Histol Histopath (1986) 1: 235-239

Histology and Histopathology

Observations on cell kinetics and viability of a human melanoma cell line exposed to dicarboxylic acids in tissue culture

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Summary. Cultures of human melanoma cell line B0008 were exposed to the disodium salts of azelaic acid (C_9,2Na), adipic acid (C_6, 2Na) and dodecanediaic acid (C_{12} 2Na) at 10^{-2}M and 5 x 10^{-3}M for 24 hrs. None of the diacid salts had a significant effect on growth rate or viability of the cells, at 10^{-4}M for 24 hrs nor had C_6 2Na any effect at 5 x 10^{-2}M. At 5 x 10^{-2}M for 24 hrs, both C_9 2Na, and C_{12} 2Na had a significant effect in reducing both growth and viability. These effects were accompanied by morphological evidence of cell death, and swelling of mitochondria and accumulation of lipid droplets within cytoplasm of still viable cells.

Key words: Melanoma - Tissue culture - Dicarboxylic acids

Introduction

Clinically, dicarboxylic acids have a cytotoxic effect on the abnormally hyperactive and malignant epidermal melanocyte (Nazzaro-Porro et al., 1979, 1980; Breathnach et al., 1984; Leibl et al., 1985) and in tissue culture, the C_9 and C_{12} diacids have been shown to affect viability and proliferation of murine melanoma cells at concentrations of and greater than 10^{-2}M. This effect is due primarily to inhibition of mitochondrial oxido-reductases (Passi et al., 1984) and of nuclear DNA synthesis (Leibl et al., 1985), and is accompanied by ultrastructural damage to mitochondria (Robins et al., 1985b; Hu et al., 1986). Here, the effect of dicarboxylic acids on the growth kinetics and ultrastructure of a human melanoma cell line has been investigated. In a previous report, (Robins et al., 1985a), a comparison between the effects of dicarboxylic acids on the ultrastructural morphology of pure cultures of "normal" human melanocytes, and cultures of the present melanoma cell line was made. Only attached, viable cells were examined, and no cell counts were made. In this study, total and differential cell counts were made on the melanoma cultures, and both attached and detached cells were examined by electron microscopy.

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Materials and methods

Cell Cultures

Cultures of B0008 human melanoma (Professor R. Mackie, Glasgow) were grown in Eagles MEM supplemented with 10% fetal calf serum, 2 m M glutamine, 100 IU/ml penicillin, and 100 mg/ml Streptomycin, 0.25 mg/ml Fungizone, in plastic petri dishes (diameter 3.5 cm). The cultures were maintained at 37" C in a humidified atmosphere of 5% CO_2 95% air at pH 7.3.-7.4.

Dicarboxylic Acids

Dicarboxylic acids used were dodecanedioic acid (C_{12}), azelaic acid (C_9) and adipic acid (C_6). The sodium salts of the diacids were prepared as previously described (Robins et al., 1985b) and added to cultures in medium to give final concentrations of 5 x 10^{-2}M and IO'M. The pH of media with added diacids was 7.2-7.4.

Cell Counts

Cells were grown in 5ml wells and inoculated with sufficient cells to produce a count of 4-6 x 10^4 cells/ml at the following day (experimental day 0). This figure is

referred to as the initial count, and reproducability of count was checked at its stage by counting in triplicate 3 randomly selected wells. Two runs of the following procedures were carried out. Diacid salts in culture media were added to the cultures on day 0, and after 24 hours exposure, cells were harvested. Detached dead cells were spun from the supernatant and pooled with live attached cells which had been removed with trypsin as previously described (Robins et al., 1985b), and these were counted using a haemocytometer. Total cell count and counts of dead cells as judged by their uptake of Trypan Blue, were carried out, and the data was analysed by Students t-test. Histograms of growth rate and viability were prepared.

