

VIII- II -3. Original Research

OR1 Effect of Tirapazamine and Mild Temperature Hyperthermia on the Recovery from Radiation-Induced Damage in Pimonidazole-Unlabeled Quiescent Tumor Cells

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BACKGROUNDS AND PURPOSES: Human solid tumors are thought to contain moderately large fractions of quiescent (Q) tumor cells, which are not involved in the cell cycle and have stopped dividing, but which are as viable as established experimental animal tumor lines. The presence of Q cells is probably due, at least in part, to hypoxia and the depletion of nutrition in the tumor core as a consequence of poor vascular supply. As a result, with the exception of non-viable Q cells at the very edge of the necrotic rim where there is diffusion-limited hypoxia, Q cells are viable and clonogenic but have ceased dividing.

A few years ago, the universal detection of hypoxic cells in both tissues and cell cultures became possible using pimonidazole, a substituted 2-nitroimidazole, and a mouse IgG1 monoclonal antibody (MAb1) to stable covalent adducts formed through reductive activation of pimonidazole in hypoxic cells. Here, we tried to selectively detect the response of the pimonidazole-unlabeled and probably oxygenated cell fraction of the Q cell population. To achieve this we combined our method for selectively detecting the response of Q cells in solid tumors with the method for detecting cell and tissue hypoxia using pimonidazole and MAb1 to pimonidazole.

The development of bioreductive agents that are particularly toxic to hypoxic cells is considered a promising approach to solving the problem of radio-resistant tumor hypoxia in cancer radiotherapy. Tirapazamine (TPZ), a lead compound in the development of bioreductive hypoxic cytotoxins, in combination with radiation has been shown to be very useful for controlling solid tumors as a whole, especially for controlling Q tumor cell populations that are rich in hypoxic region. Mild temperature hyperthermia (MTH) was reported to increase the response of tumors to radiation by improving oxygenation through an increase in tumor blood flow. Further, MTH was also shown to enhance the tumor response, especially of the intratumor Q cell population, to TPZ.

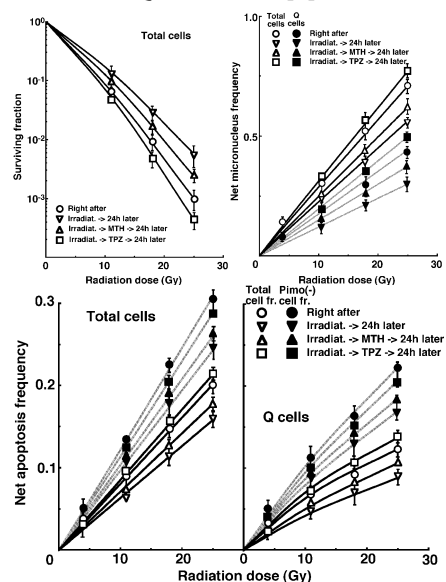
In the present study, the radio-sensitivity of the pimonidazole-unlabeled cell fraction of the Q cell population after cobalt-60 γ -ray irradiation followed with or without post-irradiation TPZ administration or MTH, was determined, to examine the effect of TPZ and MTH on the repair of radiation-induced damage in pimonidazole-unlabeled Q tumor cell population.

MATERIALS AND METHODS: Labeling of proliferating (P) cells in C57BL/6J mice bearing EL4 tumors was achieved by continuous administration of 5-bromo-2-deoxyuridine (BrdU). Tumors were irradiated with γ -rays at 1 h after the administration of pimonidazole followed by TPZ treatment or MTH. Twenty-four

hours later, assessment of the responses of Q and total (= P + Q) cells were based on the frequencies of micronucleation and apoptosis using immunofluorescence staining for BrdU. The response of the pimonidazole-unlabeled tumor cell fractions was assessed by means of apoptosis frequency using immunofluorescence staining for pimonidazole.

RESULTS: With γ -rays only, the pimonidazole-unlabeled cell fraction showed significantly enhanced radio-sensitivity compared with the whole cell fraction more remarkably in Q cells than total cells. However, a significantly greater decrease in radio-sensitivity in the pimonidazole-unlabeled than the whole cell fraction, evaluated using a delayed assay, was more clearly observed in Q cells than total cells. Post-irradiation MTH more remarkably repressed the decrease in radio-sensitivity in the Q cell than the total cells. Post-irradiation TPZ administration produced a large radio-sensitizing effect on both total and Q cells, especially on Q cells. On the other hand, in pimonidazole-unlabeled cell fractions in both total and Q cells, TPZ suppressed the reduction in sensitivity due to delayed assay much more efficiently than MTH, whereas no radio-sensitizing effect was produced.

CONCLUSION: Not only through suppressing the recovery from radiation-induced damage but also through radio-sensitizing effect, post-irradiation TPZ administration is very useful for repressing the increase in the difference in radio-sensitivity due to the delayed assay not only between total and Q tumor cells but also between the pimonidazole-unlabeled and the whole cell fractions within the total and Q tumor cells [1].



REFERENCES:

[1] S. Masunaga *et al.*, *J. Cancer Ther.*, **4** (2013) 521-528.

ピモニダゾール非標識酸素化休止期腫瘍細胞分画における放射線損傷からの回復に対するティラパザミン処置と低温度温熱処置の影響

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