FARMACIA, 2018, Vol. 66, 1

ORIGINAL ARTICLE

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

ANITA CHVATAL [1], RAMI ALZHRANI [2], AMIT K. TIWARI [2], RITA AMBRUS [1]*, PIROSKA SZABÓ-RÉVÉSZ [1], SAI HS. BODDU [2]

[1]*University of Szeged, Faculty of Pharmacy, Institute of Pharmaceutical Technology and Regulatory Affairs, Eötvös u. 6, 6720 Szeged, Hungary*
[2]*The University of Toledo, College of Pharmacy and Pharmaceutical Sciences, Frederic and Mary Wolfe Centre, Health Science Campus, 3000 Arlington Avenue, MS 1013, 43614-2595 Toledo, OH, USA*

**corresponding author: arita@pharm.u-szeged.hu*

Manuscript received: September 2017

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.

Rezumat

Scopul studiului prezentat a fost evaluarea citotoxicității sistemelor de inhalare a pulberilor uscate (DPI) cu sarea potasică a meloxicamului în monostraturile celulelor epiteliale pulmonare A549, pentru a obține informații privind utilitatea administrării medicamentului la nivel pulmonar. De asemenea, a fost caracterizat efectul excipienților utilizați (cum ar fi aditivii și polimerii de intensificare a aerosolizării) asupra citotoxicității formulărilor DPI. Am raportat pentru prima dată citotoxicitatea acestei substanțe active în studiul comparativ cu meloxicamul, iar rezultatele au arătat că nu există diferențe privind siguranța, pentru concentrațiile de 0,01 și 0,1 mg/mL. Efectul protector al L-leucinei a fost observat în unele formulări, în timp ce utilizarea polivinil-alcoolului (PVA) a redus acest avantaj. Comparând cei doi polimeri, s-a putut stabili că polivinilpirolidona (PVP) este mai puțin toxică decât PVA în aceleași concentrații.

Keywords: A459 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 (AREC.X heating magnetic stirrer, Velp Scientifica Srl, Italy). The spray drying parameters were set at lower temperature (inlet temperature 100°C) for the samples containing AC and higher temperature (inlet temperature 140°C) for the samples without the pouring agent. Büchi B-191 Mini Spray Dryer (BÜCHI Labortechnik AG, Switzerland) was used for the size reduction and particle formulation. The other spray drying parameters were similar for each sample: atomizing air flow rate 600 L/h, aspirator rate 75%, and solution feed rate 2 mL/min, nozzle cleaning interval level 5 (Table I).

Table I

Composition and spray drying properties (SPD) of DPI formulations

Samples*	MP	LEU	PVA	PVP	AC	96% Ethanol	SPD method
MP-spd	1.0	-	-	-	-	-	inlet temperature: 140°C outlet temperature: 81 - 79°C
MP-LEU	1.0	2	-	-	-	-	
MP-LEU-PVA	1.0	2	0.1	-	-	-	
MP-PVA-AC	0.1	-	-	0.05	0.25	5	inlet temperature: 100°C outlet temperature: 65 - 58°C
MP-PVP-AC	0.1	-	0.05	-	0.25	5	

*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-pirrolidon; AC = ammonium-carbonate

Cell line

The samples were tested in human epithelial A549 lung carcinoma cells (ATCC®, USA). Cells were maintained in Dulbecco's Modified Eagle's Medium and Nutrient mixture F-12 50:50 (DMEM/F-12) (Cellgro, USA) mixed with 10% foetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin in 5% carbon-dioxide environment at 37°C. The medium was changed every other day. The MTT assay [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] was carried out to examine the possible cytotoxicity of M and MP in A549 cells [10]. In brief, A549 cells were seeded in 96-well cell culture plates with lid (Corning™, NY) at a density of 10,000 cells/well, established with a haemocytometer (Fisher Scientific, PA). Cells were pre-incubated for 24 hours at 37°C, in 5% carbon-dioxide to assist cell attachment. The pure drugs (labelled as M-raw and MP-raw in Figure 1), as well as the spray dried formulations of MP (MP-spd) were dispersed in DMEM/F-12 medium to obtain final active ingredient concentrations of 0.01, 0.1, 1, 2, 5 and 10 mg/mL. The cells were then exposed to varying concentrations of M-raw, MP-raw and spray dried MP formulations for 1 hour. Negative controls were incubated in DMEM/F-12 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 H[1]plate spectrophotometer (Biotek®, VT) at 570 nm.

