Circadian Mitotic Rhythm as a Guide for the Administration of Antimetabolites* (34117)

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In the tissues of mammals, the occurrence of cell division is unevenly distributed throughout the day. This uneven distribution of mitosis is a general phenomenon and can be found in the normal tissues of animals. In contrast, some neoplastic tissues show either absence or a different mitotic rhythm, as reported by many authors (1–10).

If mitosis is synchronized, necessarily the metabolic events which lead into mitosis must also be synchronized. Work has been published indicating that a circadian rhythm indeed exists for several enzymes, as well as for nucleic acid metabolism, of both the RNA and DNA types (11–16). Thus, if the mitotic and metabolic rhythms are timed differently in normal mammalian tissues, as compared with neoplastic tissues, advantage could be gained with the selective time of administration of cancer chemotherapeutic agents. At this selective time period of the day, theoretically, they should have a minimum effect upon the normal cells which are to undergo division at peak mitosis time. Neoplastic cells dividing at their own pace, asynchronously with the normal cells, would in this way during the course of chemotherapy be relatively more exposed to the antimetabolite.

No data have yet been obtained to indicate that this therapeutic scheme is efficient in tumor-bearing animals or human patients with neoplasias. However, evidence has been presented indicating that cancer chemotherapeutic agents may cause either marked or absent mitotic inhibitiory effect upon the cells which undergo mitosis at 0700 hours of the day (17). This effect depends upon the time of the day in which the drugs are given.

Since in the cornea of rats 0700 hr represents the diurnal peak period for the occurrence of mitosis, protection of the bulk of corneal cells which normally undergo mitosis in any 24-hr period was obtained. As judged by the data obtained with this tissue, in which the mitotic ratio maximum (0700 hr)/minimum (1900 hr) is approximately 14/1 (5), a significant gain in the therapeutic index of cancer chemotherapeutic agents could be obtained with their administration at specific time periods of the day.

In this work, we are presenting: (1) additional data to indicate that a similar response to that previously reported for actinomycin D (17) can be obtained also with 5-fluorouracil (5-FU) and cytosine arabinoside (ARACY); (2) the effect of cytosine arabinoside administration, at either 1400 hr or 2300 hr of the day, upon the circadian distribution of mitosis in the corneal epithelium of rats.

Materials and Methods. Female rats of the Holtzman strain with weight ranging from 110 to 150 g were used in all experiments. Administration of the different antimetabolites was done (doses on tables) either at 1400 hr or at 2300 hr of the day, by the intraperitoneal route; the rats were sacrificed at different hours of the day as indicated in the tables. The eyes were removed, fixed, and stained as previously described (20). Mitoses were counted with the use of a microscope with a reticulum (7.5 × 7.5 mm) ocular (10×) and oil immersion lens. One hundred microscopic fields were counted in each cornea. All phases of mitosis were included

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with exception of those cells in early prophase and late telephase. All animals were kept in the Department's animal quarters for 1–2 weeks prior to their use in the experiments. They were fed a commercial diet *ad libitum*. Lights were kept on from 0600 to 1800 hr and darkness prevailed from 1800 to 0600 hr of the day.

Results and Discussion. Mitosis as well as the synthesis of both DNA and RNA, in mammals, present distinct diurnal rhythms (15). This fact led us to investigate the possible relationship between the diurnal peaks of DNA and RNA synthesis and the diurnal peak of mitotic activity. Thus we felt that it would be important to study and compare the antimitotic effect of antimetabolities which alter primarily either DNA or RNA metabolism, administering the antimetabolites at different times of the day. For this purpose, cytosine arabinoside, primarily a DNA inhibitor, 5-fluorouracil, and actinomycin D, which primarily alter RNA metabolism, were selected. Results obtained with the use of actinomycin D, 5-fluorouracil, and cytosine arabinoside, upon the 0700 h corneal epithelium mitotic peak, the respective doses, and the time of drug administration are presented in Table I.

As seen in Table I a striking time differential effect was obtained with the antimetabolites used. Actinomycin D, in the lower dose, did not inhibit the 0700 mitotic peak when administered at 2300 hr, while both ARACY and 5-FU did so. The opposite was true when these drugs were administered at 1400 hr of the day: Actinomycin D effectively inhibited mitosis, ARACY did not cause a significant inhibition and 5-FU was significantly less effective as a mitotic inhibitor than when administered at 2300 hr ($p<0.005$). We believe that this relationship—Activity: Time of day in which the drug is administered—is related both to the drug's mechanism of action and to the cells' diurnal metabolic cycle. As also seen in Table I, mitoses were inhibited in the groups of rats which received 20 μg of actinomycin D at 2300 hr while no inhibition was seen in the group of rats which received 10 μg of actinomycin at 2300 hr. Experiments in which the synthesis of both RNA and DNA are studied along with the scoring of mitosis will be necessary in order to understand better and possibly interpret the above results.

