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EFFECTS OF CYTOSINE ARABINOSIDE AND X-IRRADIATION ON THE CELL KINETICS OF DUODENAL AND MALIGNANT CELL POPULATIONS

by

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ABSTRACT

Determinations of the LI, MI, and DI were employed to ascertain the effect of x-irradiation on a normal cell population and on a malignant cell population. In addition, the morphology of the duodenum and of the B16 melanoma were examined (at the LM level) as were survival patterns of treated and untreated C57Bl/6J mice bearing the tumour. Synchrony was produced by the administration of 8 i.p. injections of 12.5 mg/kg cytosine arabinoside (ara-C) given at two-hour intervals. The ara-C-induced synchrony occurred earlier and decayed more rapidly in the tumour cell population than it did in the duodenal cell population. The regimen of ara-C administration did not exert any effect on the MST of experimental animals, but was lethal to 10% of the mice. Damage to the duodenal mucosa was severe four hours following the last injection, but recovery was almost complete by two days. Dosages of x-irradiation (500R, 1000R, 1500R, and 500R x 2) caused damage to the duodenal mucosa which, assessed on a morphological basis, was dose-dependent. Changes in the duodenal indices following x-irradiation were indicative of a general damage but did not reflect a dose-dependency. All doses of x-irradiation caused some retardation and/or regression of tumour growth, as evidenced by decreased labeling and mitotic indices and augmented degenerating index, however a dose-dependent effect was not apparent. The MST of irradiated mice appeared to be dose-dependent. Doses of 500R x 2, 1000R, and 1500R all increased the MST of mice bearing the tumour. A 1000R dose of x-irradiation was administered four, 14, 16, 18 or 20 hours following the

last injection of ara-C, when it was assumed that the cohort of tumour cells would be in the late G_1, early G_2, M, mid-G_1, and late G_1 phase of the cell cycle, respectively. The cohort of duodenal cells would likely be in late G_1 at four hours and at 14 hours and in S phase at 16, 18, and 20 hours. A 1000R dose of x-irradiation four hours following the last injection of ara-C was lethal to 75% of the animals, and a 500R dose at this time, though not nearly as lethal, was relatively ineffective in retarding tumour growth. A 1000R dose 14 hours following ara-C proved to be the most effective regimen in terms of retardation and/or regression of tumour growth, although 1000R at 16 hours or at 18 hours were also effective treatments. The degree of damage to the duodenal mucosa augmented as the time between ara-C administration and irradiation was shortened. Generally, reduction or fractionation of an irradiation dose caused less duodenal damage than the identical dose administered as a single exposure, but the therapeutic gain was offset by less retardation or regression of the tumour growth.

TABLE OF CONTENTS

Page

Page

LIST OF CHARTS

Chart

Page

LIST OF FIGURES

LIST OF TABLES

Table | Page

CHAPTER I

STATEMENT OF THE PROBLEM

Introduction

Melanoma, the malignant lesion composed of melanin-producing cells, is among the most unpredictable tumours in oncology (Allen and Spitz, 1953). This is because some are highly malignant, with very poor prognoses, while others, even though very similar histologically, may behave in a relatively benign fashion (Robbins, 1967).

As melanomas have been generally considered to be radioresistant (Desjardins, 1934; Pack, 1948; Philips, 1963), the treatment of choice is usually extensive surgical excision, combined with resection of the appropriate regional lymph nodes (Pack, 1948; McNeer and DasGupta, 1965). Despite the reluctance of many radiotherapists to irradiate pigmented tumours, several investigators reported remissions and even cures of human melanomas treated with irradiation alone or in combination with surgery (Jorgshol and Engdahl, 1955; Hellriegel, 1963; Weitzel, 1970; von Glasser, 1972).

Although various chemotherapeutic agents have been used clinically in conjunction with irradiation, the rationale in the administration of such combinations is often based solely on the status of the patient's recovery from side effects, rather than on the actual impact on the cell kinetics of the particular tumour. The growth fraction of a tumour represents a mixed population of cells, in regard to proliferation rate, and hence, the effects of irradiation may vary according to the position of a cell in the cell cycle at the time of administration. It is plausible

then, that the effect of irradiation on a tumour might be enhanced if the growth fraction of cells were more homogeneous with regard to the position in the cell cycle. If, for example, a drug could be used to synchronize the cells in a particular phase of the cell cycle, that cohort of cells could subsequently be irradiated while in the most radiosensitive phase. It would be expected that more cells would thereby be eradicated using synchrony and irradiation in combination than would be killed by mere irradiation alone of the (normally) heterogeneous asynchronous population of proliferating cells.