Morphology

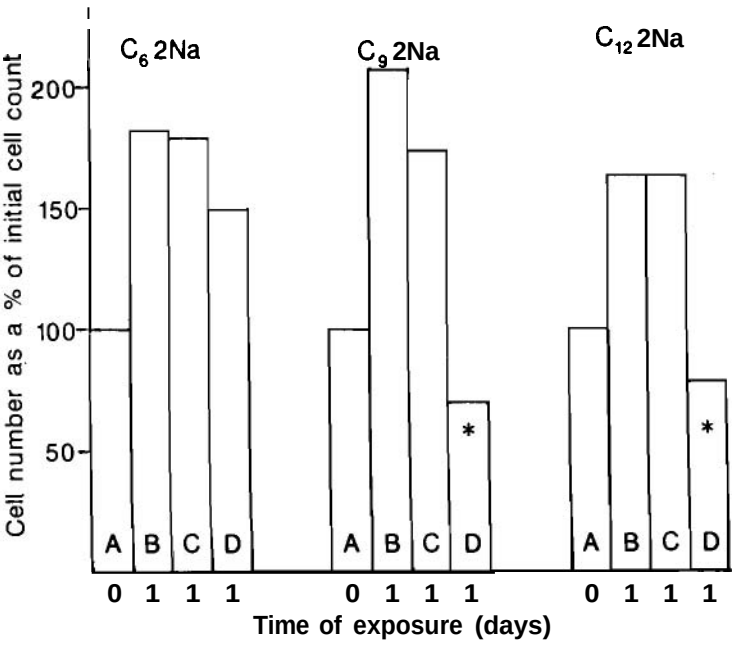
All cells both attached and detached, control and experimental were fixed in glutaraldehyde (2.5%), postosmicated, spun down into a pellet and further routinely processed for electron microscopy.

Results

Cell Counts (Fig.1)

At 10^{-2}M for 24 hours, none of the diacid salts had a significant effect on the growth rate of melanoma cells. At 5×10^{-2}M, $C_6$2Na, likewise had no significant effect. However, at this concentration, both C_9 2Na and C_{12} 2Na reduced growth significantly. As can be seen from the histograms, with both C_9 2Na and C_{12} 2Na at 5×10^{-2}M for 24 hours, there was an overall reduction in cell numbers (20-30%) compared with the initial cell count on day 0. This reduction is a measure of death and disintegration of cells, as confirmed by presence of debris and fragments in material examined by electron microscopy (Figs. 2-7). As compared with the 24 hours control, and C_6 2Na at 5×10^{-2}M there was a significant reduction in growth rate with C_{12} 2Na and C_{12} 2Na at 5×10^{-2}M.

Fig. 1. Histograms to show the effect of C_6 2Na, C_9 2Na and C_{12} 2Na at concentrations of 10^{-2}M and 5×10^{-2}M for 1 day on the mean total growth of a human melanoma line (B0008) expressed as a percentage of the initial cell count on day 0. A: initial cell count on day 0 (100%). B: control cell count after 1 day. C: cell count after exposure to 10^{-2}M diacids. D: cell count after exposure to 5×10^{-2}M diacids. *p = < .001



Viability (Table 1)

At 10^{-2}M for 24 hours, C_{12} significantly reduced viability compared to control, but C_9 2Na at the same concentration and exposure time had no significant effect. At 5×10^{-2}M for 24 hours, both C_9 2Na and C_{12} 2Na produced a significant reduction in viability. With C_{12} 2Na, for both concentrations of the diacid, no significant effect on viability was observed.

Table 1. Viability (%) of human melanoma cells in culture exposed to C_6 2Na, C_9 2Na, and C_{12} 2Na at concentrations of 10^{-2}M and 5×10^{-2}M for 24 hours. *(p = < .001).

DIACID	CONTROL	10^{-2}M	5×10^{-2}M
C_6 2Na	89.2 + 3.2	90.6 + 3.2	84.4 + 4,8
C_9 2Na	95.2 + 2.6	95.4 + 5.2	*68.1 + 11.1
C_{12} 2Na	90.0 + 3.2	*85.5 + 5.5	*75.6 + 3.1

Ultrastructure

Typical cells of control cultures are illustrated in Figs. 2 and 5; ocasional pyknotic cells were seen. Cells of cultures exposed to the diacids at 10^{-2}M for 24 hours were indistinguishable from those of controls. Cells exposed to C_{12} 2Na for 24 hours at 5×10^{-2}M (Figs. 3, 6) were likewise essentially similar to controls. Exposure to C_9 2Na and C_{12} 2Na at 5×10^{-2}M for 24 hours had a definite effect. Cells of these cultures, as compared with controls, exhibited numerous lipid droplets in the cytoplasm, and the mitochondria were evidently swollen, though not vacuolated (Fig. 7). Many more pyknotic cells were seen than in the control cultures or in those exposed to C_6 2Na at 5×10^{-2}M, and debris and cell fragments were prominent (Fig.4).

