Cell cycle time and rate of entry of cells into mitosis in the small intestine of young rats

A. C. M. Wille, F. A. Oliveira, M. A. M. Soares and J. R. Gomes

*Departamento de Biologia Estrutural, Molecular e Genética, Setor de Ciências Biológicas e da Saúde,
Universidade Estadual de Ponta Grossa, Paraná, Brazil*

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Abstract. Cell cycle time (T_C) and the rate of entry of cells into mitosis (r_M) in the jejunum and duodenum of young rats were investigated using the stathmokinetic method. The cell cycle times in the jejunum were 24.3 and 28.3 h in light and dark periods, respectively. Cell cycle times in the duodenum were 17.1 and 21.5 h in light and dark periods, respectively. Rates of entry of cells into mitosis in the jejunum were 1.2 and 1.1 cells/cell/h in light and dark periods and rates of entry of cells into mitosis in the duodenum were 1.4 and 1.8 cells/cell/h in light and dark periods, respectively. Although these changes to cell cycle time values are not statistically significant, the variation between the two periods should be considered in relation to its possible biological effects.

INTRODUCTION

The intestinal epithelium undergoes constant cell renewal and in the jejunum and duodenum there are two distinct compartments: intestinal crypts and villi. Proliferation and differentiation of cells occurs in the crypts. On the villi, cells that have migrated from the crypts execute their various functions and subsequently are lost from the tip eliminated by apoptosis.

In the adult rat, the deepest crypts and the tallest villi are found in the duodenum, while the flattest crypts and smallest villi are found in the ileum. This morphological differentiation begins during foetal development from the 14th day before birth (Ono *et al.* 1989; Yamamoto *et al.* 1992) and continues until the third week after birth, when the morphofunctional pattern of the upper gut is established (Herbst & Sunshine 1969; Klein & McKenzie 1983).

Cell kinetic processes in the intestinal epithelium are in flux during the third week after birth (Herbst & Sunshine 1969; Yeh 1977; Trahair 1989) which is the weaning period. During this phase, there is an increase in the number and size of intestinal crypts and villi, in the rate of cell migration from crypt to villus and in a gradual reduction of the cell cycle time (Yeh 1977, 1991). Alterations in cell kinetic processes in the gastrointestinal epithelium during the suckling and weaning period could be caused by changes in circadian rhythm, intake of food, hormonal activity and/or effects of growth factors.

Studies related to the proliferative behaviour of cell populations in the intestinal epithelium of rats during suckling and weaning phases have contributed to the understanding of intestinal development during the young-to-adult transition.

Previous investigations carried out by our group have demonstrated that the metaphasic index (MI) in the jejunal epithelium of 18-day-old rats is higher in the dark daily period than in the light period (data not published).

Thus, the main aim of this work has been to verify whether there is a relationship between the circadian rhythm, of MI and cell cycle time in the jejunum. In addition, we have obtained the rate of entry of cells into mitosis (r_M) in the jejunal epithelium (T_C) during dark and light periods of the day. In this work we have also obtained the cell cycle time and rate of entry of cells into mitosis (r_M) in the duodenal epithelium of 18-day-old rats in light and dark periods.

We have found in the jejunum and duodenum that highest T_C values occurred during darkness; for r_M values, however, in both jejunum and duodenum there was no statistical difference between values of light and dark periods. These results have demonstrated no unequivocal relationship between the circadian rhythm of MI and cell cycle time in jejunum.

MATERIALS AND METHODS

Five groups, each of three 18-day-old Wistar rats, both male and female, were used in each period (light and dark). All animals were maintained with a 12-h light/dark cycle (06.30–18.30 h) and temperature variation between 23 °C and 25 °C only. Food and water were provided *ad libitum*.

All animals received an intraperitoneal injection of vincristine sulphate (Oncovin, Lilly do Brasil Ltda, São Paulo, Brasil) 0.5 mg/kg body weight at 13.30 h (light) or 01.00 h (dark).

Cohorts were killed at each of the following time points: 15 min, 30 min, 1 h, 1.5 h and 2 h after vincristine injection. To obtain the metaphasic index, 2-cm tissue samples were collected and processed from each duodenum and jejunum. Cell counting was performed in selected longitudinally sectioned crypts showing the lumen. Approximately 3000 cells per animal were counted and the MIs were obtained as the ratio between arrested metaphases and total number of cells.

For each time point, the MI was adjusted by 0.62 – the Tannock Constant (Tannock 1967; Aldewachi *et al.* 1975). MI factors were calculated according to the method originally proposed by Puck & Steffen (1962), converting MIs to a logarithmic scale following formula: log value of $1 + MI$. MI factors obtained were used to construct regression curves. Where 'b' is the slope of the regression curve, calculation of cell cycle time (T_C) and rate of entry of cells into mitosis (r_M) were performed using the following formulae:

$$b = \log(1 + MI)/t$$

$$r_M = 1/T_C$$

$$T_C = \log 2/b(\log 2 = 0.3010)$$

The r_M values were expressed in cells/cell/h.

Slopes of the regression curves obtained for light and dark time periods were analysed by the Student *t*-test, using the statistics program BioEstat 2.0 (Belém, Brasil).

