Repair and fixation of potentially lethal damage (PLD) as demonstrated by delayed plating or incubation with araA in contact inhibited refed plateau-phase C3H mouse embryo 10 T₁/₂ cells grown in the presence of BrdUrd

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Summary. C3H mouse 10 T₁/₂ cells showing strong inhibition of growth at confluency were grown under daily refeeding in the presence of BrdUrd (from 0 to 1 μM) and exposed to γ-rays either while exponentially growing or in the plateau phase. An increase in radiosensitivity was observed in both growth conditions mainly reflected by a reduction in Dq. Greater radiosensitization was observed in exponentially growing than in plateau-phase cells, and 3–4 times higher BrdUrd concentrations were required in plateau-phase cells for similar potentiation in killing. This effect could not be entirely attributed to a reduction in BrdUrd incorporation since measurements with ³H-BrdUrd showed reductions in incorporation between only 17–47% in plateau-phase cells. The rate of repair of potentially lethal damage (PLD) as demonstrated by delayed plating was not affected by the incorporation of BrdUrd, but the amount of repair (measured as the relative increase in cell survival) was higher for BrdUrd-containing cells. Post-irradiation treatment of cells in the plateau-phase (no BrdUrd) with 9-β-D-arabinofuranosyladenine (araA) caused fixation of radiation-induced PLD. AraA treatment of cells grown in the presence of various amounts of BrdUrd also caused fixation of PLD, but resulted in survival levels similar to those observed with cells growing in BrdUrd-free medium. This result indicates that BrdUrd mediated radiosensitization cannot be observed when cells are prevented from repairing PLD by postirradiation incubation with araA. Based on these findings we propose that the mechanism of radiosensitization by BrdUrd incorporation might be, by increasing probability of fixation,

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mediated by the postirradiation progression of cells through the cycle, of a sector of PLD also sensitive to post-irradiation treatment with araA. For this sector of PLD the term α-PLD has been proposed.

Introduction

Partial replacement of thymidine by BrdUrd in the DNA of mammalian cells confers an increase in sensitivity to a subsequent exposure to low LET ionizing radiations (Szybalski 1961; Erikson and Szybalski 1961; Delihas et al. 1962; Dewey and Humphrey 1965; Dewey et al. 1974). The degree of radiosensitization increases with increasing thymidine replacement (Dewey and Humphrey 1965; Mohler and Elkind 1963) which is higher in the presence of FdUrd (Cohen et al. 1958; Harbers et al. 1959). It is reflected by alterations either in the survival curve slope and/or in the shoulder width (Szybalski 1961; Erikson and Szybalski 1961; Delihas et al. 1962), and is regarded as indicating DNA as the target for radiation killing (Szybalski 1961; Erikson and Szybalski 1961). The mechanism of cell sensitization to radiation by BrdUrd is not exactly known. However, based on the differences observed in the physical properties of BrdUrd-containing DNA (Szybalski 1961) an effect in the induction of radiation lesions has been proposed (Shipley et al. 1971; Lehman et al. 1972). Alternatively incorporation of BrdUrd has also been assumed to affect the cellular repair system (Lett et al. 1964).

Halogenated pyrimidines have been considered as radiosensitizers in the treatment of human tumors (Bagshaw et al. 1967; Hoshino and Sano 1969; Szybalski 1974; Goffinet and Bagshaw 1974; Kinsella et al. 1984) and encouraging results recently obtained (Kinsella et al. 1984; Kinsella et al. 1985). Although a significant amount of data has been accumulated on the BrdUrd mediated radiosensitization of actively proliferating cells no information is available on the radiation sensitivity of BrdUrd containing non-growing (plateau-phase) populations. However, there is evidence that a large fraction of cells comprising a tumor rest in a non-growing state, and transitions of cells from a growing to a non-growing state (Mendelsohn 1965) are possible during treatment. Furthermore, relatively little is known about the possible modification BrdUrd incorporation may have on cellular repair reactions. Although split-dose recovery was not significantly affected by BrdUrd incorporation (Shipley et al. 1971; Kim et al. 1964; Fox 1968), the effect of BrdUrd on the ability of cells to repair potentially lethal damage (PLD) has not been studied, despite the possible importance of this type of repair in radiation therapy.

In this paper, we report results of experiments conducted to study the radiosensitivity of cells grown in the presence of BrdUrd, and irradiated either while still logarithmically growing or after reaching a plateau-phase. C3H mouse embryo 10 T1/2 cells were used (Rezninoft et al. 1973) showing strong contact inhibition of growth. The ability of BrdUrd-containing cells to repair PLD in the plateau phase was studied by using two different experimental approaches. First, cells were kept in the plateau-phase of growth for few hours after irradiation and before plating to measure surviv-