Fig. 2. Typical field of control human melanoma (B0008) cells in culture. Compare with Figures 3 and 4 x 2,400

Fig. 4. Human melanoma (B0008) cells exposed to C_9 2Na at 5×10^{-3}M for 24 h in culture. Pyknotic and degenerate cells and cellular debris were a feature, as also with cultures exposed to C_{12} 2Na. Compare with Figures 2 and 3. x 2,400

Fig. 3. Human melanoma (B0008) cells exposed to C_6 2Na at 5×10^{-3}M for 24 h in culture. Essentially similar to control (Figure 1). x 2,400

Fig. 5. Cell of control human melanoma (B0008) culture. Compare with Figures 6 and 7. x 4,950

Fig. 6: Cell of human melanoma (B0008) culture exposed to C_6 2Na at 5 x 10^{-2}M for 24 h. General appearance and mitochondrial size essentially similar to control. (Figure 5). x 4,950

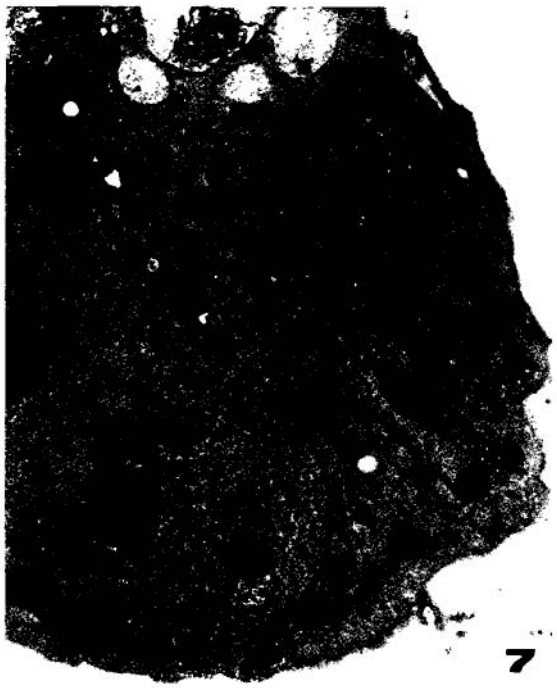


Fig. 7. Cell of human melanoma (B0008) culture exposed to C_9 2Na at 5 x10^{-2}M for 24 h. Note, in comparison with Figures 5 and 6 taken at same magnification, evident swelling of mitochondria, and lipid droplets in the cytoplasm. x 4,950

Discussion

This study has shown that the sodium salts of azelaic acid (C, 2Na) and dodecanedioic acid (C_{12} 2Na) have a significant effect upon proliferation and viability of human melanoma cells at a concentration of 5 x 10^{-2}M for 24 hours; C_{12} 2Na had a significant effect on viability at I x 10^{-2}M. That these effects are specific for the two diacids is shown by the fact that another dicarboxylic acid, adipic acid (C, 2Na) had no significant effect on either proliferation or viability at the same concentrations. Swelling of mitochondria and an increase in the number of cytoplasmic lipid droplets of viable cells was observed with C_9 2Na and C_{12} 2Na, but not with C_6 2Na. These results confirm previous observations on the effect of dicarboxylic acids on cell kinetics and morphology of murine melanoma cells in culture (Robins et al., 1985b; Leibl et al., 1985; Hu et al., 1986). The morphological effect on mitochondria was very much less than that observed in a previous experiment (Robins et al., 1985a) when cells of the same human melanoma line were exposed to the diacids at a concentration of 10^{-1}M for 1 to 6 hours, clearly indicating that these changes are dose-dependent. The fact that Hu et al. (1986) found equally massive swelling and vacuolation of mitochondria of B 16 mouse melanoma cells exposed for 6 days to 5 x 10^{-4}M and I x 10^{-3}M azelaic acid also indicates that they are time dependent. In the previous study, (Robins et al., 1985a) vesiculation of Golgi cisternae was observed at 10^{-1}M for one hour but this was not a feature of the present cells exposed to 5 x 10^{-2}M for 24 hours. Taking morphologic and cell kinetic observations together, the present observations confirm previous conclusions (Passi et al., 1984; Robins et al., 1985a, b) that the mitochondrion is a prime target for the biological effect of dicarboxylic acids.

Acknowledgements. This work was supported by a grant from Schering A G, Berlin, and by grant, No 850227044, project Oncologia, C.N.R., Italy. Electron microscopes supplied by the Wellcome Trust.

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Accepted May 6, 1986