

Cytotoxicity of inhalable dry powders in A549 human lung cancer cell line

*Citotoxicitatea pulberilor uscate inhalabile în linia celulară de cancer pulmonar
A549 uman*

**Anita Chvatal¹, Rami Alzhrani², Amit K.Tiwari², Rita Ambrus¹, Piroska Szabó-
Révész¹, Sai HS. Boddu²**

¹University of Szeged, Faculty of Pharmacy, Institute of Pharmaceutical Technology
and Regulatory Affairs, Eötvös u. 6, 6720 Szeged, Hungary

²The University of Toledo, College of Pharmacy and Pharmaceutical Sciences,
Frederic and Mary Wolfe Center, Health Science Campus, 3000 Arlington Avenue,
MS 1013, Toledo, OH, USA 43614-2595

*Correspondence: Rita Ambrus PhD

Institute of Pharmaceutical Technology and Regulatory Affairs, University of Szeged,
Faculty of Pharmacy, H-6720 Szeged, Eötvös u. 6, Hungary

Tel +36 62 545 575

Fax +36 62 545 571

E-Mail: arita@pharm.u-szeged.hu

KEYWORDS:

A459 cell line, meloxicam, meloxicam potassium, dry powder inhalation

Linia celulară A459, meloxicam, potasiu meloxicam, inhalare cu pulbere uscată

ABSTRACT

The overall aim of the presented project is 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 study with meloxicam (M) and the results show 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.

Scopul general al proiectului prezentat este studierea citotoxicității sistemelor de inhalare a pulberilor uscate (DPI) cu meloxicam-potasiu în monostraturile celulelor epiteliale pulmonare A549, pentru a obține informații despre adecvarea lor pentru administrarea medicamentului la nivel pulmonar. De asemenea, am caracterizat efectul excipienților utilizați (cum ar fi aditivii și polimerii de intensificare a aerosolizării) asupra citotoxicității formulărilor DPI. Am raportat pentru prima dată

citotoxicitatea meloxicam-potasiu în studiul comparativ cu meloxicam, iar rezultatele arată că nu se poate determina nici o diferență în siguranță la 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) scade acest avantaj. Comparând cei doi polimeri, se poate stabili că polivinilpirolidona (PVP) este mai puțin toxică decât PVA în aceleași concentrații.

INTRODUCTION:

With the carrier-free dry powder inhaler (DPI) formulations active ingredients can inhaled with higher lung deposition even at a 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) (Healy et al. 2014).

In our previous work, we discussed different preparation methods of carrier-based and carrier-free formulations of M and MP as a possible treatment of non-small cell lung carcinoma or cystic fibrosis (Pomázi, Ambrus, and Szabóné Révész 2014). M is a non-steroidal anti-inflammatory (NSAID) drug, conventionally used orally for the treatment of rheumatoid diseases (Ghorab et al. 2004)(Mezei et al. 2009)(Mezei et al. 2006). 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 (Ambrus et al. 2011).

In the present work, we studied the cytotoxicity of MP, a novel potassium salt of M prepared by the Egis Pharmaceutical Plc. (Chvatal et al. 2017) 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 (Foster et al. 1998), 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). Polyvinylpyrrolidon 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 (Pilcer and Amighi 2010). In some preparations ethanol 96% (AppliChem, Germany) was used in 10 v/v% to enhance the solubility of the MP (Table 1.).

Sample preparation

The samples preparation of each concentrations were described in our previous work (Chvatal et al. 2017). 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, presented in Table I., were similar for each sample.

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 temp. 140°C
MP-LEU	1.0	2	-	-	-	-	aspirator 75 % pump 0.05 rpm
MP-LEU-PVA	1.0	2	0.1	-	-	-	inlet temp. 100°C
MP-PVA-AC	0.1	-	-	0.05	0.25	5	aspirator 75 % pump 0.05 rpm
MP-PVP-AC	0.1	-	0.05	-	0.25	5	

*Amounts presented in 50 g of purified water.