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).

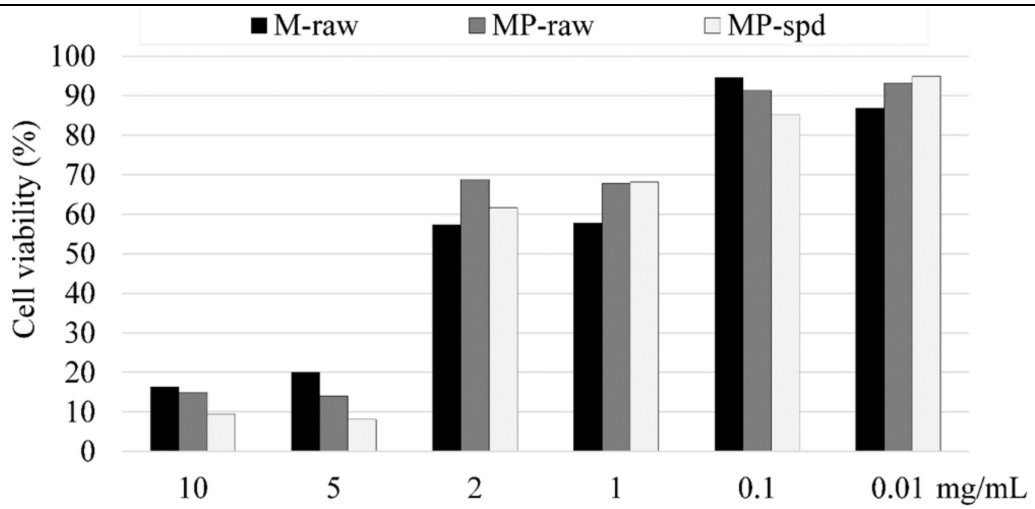

Figure 1.
Cytotoxicity of raw meloxicam (MP-raw), raw meloxicam potassium (MP-raw) and the spray dried meloxicam potassium (MP-spd) (values presented are means, SD were less than 0.1% for each concentration)

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.

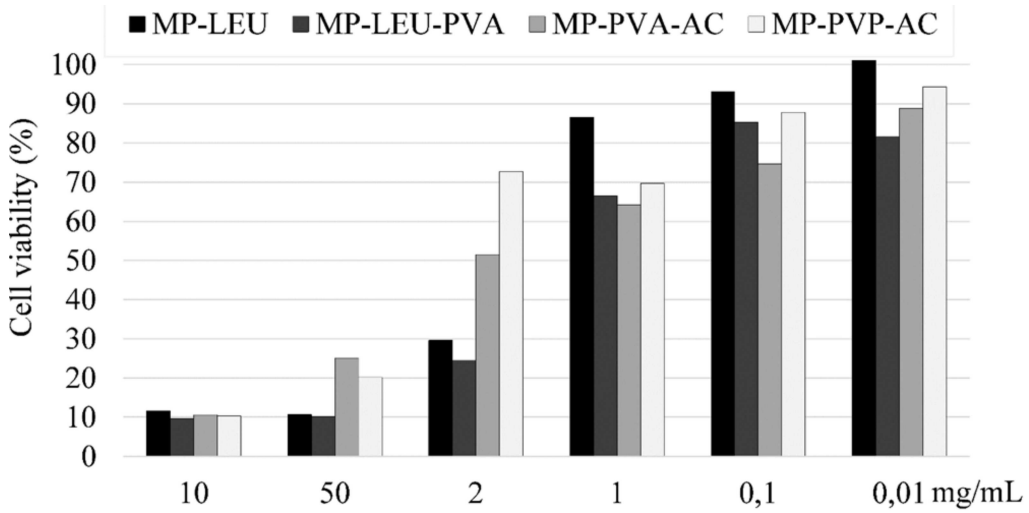

Figure 2.
Cytotoxicity of spray dried meloxicam potassium formulations at varying concentrations (values presented are means, SD were less than 0.1% for each concentration)