With the above results in hand, the effect of ARACY upon the circadian distribution of mitosis in the corneal epithelium was sought. The data obtained are presented in Table II. The rats that received injections at 1400 hr were sacrificed at 4-hr intervals from 2300 hr of the same day (group 1) of drug administration to 1500 hr of the following day (group 5). The rats injected at 2300 hr

TABLE I. Cells in Mitosis in the Cornea of Rats at 0700 hr of the Day.

Group		Drug administration		Mitosis/100 fields ± SE[a]
		Time (hr)	Dose	
I	Actinomycin D	2300	10 μg	238 ± 23 (5)[b]
II	Actinomycin D	2300	20 μg	179 ± 27 (5)
III	Actinomycin D	1400	10 μg	161 ± 19 (5)
IV	Actinomycin D	1400	20 μg	154 ± 10 (5)
V	5-Fluorouracil	2300	60 mg/kg	107 ± 20 (8)
VI	5-Fluorouracil	1400	60 mg/kg	195 ± 13 (8)
VII	Cytosine arabinoside	2300	8.5 mg/kg	155 ± 16 (7)
VIII	Cytosine arabinoside	1400	8.5 mg/kg	257 ± 11 (7)
IX	Control	—	—	239 ± 10 (20)

[a] SE, standard error of the mean value.

[b] Figures in parentheses represent the number of rats in the group.

The p values for the different experimental groups, when compared with the control group, were as follows: Group I $>.05$; Group II $<.05$; Group III $<.005$; Group IV $<.001$; Group V $<.001$; Group VI $<.02$; Group VII $<.001$, and Group VIII $>.05$.

TABLE II. The Effect of Cytosine Arabinoside upon the Circadian Distribution of Mitosis in the Cornea of Rats.

Groups	Mitoses/100 microscopic fields ± SE[a]				
	2300[b]	0300[b]	0700[b]	1100[b]	1500[b]
Control I	18.0 ± 5.5 (11)	63 ± 12.7 (11)	186 ± 11 (26)	106 ± 7.7 (16)	74 ± 15 (9)
ARACY[c]					
1400	25 ± 5.5 (5)	40 ± 10.5 (6)	198 ± 29 (6)	69 ± 9.1 (6)	57 ± 10.4 (5)
2300	—	22 ± 8.2 (5)	69 ± 13.6 (5)	129 ±13.3 (5)	59 ± 8.7 (7)

[a] SE, standard error of the mean values.

[b] Hour of the day at which the different groups of rats were sacrificed; figures in parentheses represent the number of rats sacrificed in each group.

[c] ARACY, Cytosine arabinoside injected at either 1400 or 2300 hr by the intraperitoneal route (0.3 ml) at the dose of 30 mg/kg of body weight.

were also sacrificed in several separated groups at 0300, 0700, 1100, and 1500 hr of the immediate day after drug administration. The control groups were obtained from several experiments, over a space of 4 months, under similar environmental conditions (light and temperature). The average weight of the control as well as the experimental rats was 135 g. The expected results were obtained as only the animals injected at 2300 hr of the day showed a significant alteration in the diurnal distribution of mitosis, when compared to normal untreated controls. Remarkable inhibition of mitosis at 0300 hr ($p<.02$) and at 0700 hr ($p<.001$) was seen in the group of rats treated with ARACY at 2300 hr when compared with the 0300 hr and 0700 hr control groups.

Even more encouraging from the overall therapeutic point of view were the results found with the group of rats treated with ARACY at 1400 hr of the day. In this group of rats, the effect of the antimetabolite was apparently lost in the naturally decreased period of diurnal mitotic activity.

The effects obtained with this particular drug administration schedule upon the corneal epithelium are not necessarily applicable to other area of the organism such as bone marrow and gastrointestinal epithelium. However, a significantly different pattern is not expected to be found.

Additional experiments will be carried out to determine the specific hour of the day in which the administration of ARACY, 5-FU, and actinomycin D will cause maximum mitotic inhibitory effect. In these experiments, the uptake and incorporation of radioactive precursors into either DNA or RNA of the corneal epithelium will also be studied.

In summary, the presented results indicate: (1) the possible usefulness of this method in the screening of potential cancer chemotherapeutic agents, and (2) the need for a greater care in the selection of the time of day in which the drug is administered as it might definitely determine increased or decreased toxicity and/or therapeutic effects.

Summary. Results are presented to indicate that the time of day in which the administration of cytosine Arabinoside, 5-fluorouracil, and actinomycin D is made, is critical insofar as their capacity to inhibit mitosis in the corneal epithelium of rats is concerned. The possible usefulness of these results in cancer chemotherapy is discussed.

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