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% fetal 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 (Gerlier and Thomasset 1986). 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 hemocytometer (Fisher Scientific, PA). Cells were pre-incubated for 24 hours at 37°C, in 5% carbon-dioxide to assist cell attachment. The pure drugs as M-raw and MP-raw as well as the spray dried MP (MP-spd) and the formulations of MP 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, MP and

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 H¹plate spectrophotometer (Biotek[®], VT) at absorbance 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 and formulations of MP exhibited significant cytotoxicity at higher concentrations of 1, 2, 5 and 10 mg/mL compared to the negative control. No difference in cytotoxicity was observed in case of the ionic (MP) and nonionic (M) 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) (Horváth et al. 2016). At higher concentrations of 1 mg/mL, the active ingredient remains suspended and this could cause low cell viability (Fig. 1).

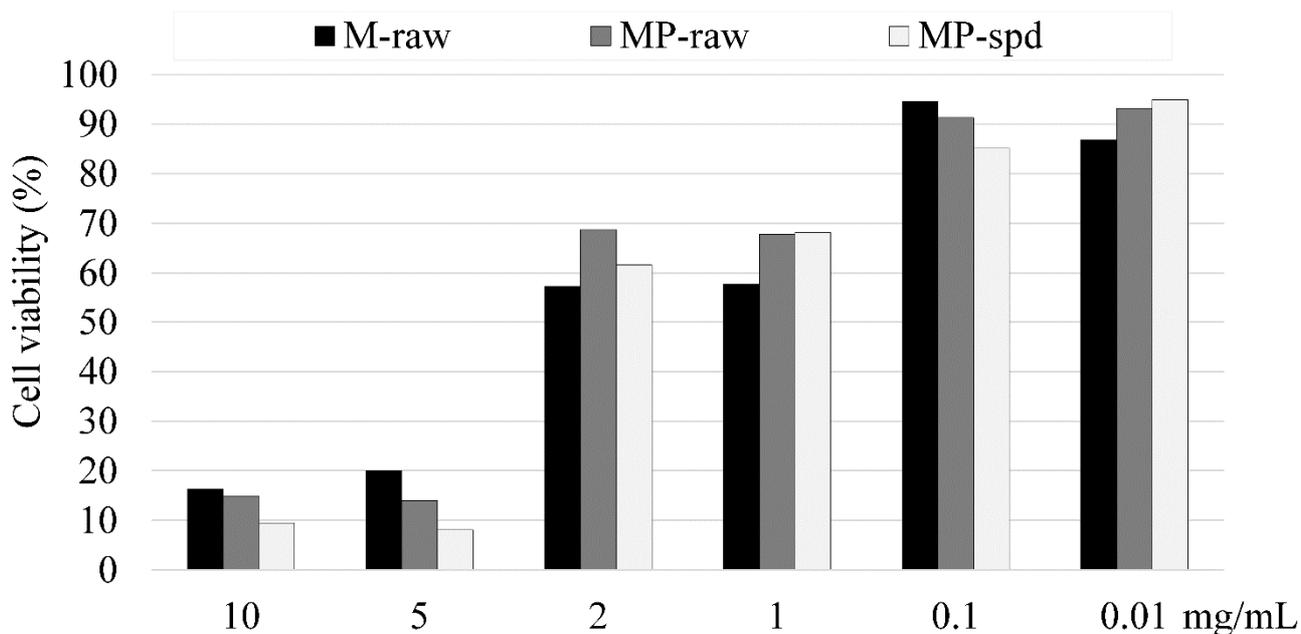


Figure 1: Cytotoxicity of meloxicam, meloxicam potassium and the spray dried meloxicam potassium (Values presented are means, SD were less than 0.1% at each concentration).

The cytotoxicity profile of MP-LEU was found to be same as the raw material (MP-raw) and less cytotoxic when compared to other formulations with other excipients at the tested concentrations (Fig. 2). Higher viability of the cells can be related to the effect of leucine is improving cell proliferation and metabolism of bronchial epithelial cells (Prota et al. 2011). 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 shows lower cell

viability than those with PVP. As the PVP is hydrophilic and more water soluble than the PVA, it can be cause the difference in the results prepared the same way.

