5 (26), the effect of these signals should not be considered as a cause to explain differences concerning PS and CS mitotic activity. Results from the present study suggest a paracrine and autocrine control hypothesis on the preputial gland proliferation and also the existence of differences in regard to skin sebaceous glands. Differential organ response to the androgens' effects on cell- cycle were previously reported (28); these variations could be related to the presence of different enzymes isotypes that catalyze the conversion of sexual steroids in the PS and in CS (5). Another plausible explanation would be a differential enzymatic activity (3, 19).

The preputial glands of several rodents' species have been long employed as a model to investigate a wide spectrum of topics e.g. the rat preputial system is used as an experimental model in sebaceous gland physiology (26). More recently, preputial glands have been used in numerous assessments on toxicological aspects (12, 22), and sex, individuality, and/or the genetic background differences between mice strains (32). Our work strongly suggests that the extrapolation of PS findings to CS may lead to erroneous interpretations. The existence of circadian rhythms in these glands could be extended to other aspects of the organs`

Figure 1

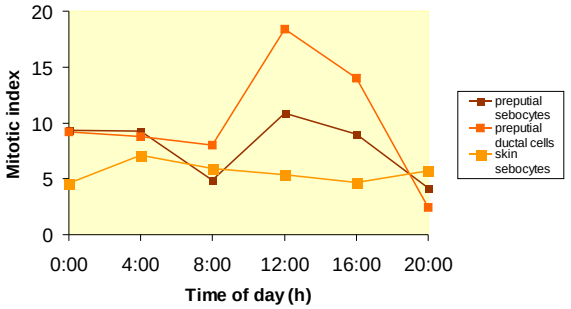

Table 1. Mitotic activity of preputial glands and sebaceous skin

	Mitotic index			
Hour	Preputial sebocytes (A)	Preputial ductal cells (B)	Skin sebocytes (C)	P
0:00	9.33 ± 1.71	9.18 ± 0.79	4.6 ± 0.60	
4:00	9.27 ± 2.01	8.75 ± 1.75	7.1 ± 0.50	
8:00	4.91 ± 0.92	8.04 ± 2.16	5.9 ± 0.50	
12:00	10.88 ± 2.29	18.39 ± 3.05	5.4 ± 0.70	
16:00	9.02 ± 1.01	13.98 ± 2.03	4,7 ± 0.79	
20:00	4.16 ± 0.80	2.47 ± 0.62	5.7 ± 1.00	
X 24 h	7.93 ± 0.68	10.19 ± 1.07	5.60 ± 0.30	A-B < 0.01; B-C <0.05
P	00:00 - 20:00 <0.05	12:00 - 20:00 < 0.001	00:00 - 04:00 < 0.01	
	08:00 - 16.00 < 0.05			
	20:00 - 12:00 < 0.05			

a Results are expressed as mean ± standard error

X 24 h: mean of the whole sample period; P: significance of differences in mean values.

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