Vanillin induces apoptosis in floating colorectal cancer cells under a metastasis-associated condition

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INTRODUCTION

Drug resistance is considered as a major cause of treatment failure in 90% of cancer patients with metastatic stage [1]. Metastasizing cancer cells can be studied in vitro by means of non-adherent cell culture. Our previous study revealed that SW480 human colorectal cancer cells cultivated under non-adherent culture condition naturally developed drug resistance. Finding new compounds that are safe and effectively kill cancer cells, especially metastasizing cancer cells, is an important research topic nowadays. Vanillin (Fig.1) is indicated as safe and used as flavoring agent in a variety of food products. Moreover, vanillin has been reported for its potent antioxidant, anti-inflammatory, and anticancer activities [2].

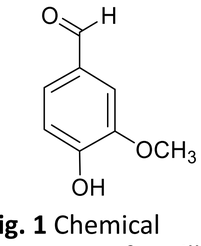

Fig. 1 Chemical structures of vanillin

OBJECTIVE

To investigate the cytotoxicity of vanillin on SW480 colorectal cancer cell line.

MATERIALS and METHODS

SW480 colorectal cancer cells were seeded in normal plates for adherent condition or poly(2-hydroxyethyl methacrylate)-coated plates for non-adherent condition (Fig.2) . Cells in both culture conditions were studied:

-Cell viability was determined by modified MTT assay [3] after 72 treatment with test compounds.

-Apoptosis was determined by Muse Annexin V & Dead Cell after treatment with vanillin for 72 h.

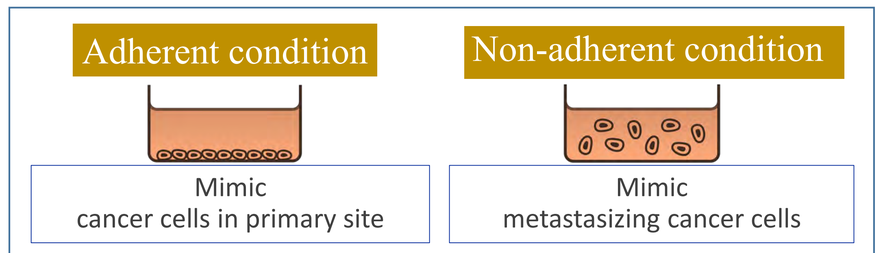

Fig. 2 Cell culture conditions

RESULTS and DISCUSSION

1.Differential cytotoxicity of test compounds between attached and floating cancer cells

To study the response to anticancer drugs and vanillin, attached and floating cells are treated for 72 h before MTT assay. MTT results indicated that floating cells acquired multidrug resistant property as seen from the resistance index > 1.0. However floating cells were more sensitive to vanillin because of sensitivity index > 1.0 (Table 1).

Table 1 IC_{50} values of anticancer drugs and vanillin on attached and floating cells.

Compound (concentration unit)	IC_{50} [a]		Resistance index [b]	Sensitivity Index [c]
	Attached cells	Floating cells		
5-fluorouracil (μM)	9.1 ± 0.3	19.6 ± 7.2	2.2*	-
Oxaliplatin (μM)	2.8 ± 0.2	4.3 ± 0.2	1.5*	-
Paclitaxel (nM)	69.2 ± 8.4	83.5 ± 2.1	1.2	-
Vanillin (mM)	4.7 ± 1.0	2.7 ± 0.4	0.6**	1.7**

[a] Average ± SD from three independent experiments. [b] Resistance index = IC_{50} value of floating cells / IC_{50} value of attached cells. [C] Sensitivity Index = IC_{50} value of attached cells/ IC_{50} value of floating cells
*P < 0.05 and **P < 0.01, attached vs. floating cells.

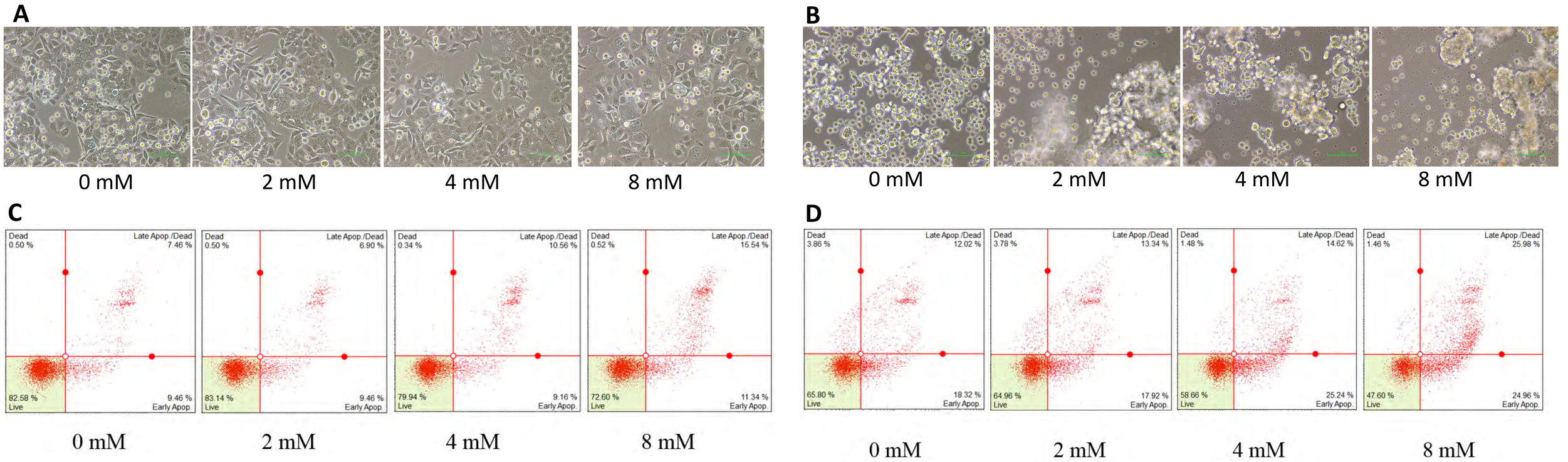

Fig. 3 Apoptosis-inducing effect of vanillin on attached and floating cells. Representative photographs of attached cells (A) and floating cells (B), magnification: 200×. Representative scatter plots of apoptosis analysis of attached cells (C) and floating cells (D). The percentage of cells undergoing apoptotic cell death of attached cells and floating cells (E) are the mean ± SD from three separate experiments. Significant differences are shown by * ($p<0.05$) and *** ($p<0.001$).

2.Apoptosis-inducing effect of vanillin on attached and floating cells

To investigate mode of cell death induced by vanillin, after 72h treatment with vanillin cells from both culture conditions were studied apoptotic rate by Muse apoptosis. Compared with attached cells, floating cells were more sensitive to apoptosis inducing effect of vanillin (Fig.3).

CONCLUSION

In conclusion, SW480 floating cells under non-adherent conditions were more susceptible to cytotoxicity of vanillin. Our results suggest that vanillin might be an effective anticancer agent for metastatic colorectal cancer treatment.

ACKNOWLEDGEMENTS

This research was supported by the Thailand Science Research and Innovation, the Chulabhorn Research Institute (grant no. 302/2205), and the Center of Excellence on Environmental Health and Toxicology (EHT).

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