In any investigation dealing with the effects of therapy on malignant cell populations, the therapeutic ratio is of primary importance, that is, the effect of the treatment on the malignant cells compared to the effect of the treatment on normal proliferating cell populations. Indeed, the success or failure of any treatment schedule is dependent on the amount of damage sustained by normal tissues adjacent to the tumour, and the ability of those normal tissues to recover from any such injury.

Statement of the Problem

The main purpose of this investigation was to determine the effect of x-irradiation on the synchronized growth fraction of the B16 mouse melanoma. Subsidiary problems were as follows:

1. To determine the effect of cytosine arabinoside (ara-C) alone, x-irradiation alone, and ara-C in combination with x-irradiation on the cell kinetics of the duodenum of the C57Bl/6J mouse.

2. To determine the effect of ara-C alone, x-irradiation alone, and ara-C in combination with x-irradiation on the cell kinetics of the B16 malignant mouse melanoma.

3. To determine the effect of ara-C alone, x-irradiation alone, and ara-C in combination with x-irradiation on the survival rates of C57B1/6J mice bearing the B16 melanoma.

Definitions of Terms

Ara-C--as used in this study, is the abbreviation of 1-B-D-arabinofuranosylcytosine, or cytosine arabinoside.

Melanoma (pl. melanomas)--refers to the malignant lesion of melanin-producing cells, also melanocarcinoma or malignant melanoma (Robbins, 1967).

Synchrony--as used in this study, synchrony refers to the condition whereby a significantly greater than normal proportion of the growth fraction of cells of a population are in one particular phase of the cell cycle. A synchronization procedure is, therefore, any procedure which produces synchrony.

X-irradiation--as used in this study, refers only to that ionizing radiation emitted by an x-ray device.

CHAPTER II

REVIEW OF RELATED LITERATURE

INTRODUCTION

The Cell Cycle

The term cell cycle refers to the phases intervening between the completion of one mitosis of a cell until the completion of the subsequent mitosis of one or both daughter cells (Baserga, 1968). Howard and Pelc (1953) divided the cell cycle into four phases: M, G_1, G_2, and S. The latter three phases are referred to, collectively and morphologically, as interphase.

Mitosis approximates one hour with the majority of cells, and constitutes that part of the cell cycle when actual cell division occurs. Following its completion, the yield is two daughter cells, morphologically and genetically identical to one another and to the original parent cell. Cells in any one of the four stages of mitosis, namely prophase, metaphase, anaphase, and telophase, can be readily recognized at the light microscope level and are generally referred to as "mitotic figures" (MF's).

Between the termination of mitosis and the commencement of S-phase is the G_1 phase, or postmitotic gap. During that phase the cell is engaged in the synthesis of RNA and proteins (Baserga, 1968). The duration of the G_1 phase varies widely among different cell types. Indeed, the main variability in the length of a cell cycle is determined by the length of the G_1 phase (Mueller, 1969).

The S-phase (DNA synthesis), when DNA is replicated preparatory to division, follows G_1 and may last from 4 to 10 hours (Patt and Quastler,

1963). Thymidine, a natural precursor of thymine, is used by cells in the synthesis of DNA. In the complex process of autoradiography, thymidine, "labeled" with the radioactive isotope tritium, is administered. It can subsequently be visualized by reduced silver grains in the emulsion, overlying the nuclei of cells which were in S phase at the time the tritiated thmidine (H^3-TdR) was available. In this manner, labeled cells can be readily recognized at the light microscope level.

The G_2 phase (premitotic gap) usually lasts between one and two hours--from the termination of DNA synthesis to the morphologically discernible onset of mitosis (prophase). G_2 cells apparently synthesize RNA with the spindle fiber proteins requisite for cell division (Baserga, 1968).

In summary, of the four phases of the cell cycle, two phases, the M phase and S phase, are discernible at the light microscope level. Although nuclei of G_2 cells differ from those of G_1 inasmuch as they contain double the amount of DNA, G_2-phase cells cannot be distinguished morphologically from cells in G_1. Moreover, G_1 and G_2 cells cannot be morphologically discerned from cells which are are not in the proliferating cycle.

Cell Renewal Systems

A population of cells in which cells are lost continuously by exfoliation and replaced simultaneously by cells newly formed by mitosis is termed a cell renewal system (Leblond and Walker, 1956). This is in contrast to a system in which more cells are added by mitosis than are extruded or die. In the latter expanding cell populations, growth is the consequence. In a renewing cell population, a steady state is maintained, as cell production precisely balances cell loss.

Cell renewal systems have been divided into compartments, based on the morphology, function, or location of the constituent cells. Proliferation and functional activity of the cells are thereby separated. The proliferating compartment consists of those cells which pass through the cell cycle, and hence will eventually divide. It has also been designated the "growth fraction". The functional compartment comprises those cells which left the cell cycle, matured or differentiated, and are no longer capable of division. That compartment represents the "differentiating cell fraction". Not all cells remain in the proliferating compartment and divide indefinitely. As cells are lost, or extruded, from the functional compartment, new cells leave the proliferating compartment to differentiate and become functional cells. Such cells, in the process of differentiation, make up a third compartment--the maturation compartment (Patt and Quastler, 1963). It must be realized that, just as the entire population of cells in a cell renewal system is in a steady state, so are the cells within the individual compartments.

