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Short communication

Fluorous solvent for cell culture

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ABSTRACT

Incubation of mouse melanoma B16 cells in fluorous solvents with low boiling point such as perfluoromethylcyclohexane, 1,1,1,3,3,3-hexafluoro-2-propanol, ethylpentafluoropropionate resulted in cell death. However, cells lived up to 2 days in fluorous alcohols such as 2,2,3,3,4,4,5,5-octafluoro-1-pentanol and 3,3,4,4,5,5,6,6,6-nonafluoro-1-hexanol with relatively higher fluorine content. Remarkably, cells survived deprived of nutrition up to 4 days when incubated in 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-1-hexanol or in 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptanol that have the most number of fluorine atoms (11 and 12, respectively) among the perfluoroalkyl alcohols used, and with boiling points of 128 °C and 169 °C, respectively.

1. Introduction

Perfluorocarbons are characterized by their outstanding biological inertness [1] and hydrophobicity that serve as basis for their biomedical potential in targeted drug delivery, pulmonary disease therapy, treatment of neonatal respiratory distress syndrome and in molecular imaging using ultrasound and magnetic resonance. Perfluorooctyl bromide ($C_8F_{17}Br$) has been approved and widely used as contrast agent for bowel delineation by magnetic resonance imaging (MRI) [2]. Highly fluorinated polymers are inert materials that have found useful application for contact lenses [2] and other devices. The unique properties due to carbon-fluorine linkage, low surface tension and high gas solubility are among the characteristics that make these compounds interesting in clinical application such as oxygen carrier [3] in blood substitutes.

In our work on the synthesis of glycolipids via saccharide primer strategy [4], perfluoroalkyl alcohols have been employed for the chemical synthesis of fluorous-tagged saccharide primers that were used as substrates for glycosylation by cellular enzyme to afford biologically important glycolipids [5]. Incubation of mouse melanoma B16 cells in the presence of saccharides with perfluoroalkyl chains did not adversely affect morphology and viability at 50 μM concentration. The presence of the perfluoroalkyl tail was not harmful to cells. This ignited the interest to incubate mouse melanoma B16 cells in fluorous solvents only to determine their direct effect on cells.

In this research, mouse melanoma B16 cells were cultured in various fluorous solvents such as perfluoromethylcyclohexane, 1,1,1,3,3,3-hexafluoro-2-propanol, ethylpentafluoropropionate, 4,4,5,5,5-pentafluoropentanol, 2,2,3,3,4,4,5,5-octafluoro-1-pentanol, 3,3,4,4,5,5,6,6,6-nonafluoro-1-hexanol, 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-1-hexanol and 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptanol to determine the direct effect of fluorous solvents. The effect on cells was assessed after 1 day and after 4 days. For comparison, B16 cells were also incubated in water (Milli Q), 17.5 mM glucose in water, or phosphate buffered saline (PBS) under the same conditions.

2. Materials and methods

Mouse melanoma B16 cells (5×10^5 cells per well, 24-well dish) were cultured in 1.5 ml 1:1 DMEM-F12 (from Gibco) supplemented with 10% fetal bovine serum (FBS). After 48-h incubation, the cells were washed with DMEM/F12 (without FBS) containing 1% insulin–transferrin–selenium X (ITS-X) solution and then washed with corresponding fluorous solvent (commercially available from Daikin). Then, the cells were incubated in various fluorous solvents such as perfluoromethylcyclohexane, 1,1,1,3,3,3-hexafluoro-2-propanol, ethyl pentafluoropropionate, 4,4,5,5,5-pentafluoropentanol, 2,2,3,3,4,4,5,5-octafluoro-1-pentanol, 3,3,4,4,5,5,6,6,6-nonafluoro-1-hexanol, 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-1-hexanol and 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptanol. Cells were also incubated in phosphate buffered saline solution, in water (Milli Q) only and in glucose (17.5 mM) having the same concentration as the commercially available culture medium (DMEM-F12). The cells were monitored overnight and after 4 days.