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 pharmaco-therapeutic potential in chronic obstructive pulmonary disease (COPD) or other lung disease.

Acknowledgement

This work was supported by ÚNKP-17-4-III-SZTE-3 New National Excellence Program of the Ministry of Human Capacities.
This project partly was made as part of the Exchange Agreement between The University of Toledo College of Pharmacy and Pharmaceutical Sciences and the University of Szeged, Faculty of Pharmacy.

References

1. Healy AM, Amaro MI, Paluch KJ, Tajber L, Dry powders for oral inhalation free of lactose carrier particles. *Adv Drug Deliv Rev.*, 2014; 75: 32-52.
2. Pomázi A, Ambrus R, Szabó-Révész P, Physicochemical stability and aerosolization performance of mannitol-based microcomposites. *J Drug Deliv Sci Technol.*, 2014; 24(4): 397-403.
3. Ghorab MM, Abdel-Salam HM, El-Sayad M, Mekhel MM, Tablet formulation containing meloxicam and beta-cyclodextrin: mechanical characterization and bioavailability evaluation. *AAPS Pharm Sci Tech.*, 2004; 5(4): 63-68.
4. Mezei T, Mesterházy N, Bakó T, Porcs-Makkay M, Simig G, Volk B, Manufacture of high-purity meloxicam *via* its novel potassium salt monohydrate. *Org Process Res Dev.*, 2009; 13: 567-572.
5. Mezei T, Simig G, Molnár E, Lukács G, Porcs-Makkay M, Volk B, Fekete HV, Nagy K, Mesterhazy N, Krasznai Gy, Vereczkeyné DGy, Kortvélyessy Gy, Pécsi É, Process for preparation of high-purity meloxicam and meloxicam potassium salt. 2006; Pub. No.: US 2012/0035162 A1.
6. Ambrus R, Pomázi A, Réti-Nagy K, Fenyvesi F, Vecsernyés M, Szabó-Révész P, Cytotoxicity testing of carrier-based microcomposites for DPI application. *Pharmazie*, 2011; 66(7): 549-550.
7. Chvatal A, Farkas Á, Balásházy I, Szabó-Révész P, Ambrus R, Aerodynamic properties and *in silico* deposition of meloxicam potassium incorporated in a carrier-free DPI pulmonary system. *Int J Pharm.*, 2017; 520(1-2): 70-78.
8. Foster KA, Oster CG, Mayer MM, Avery ML, Audus KL, Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism. *Exp Cell Res.*, 1998; 243(2): 359-366.
9. Pilcer G, Amighi K, Formulation strategy and use of excipients in pulmonary drug delivery. *Int J Pharm.*, 2010; 392(1-2): 1-19.
10. Gerlier D, Thomasset N, Use of MTT colorimetric assay to measure cell activation. *J Immunol Methods*, 1986; 94(1-2): 57-63.
11. Horváth T, Bartos C, Bocsik A, Kiss L, Veszelka S, Deli MA, Újhegyi G, Szabó-Révész P, Ambrus R, Cytotoxicity of different excipients on RPMI 2650 human nasal epithelial cells. *Molecules*, 2016; 21(5): 1-5.
12. Prota L, Santoro A, Bifulco M, Aquino RP, Mencherini T, Russo P, Leucine enhances aerosol performance of naringin dry powder and its activity on cystic fibrosis airway epithelial cells. *Int J Pharm.*, 2011; 412(1-2): 8-19.