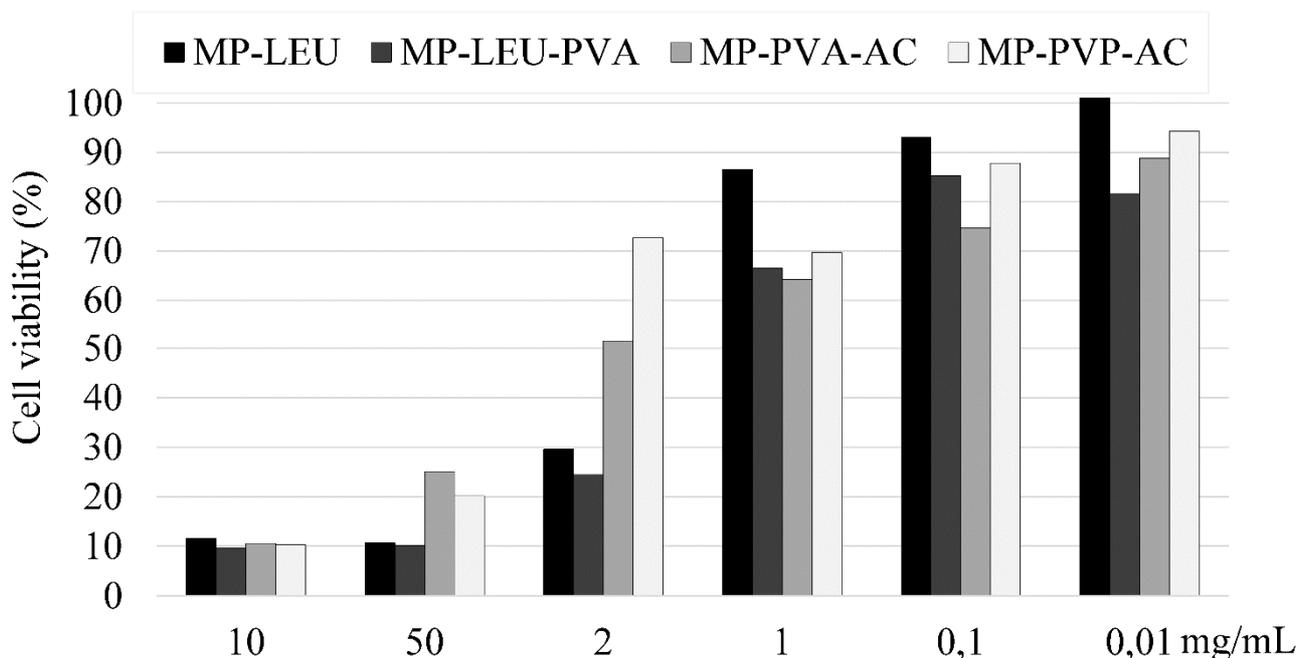


Figure 2: Cytotoxicity meloxicam potassium formulations at varying concentrations (Values presented are means, SD were less than 0.1% at each concentration).

CONCLUSION:

To conclude, it was clarified that the MP has similar cytotoxic effect as M on A549 cells and they can be safely used at 0.1 and 0.01 mg/mL concentrations. The presence of additives modified the cytotoxicity of the samples too. The presence of PVA increased the toxic effect compared to other samples at the same concentrations, while PVP is less toxic than PVA. While LEU does not seems to be toxic under 0.1 mg/mL. To conclude, we report for the first time the cytotoxicity of MP and its formulations in

A549 lung epithelial cells. It is very important to identify the toxic effect and doses of MP as it has the potential to treat inflammation in 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

- 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): 549–50.
- 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): 70–8.
- 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): 359–66.
- Gerlier D, Thomasset N. Use of MTT colorimetric assay to measure cell activation. *J Immunol Methods*. 1986, 94(1–2): 57–63.
- Ghorab MM, Abdel-Salam HM, El-Sayad M a, Mekhel MM. Tablet formulation containing meloxicam and beta-cyclodextrin: mechanical characterization and bioavailability evaluation. *AAPS Pharm Sci Tech*. 2004, 5(4): 59.
- 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.
- Horváth T, Bartos C, Bocsik A, Kiss L, Veszélka S, Deli MA, et al. Cytotoxicity of different excipients on RPMI 2650 human nasal epithelial cells. *Molecules*. 2016, 21(5): 1–5.

- 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: 567–72.
- Mezei T, Simig G, Molnár E, Lukács G, Porcs-Makkay M, Volk B, et al. Process for preparation of high-purity meloxicam and meloxicam potassium salt. 2006.
- Pilcer G, Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. *Int J Pharm.* 2010, 392(1–2): 1–19.
- Pomázi A, Ambrus R, Szabóné Révész P. Physicochemical stability and aerosolization performance of mannitol-based microcomposites. *J Drug Deliv Sci Technol.* 2014, 24(4): 397–403.
- 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.