CYTOTOXICITY OF INHALABLE DRY POWDERS IN A549 HUMAN LUNG CANCER CELL LINE

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Abstract

The aim of the present work was to study the cytotoxicity of meloxicam potassium (MP) containing dry powder inhalation systems (DPIs) in monolayers of A549 lung epithelial cells, in order to acquire information on its suitability for pulmonary drug delivery. We also characterized the effect of the used excipients (such as aerosolization enhancer additives and polymers) on the cytotoxicity of the formulated DPIs. We reported for the first time the cytotoxicity of MP in comparison with meloxicam (M) and the results showed that no difference in the safety can be determined at 0.01 and 0.1 mg/mL concentrations. The protective effect of L-leucine was observed in some formulations, while the use of poly-vinyl-alcohol (PVA) decreases this advantage. Comparing the two polymers it can be established that the poly-vinyl-pirrolidon (PVP) is less toxic than the PVA in the same concentrations.

Keywords: A549 cell line, meloxicam, meloxicam potassium, dry powder inhalation

Introduction

With the carrier-free dry powder inhaler (DPI) formulations, active ingredients can be inhaled with higher lung deposition, even at lower inhalation flow rates. These new carrier-free formulations can offer an alternative local or systemic treatment of pulmonary and other diseases (e.g. inhalable insulin for diabetes or tobramycin for cystic fibrosis) [1].

In our previous work, we discussed different preparation methods of carrier-based and carrier-free formulations of meloxicam (M) and meloxicam potassium (MP) as a possible treatment of non-small cell lung carcinoma or cystic fibrosis [2]. M is a non-steroidal anti-inflammatory (NSAID) drug, conventionally orally used for the treatment of rheumatoid diseases [3-5]. In our previous studies, we presented the cytotoxicity of M-containing microcomposites in monolayers of Calu-3 cells. The results showed that M can be used safely at a maximum concentration of 5 mg/mL [6].

In the present work, we studied the cytotoxicity of MP, a novel potassium salt of M [7] in comparison with M. The MP shows a better water solubility than M, which gives the option of a one-step DPI preparation by co-spray drying procedure. The present study focuses on the cytotoxicity of MP containing DPIs in monolayers of A549 lung epithelial cells [8], in order to acquire information on its suitability for pulmonary drug delivery.

Materials and Methods

Materials

We acquired MP and M as active ingredients from Egis Company (Egis Pharmaceutical Plc, Hungary). Poly-vinyl-pirrolidon K25 (PVP) (ISP Customer Service GmBH, Germany) and poly-vinyl-alcohol 3-88 (PVA) (ISP Customer Service GmBH, Germany),
L-leucine (LEU) (AppliChem, Germany) and ammonium-carbonate (AC) (AppliChem, Germany) were used to enhance the aerodynamical properties of the particles [9]. In some preparations ethanol 96% (AppliChem, Germany) was used in 10 v/v % to enhance the solubility of MP.

Sample preparation

The samples preparation was described in our previous work [7]. In case of MP-PVP-AC and MP-PVA-AC, AC and 10% of ethanol was added three hours after the solution cooled down. For mixing, a magnetic stirrer was used at 300 rpm for 10 min.

The cells were then exposed to varying concentrations of 0.01, 0.1, 1, 2, 5 and 10 mg/mL. DMEM/F12 medium and 100% dimethyl-sulfoxide (Fischer Scientific, PA) was used as a positive control. After 1 hour of incubation, cells were washed with DMEM/F-12 medium and then MTT solution was added to each well and incubated at 37°C for 3 hours. The formazan crystals formed were dissolved with 100% DMSO and the viable cells were measured via Synergy H1 plate spectrophotometer (Biotek®, VT) at 570 nm.

Table I

<table>
<thead>
<tr>
<th>Samples*</th>
<th>MP</th>
<th>LEU</th>
<th>PVA</th>
<th>PVP</th>
<th>AC</th>
<th>96% Ethanol</th>
<th>SPD method</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP-spd</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MP-LEU</td>
<td>1.0</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MP-LEU-PVA</td>
<td>1.0</td>
<td>2</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MP-PVA-AC</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>0.25</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>MP-PVP-AC</td>
<td>0.1</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
<td>0.25</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

*Amounts presented in gram, dissolved in 50 g of purified water; MP-spd = meloxicam potassium spray dried; LEU = L-leucine; PVA = poly-vinyl-alcohol; PVP = poly-vinyl-pirrolidin; AC = ammonium-carbonate

Results and Discussion

In this cytotoxicity study, the culture medium and DMSO were used as negative and positive controls, respectively. The cytotoxicity of pure drug samples and formulations were compared with negative and positive controls. In the presence of DMSO, only 10% cell viability was observed. M-raw, MP-raw and formulations of MP exhibited significant cytotoxicity at higher concentrations of 1, 2, 5 and 10 mg/L compared to the negative control. No difference in cytotoxicity was observed in case of the ionic (MP-raw) and nonionic (M-raw) form at 0.1 and 0.01 mg/mL concentrations, as the solubility of the two forms are almost the same (M: 0.933 ± 0.054 mg/mL, while MP: 0.729 ± 0.0005 mg/mL, measured at 37°C, in 7.4 pH buffer) [11]. At concentrations higher than 1 mg/mL, the active ingredient remains suspended and this could cause low cell viability (Figure 1).
The cytotoxicity profile of MP-LEU was found to be the same as the raw material (MP-raw) and less cytotoxic when compared to other formulations with other excipients at the tested concentrations (Figure 2). Higher viability of the cells can be related to the effect of leucine of improving cell proliferation and metabolism of bronchial epithelial cells [12]. MP-LEU-PVA was acceptable up to 0.01 mg/mL concentration as the polymer forms a protective layer on the surface of the drug. MP-PVP-AC and MP-PVA-AC did not show cytotoxicity at 0.01 mg/mL. This indicates that AC has no effect on the safety of powders, as it totally evaporates from the solutions during the spray drying process. Samples containing PVA showed lower cell viability than those with PVP. As the PVP is hydrophilic and more water soluble than the PVA, this may cause the difference, even though, the preparation method was the same.

**Conclusions**

It was clarified that the MP has similar cytotoxic effect as M on A549 cells and both can be safely used at 0.1 and 0.01 mg/mL concentrations. The presence of additives also modified the cytotoxicity of the samples. The presence of PVA increased the toxic effect compared to other samples at the same concentrations, while PVP is less toxic than PVA. LEU has no toxic effect under 0.1 mg/mL. We report for the first time the cytotoxicity of MP and its formulations on A549 lung epithelial cells. It is very important to identify the toxic effects and doses of MP as it possess a possible pharmacotherapeutic potential in chronic obstructive pulmonary disease (COPD) or other lung disease.
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