Sensitization to Heat by Amiloride Analogue in Chinese Hamster Cells

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Abstract—Effects of five amiloride analogues on thermal cell killing were examined using Chinese hamster V-79 cells. When cells were exposed to 42°C hyperthermia in the presence of 0.2 mM amiloride or its analogue, cell survival decreased with increasing exposure time, as compared with that for exposure to 42°C alone. The degree of the thermosensitizing effect varied among the test compounds, and three analogues were found to be more potent than amiloride. The results suggest that some of the amiloride analogues may be useful as hyperthermic sensitizers in the clinical treatment of cancer.

Hyperthermia has been actively investigated as a new modality of cancer treatment. In in vitro studies, the thermal cell killing is reported to be enhanced by lowering the pH of the medium (1-3). The intracellular pH of mammalian cells is regulated mainly by the Na⁺/H⁺ exchange system in plasma membranes (4, 5), and a diuretic drug, amiloride, is known to inhibit this system (6-8). We previously reported the thermosensitizing effect of amiloride on cultured Chinese hamster V-79 cells (9), whose thermosensitivity is similar to those of human HeLa-S3 and murine Ehrlich ascites tumor cells (10, 11). In the present study, the effects of five amiloride analogues on thermal cell killing were examined using the same cell line.

Details of the culture conditions have been described elsewhere (9, 12). A water bath was used for hyperthermic treatment, and the temperature was maintained at 42±0.05°C. Amiloride and five of its analogues (compounds A to E) were synthesized by previously described methods (13). The structural formulas of these six drugs are shown in Table 1. The drugs were dissolved in dimethyl sulfoxide, treated with Dulbecco’s phosphate-buffered saline, and then filtered immediately before the experiments. The solutions were then diluted 1:10 and added to the culture medium to give a final concentration of 0.2 mM. Heating procedures were previously described (9). In brief, exponentially growing cells in Pyrex glass flasks were heated in the presence or absence of the drugs by immersing the flasks in a water bath. After the treatments, cells were trypsinized to obtain a single cell suspension, and then the cell number was determined using a Coulter Counter (Coulter Electronics Inc., model ZBI). After the serial dilution of the cells, 1 ml from each cell suspension was seeded in Falcon plastic plates containing fresh medium. In all experiments, the pH of the culture medium was kept at approximately 7.3. Colony forming ability of cells after the treatment was used as the survival criterion. The replicate experiments were repeated two or three times. The data points represent the mean of at least 12 plates, and the standard deviation is given for each point.

Cells were exposed to 42°C hyperthermia for up to 5 hr in the presence or absence of amiloride and five of its analogues. Treatment of the cells with these drugs at 37°C for 5 hr had no effect on their survival (data not shown). However, the hyperthermic treatment in the presence of amiloride or its analogue...
decreased cell survival with increasing exposure time more sharply than that with hyperthermia alone (Fig. 1). The order of sensitizing potency was E > D > C > amiloride > B > A.

The thermosensitizing activity appeared to increase, compared with that of amiloride, by introduction of alkyl substituents on the 5-aminonitrogen atom (R₂) such as tert-butylamino (E). In contrast, the activity decreased when the terminal guanido nitrogen atoms were substituted by methyl groups (A) or when the 5- and 6-position bore hydrogen atoms (B).

Compounds A, B, C and D have less Na⁺/H⁺ exchange-inhibiting activities than amiloride. The Kᵢ values in the Na⁺/H⁺ exchange inhibition, as estimated by the inhibition of FMLP (N-formyl-methionyl-leucyl-phenylalanine)-activated ²²Na⁺ efflux are 83.8 μM for amiloride (14), 938 μM for B, and over 1 mM for C and D, respectively (E. J. Cragoe, Jr., unpublished observations). On the other hand, compound E is a much more potent inhibitor of Na⁺/H⁺ exchange than amiloride. The Kᵢ for amiloride in Na⁺/H⁺ inhibition in neutrophils is 83.8 μM (14), 4 μM in A431 cells (15) and 3.5 μM in the pig kidney cells LLC-PK₁ (16). The corresponding values for compound E are 3.2, 0.12 and 0.06 μM (E. J. Cragoe, Jr., unpublished observations) Thus, the most potent compound in this study (E) is the one possessing the greatest Na⁺/H⁺ exchange inhibiting activity.

![Fig. 1. Time-survival curves of cells exposed to 42°C in the presence or absence of amiloride and its analogues (compounds A, B, C, D and E). The final concentration of the drugs was 0.2 mM. After the treatments, the cells were incubated in normal growth medium for 6 to 8 days to obtain macroscopic colonies composed of 50 cells or more. The cell survival fraction was estimated as the ratio of the number of colonies formed and the number of cells inoculated. The estimated fractions were normalized to the surviving fraction of the control (no heat). The data points represent the mean of at least 12 plates, and the standard deviation is given for each point.](image-url)
The present findings demonstrated that some of the amiloride analogues more potently sensitize cells to heat than the parent compound. Such thermosensitizers with greater Na\(^+\)/H\(^+\) exchange-inhibiting activity and with little or no diuretic activity as compared to amiloride may be useful in the clinical treatment of cancer.

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