For example, the principal cells of the epithelium of the small intestine comprise a cell renewal system (Leblond and Stevens, 1948). The proliferating compartment is confined to the area at the bottom of the crypts. As the cells mature, they move upward along the walls of the crypts and become the functional cells by the time they have reached the upper parts.

Growing Proliferative Systems

Growing proliferative systems are defined as those cell communities in which growth occurs, that is systems in which more cells are added to the

population by mitotic division than are lost by death or exfoliation. A simple proliferative system is in an *in vitro* system composed of stem cells, or cells which do not differentiate but simply continue division. Initially, the growth of such a cell culture is logarithmic, as virtually all cells are proliferating and cell death is negligible. Eventually, however, such cell cultures attain a density where growth begins to slow and, finally, a "critical density" when growth ceases altogether (Clarkson and Fried, 1971).

Tumours also often comprise growing proliferative systems, although more complicated than the aforementioned *in vitro* system. In contrast to stem cells in culture, tumours are composed of mixed cell fractions, comparable to population compartments of the cell renewal system described previously. A proportion of tumour cells do divide, these comprising the proliferating or growth fraction of the tumour. The other nonproliferating fraction is composed of cells which rarely, if ever, divide, and of cells which no longer divide because they are either degenerating or are already dead. A nonproliferating fraction may also contain G_0 type cells; that is, cells which do not normally divide but which may be stimulated by suitable means to do so under specific circumstances. The growth characteristics of any particular tumour will vary according to the proportion of cells in these compartments. As these proportions may become modified during the course of growth of a tumour, so will the growth characteristics of the tumour.

In vivo growth curves, analagous to growth curves of *in vitro* systems, have been developed for various *in situ* tumours. Such curves are most often based on measures of tumour volume or weight relative to

time. The growth curves of most tumour cell populations resemble the growth curves of cells growing in culture, that is, an initial rapid growth rate followed by its gradual deceleration (Laird, 1964; McCredie et al, 1965). Several factors were postulated as being responsible for that pattern, either wholly, or in part, especially for the decline of growth rates in cell cultures. Clarkson and Fried (1971) suggested that a lengthening of the mitotic cycle (especially the G_1 phase), augmented death rate, or failure of daughter cells to re-enter the cycle following completion of mitosis could all contribute to the stationary phase of the growth curve. Similar factors might also be responsible for the retardation in the in vivo growth rate of in situ tumours (Dethlefsen et al, 1968).

THE DUODENUM

Introduction

The small intestine has been the object of a great many investigations, at the light microscope level (Leblond and Stevens, 1948; Thrasher and Greulich, 1966; Merzel and Leblond, 1969), at the electron microscope level (Toner, 1968; Staehlin, 1972), and in terms of cell kinetics (Leblond and Stevens, 1948; Bertalanffy, 1960). Inasmuch as morphological and cell kinetic differences occur in the 3 segments of the small intestine, as well as species variations, this review is confined to a description of the characteristics of the mouse duodenum.

Morphology of the Duodenum (Figure 1)

The duodenum, the most proximal part of the small intestine, pursues a horseshoe-shaped course around the head of the pancreas. It extends from the pyloric sphincter to the jejunum, a distance of 5 to 10mm in the mouse (Montagna and Wilson, 1955). Just beyond the pyloric sphincter, the mucous membrane is thrown into large folds, the folds of Kerckring.

The wall of the duodenum is comprised of four layers, the mucosa, lining the lumen, submucosa, muscularis externa, and the serosa. The mucous membrane, the innermost layer of the small intestine, consists of three components: the epithelial lining, the lamina propria, and the muscularis mucosae. The epithelium is simple columnar and supported by a reticulin basement membrane. Beneath lies the lamina propria, a layer of loose connective tissue, where lymphocytic elements abound. The lamina propria of the C57Bl mouse is devoid of lymphatic nodules (Montagna and Wilson, 1955). Reticular, collagenous and elastic connective tissue fibres are present in this layer, as well as thin bundles of smooth muscle. Blood and lymphatic capillaries (lacteals) are numerous.

The deep layer of the mucosa, the muscularis mucosae, generally consists of two ill-defined and interwoven layers of smooth muscle, the inner layer being circularly disposed, the outer longitudinally. Some elastic fibres may be present in this layer.

The submucosa intervenes between the mucous membrane and the muscularis externa. It is predominantly a loose connective tissue layer containing blood vessels larger than those of the mucosa, and a