Figure 1. Regression curve obtained from averages of the metaphysic index as function of time post vincristine during the light period (13.45, 14.00, 14.30, 15.00 and 15.30 h). (a) Jejunum, (b) duodenum.

RESULTS

Figure 1 demonstrates an increase in the number of arrested metaphases as a function of time post vincristine in the jejunum (Fig. 1a) and in the duodenum (Fig. 1b) during the light period. Figure 2 indicates an increase in the number of arrested metaphases as a function of time post vincristine in the jejunum (Fig. 2a) and duodenum (Fig. 2b) during the dark period. Table 1 collates values of the cell cycle time (T_C) and rates of entry of cells into mitosis (r_M) in the jejunum and duodenum at the light and dark periods.

Values of T_C in the jejunum were 24.3 and 28.3 h at the light and dark periods, respectively, and values of T_C in the duodenum were 17.1 and 21.5 h at the light and dark periods, respectively. Values of r_M in the jejunum were 1.2 and 1.1 cells/cell/h at the light and dark periods, respectively, and values of r_M in the duodenum were 1.4 and 1.8 cells/cell/h at the light and dark periods, respectively.

Statistical analysis comparing slopes of the regression curves obtained from MIs (Fig. 1a and b, Fig. 2a and b) showed no significant difference ($P < 0.05$) between the T_C values at the light and dark periods in the jejunum and duodenum. Also, no statistical differences were observed in T_C values between the two regions.

DISCUSSION

In this present work we carried out experiments in weaning rats to determine the cell cycle time (T_C) in the jejunum during darkness, to verify whether there was a relationship between peak MI observed at this time of day (previous data not published) and duration of the cell cycle.

Figure 2. Regression curve obtained from averages of the metaplastic index as function of time post vincristine during the dark period (01.45, 02.00, 02.30, 03.00 and 03.30 h). (a) Jejunum, (b) duodenum.

Table 1. Cell cycle time (T_C) and the rate of cells entry into mitosis (r_M) in jejunum and duodenum of 18-day-old rats

	Light period	Dark period
Jejunum		
T_C /h	24.3	28.3
r_M (cells/cell/h)	1.2	1.1
Duodenum		
T_C /h	17.1	21.5
r_M (cells/cell/h)	1.4	1.8

The values of T_C obtained in the jejunum were 24.3 and 28.3 h at the light and dark periods, respectively, but these differences are not statistically significant. Our analysis concludes that there is no relationship between circadian variation, of MI and T_C the jejunum of weaning rats.

Gomes & Alvares (1998) studied cell proliferation in the jejunum of 18-day-old rats after 24 h of progressive fasting, by labelling the cell population with [3 H]dT at 10.00 h. They calculated T_C by the modified method of grain count halving (Bassukas & Schultze 1987). Values of T_C in the fed (control group) and fasting groups were 17.2 and 19.5 h, respectively. In a further experiment, a different cell population was labelled with [3 H]dT at 23.00 h. Values of T_C in the fed and fasting groups were 16.5 and 17.7 h, respectively. Statistical analysis showed that cell cycle times did not differ between both times of the day and treatment studied.

Differences between the values of T_C obtained by Gomes & Alvares (1998) and ours in the jejunum could be plausible if there were distinct cell populations cycling through the day. Also, such differences might be explained by methodology used in the study, where kinetic parameters were calculated from separate phases of the cell cycle. However, the value of T_C obtained at the light period (17.1 h) in the duodenum here is similar to those obtained by Yeh (1977)

with 16-day-old rats (T_C 17.7 h) and by Al-Nafussi & Wright (1982) with 18-day-old rats (T_C 16 h).

A further kinetic parameter measured in this study was the number of cells that entered into mitosis (r_M), which is similar between the light (1.2 cells/cell/h) and dark periods (1.1 cells/cell/h) in the jejunum. However, values of r_M are higher in the dark period (1.8 cells/cell/h) than in the light period (1.4 cells/cell/h) in the duodenum. Comparison between these regions shows that r_M in the jejunum (1.2 cells/cell/h) is similar to that of the duodenum (1.4 cells/cell/h) during the light. Nevertheless, r_M in the duodenum (1.8 cells/cell/h) is higher than that in the jejunum (1.1 cells/cell/h) during darkness. The highest r_M value at dark in the duodenum during the weaning phase could explain morphological differences between villi and crypts observed in the intestinal regions of adult rats. Gomes & Alvares (1998) concluded that the mechanisms of control of the cell proliferation and migration might be different in young rats and adult mice (Kaur & Potten 1986a,b). In addition, we suggest, as shown for some parts of the gastrointestinal epithelium, that mechanisms that regulate cell proliferation and cell migration involving hormones (Goldfeder & Alvares 1995; Gama & Alvares 1996), prostaglandin (Koelz *et al.* 1986; Uribe *et al.* 1987; Uribe & Garberg 1990; Uribe *et al.* 1992, 1995; Kunika *et al.* 2001) and growth factors (Foltzer *et al.* 1993; Puccio & Lehy 1988) are already present in the intestinal epithelium leading to the steady-state of cell proliferation observed in adult rats.

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