

Human Cell Cultures as an Assay for the Toxicity of Antioxidants

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Butylated hydroxyanisole(BHA), butylated hydroxytoluene (BHT), propyl gallate and dl- α -tocopheryl are used as lipid-soluble antioxidants of food additives. Metcalfe(1971), Milner(1967) reported the effects of BHT on monkey kidney cells, however, the toxicity of various antioxidants apparently has not been determined in cultured human cells.

We now report the toxicity of these antioxidants using KB cells of human epidermoid carcinoma.

MATERIALS AND METHODS

The cells used throughout this work were the KB cells of human epidermoid carcinoma(Mochida et al. 1983). Toxicity test methods used were the same as described previously(Mochida et al. 1983). Propyl gallate, butylated hydroxyanisole, butylated hydroxytoluene and dl- α -tocopheryl were obtained from Wako Pure Chemical. Ind., Ltd., Osaka, Japan. The ID₅₀ values(50% inhibitory dose to growth of cells) was used as an index of the toxicity of the antioxidants.

RESULTS AND DISCUSSION

Dose-response curves obtained with BHA, BHT, propyl gallate and dl- α -tocopheryl for KB cells are shown in Figure 1. The KB cell number showed an inhibition proportional to the concentration of the antioxidant used. Cells treated for 72 h showed a greater inhibition than those treated for 24 h or 48 h.

The ID₅₀ values for the antioxidants are presented in Table 1. The ID₅₀ values of BHA, BHT, propyl gallate and dl- α -tocopheryl were 12.5 μ g/ml, 43.0 μ g/ml, 1.3 μ g/ml and 110.0 μ g/ml, respectively, suggesting the differential toxicity of these antioxidants to KB cells.

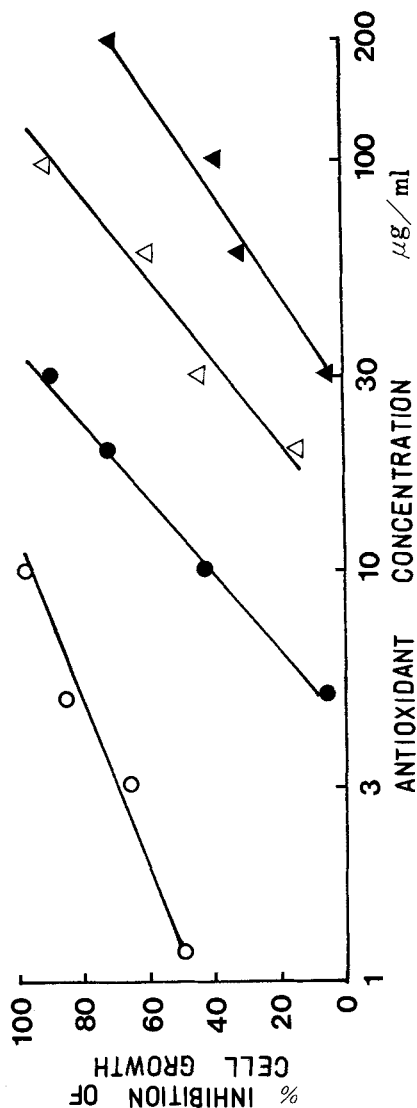


Figure 1. Dose-response curves obtained after 72 h exposure of KB cells in culture to various concentrations of antioxidants. The compounds were propyl gallate(O), butylated hydroxyanisole(●), butylated hydroxytoluene(Δ) and dl-α-tocopheryl(▲).

Table 1. Inhibitory effects of antioxidants on growth of KB cells.

compounds	ID50 (μg/ml) ¹
butylated hydroxyanisole	12.5
butylated hydroxytoluene	43.0
propyl gallate	1.3
dl-α-tocopheryl	110.0

¹ Concentration of antioxidants in growth medium that caused a 50% reduction in viable cell number after 72 h of incubation.

Of the four antioxidants, propyl gallate was the most toxic followed by BHA, BHT and dl- α -tocopheryl. The 50% inhibition concentration of KB cells growth was no remarkable difference from the results of Metcalfe (1971) and Milner(1967) who exposed monkey kidney cells to BHT. These data should serve as a basis for development of toxicity tests using cell culture systems.

REFERENCES

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