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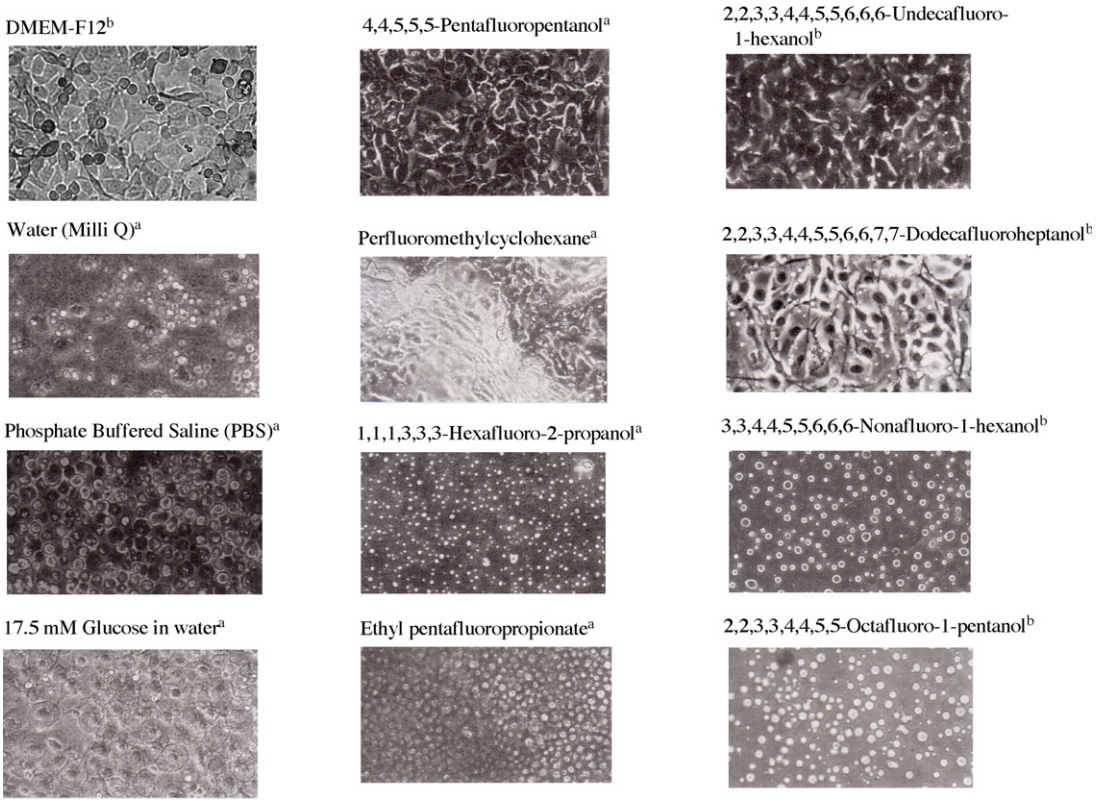

Fig. 1. Effect on mouse melanoma B16 cells after incubation in various solvents after 1 day[a] and after 4 days[b].

3. Results and discussion

Results of preliminary investigation are summarized in Fig. 1 and Table 1. As expected cells incubated in water only or in phosphate buffered saline died due to nutrient depletion and deprivation. Similarly, overnight incubation in perfluoromethylcyclohexane, 1,1,1,3,3,3-hexafluoro-2-propanol, ethyl pentafluoropropionate resulted in cell death. These fluorous solvents have relatively low boiling points of 76 °C, 59 °C, 76 °C, respectively. Except for perfluoromethylcyclohexane, these solvents have similar number of fluorine atoms (6 and 5, respectively). On the other hand, cells remarkably survived but swelled in 17.5 mM glucose in water that has the same concentration as Dulbecco's Modified Eagle's Medium/Ham's F12 (DMEM F12), the recommended culture medium for growth and proliferation of mouse melanoma B16 cells.

In 4,4,5,5,5-pentafluoropentanol, cells lived overnight but the experiment was terminated thereafter because the solvent evaporated owing to its low boiling point of 62–64 °C. However, cells lived up to 2 days in fluorous alcohols such as 2,2,3,3,4,4,5,5-octafluoro-1-pentanol and 3,3,4,4,5,5,6,6,6-nonafluoro-1-hexanol with relatively higher fluorine content and the same boiling point (141 °C). Remarkably, cells lived up to 4 days when incubated in 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-1-hexanol and in 2,2,3,3,4,4,5,5, 6,6,7,7-dodecafluoroheptanol that have the most number of fluorine atoms (11 and 12, respectively) and with boiling points of 128 °C and 169 °C, respectively. Moreover, cell attachment and cell spreading were not sacrificed. Despite the absence of nutrient for a certain period of time, this work revealed that cells could survive in the presence of fluorous solvents only.

To grow cells *in vitro*, culture conditions must mimic *in vivo* conditions with respect to CO_2 concentration, temperature, pH and nutrition. The main function of all culture media is to maintain pH and osmolality that are essential for cell viability and to provide nutrient necessary for growth and proliferation. Glucose is the main energy source of cells in culture. Based on results, glucose alone was not sufficient for the cells to thrive normally and in fact, its presence in the absence of other nutrients is detrimental and resulted to swelling of cells.

Cells have been reported to be cultured in non-aqueous medium such as toluene, ethanol and methanol. Results showed that these organic solvents exhibit cytotoxicity [6]. On the other hand, animal cells continued living up to 4 days in the presence of fluorous solvents such as 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-1-hexanol or 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptanol. This is significant because fluorous solvents did not inflict damage to cells leading to morphological change or death. Remarkably, cells survived up to 4 days deprived of nutrients and supplements. Moreover, B16 cells that are anchorage-dependent cells exhibited attachment and spreading.

Recently, fluorous solvents have gained wide attention from the perspective of green chemistry and in many aspects of synthetic chemistry and life sciences. Substantial progress has been achieved since fluorous techniques have been employed considering that fluorous solvents are immiscible in aqueous and most organic compounds. Regardless of the molecular weight, their boiling points are almost equal to corresponding hydrocarbons. Hydrophobicity is related to the number of fluorine atoms such that compounds with more fluorine atoms are more hydrophobic. Hydrophobicity contributes to the biological inertness of fluorous compounds since molecules tend to segregate rather than interact with other materials such as membrane lipids. Based on this premise, hydrophobicity could account for the survival of cells in fluorous medium only. The interaction that is seemingly absent between the fluorous solvents and the cell membrane components shielded the cells from any detrimental effects by the external fluorous media.