Journal of Experimental Biology and Agricultural Sciences, August - 2022; Volume – 10(4) page 812 – 817

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Madin-Darby Canine Kidney (MDCK) Cell line permeability of Curcumin loaded Phycocyanin nanosponges - *In-Vitro* study

Manjuladevi Kasirajan*, Ramaiyan Velmurugan, A.Vijayalakshmi

School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai- 600 117, Tamil Nadu

Received – November 01, 2021; Revision – January 14, 2022; Accepted – March 28, 2022
Available Online – August 30, 2022

DOI: http://dx.doi.org/10.18006/2022.10(4).812.817

KEYWORDS

BBB

MDCK

Nanosponges

Curcumin

Phycocyanin

ABSTRACT

Blood Brain barrier (BBB) is a natural protective wall in the brain to restrict the invasion of xenobiotics or toxic chemicals. This, in turn, becomes a major obstacle for researchers and industry people in formulating new drugs to treat brain disorders like brain tumors, Alzheimer's disease, multiple sclerosis, meningitis, and so on. The purpose of this research is to study the *in-vitro* cytotoxicity & BBB permeation of curcumin-loaded phycocyanin nanosponges (Cur-PC NS) using Madin-Darby Canine Kidney (MDCK) cell lines. Cell viability of Cur-PC NS was performed using 3-(4,5-dimethylthiazol-2-yl)-2.5- diphenyltetrazolium bromide (MTT) assay, the transepithelial electrical resistance (TEER) values, and permeability coefficient were measured to test the integrity of monolayer of MDCK cell line. Results of the current study showed that Cur-PC NS at 50µM, 85% of MDCK cells are more viable and there was a significant ($p<0.01$) reduction in TEER values up to 48 hours when compared to the curcumin. The permeability coefficient of nanosponges produced a 2.5-fold increase in enhancement ratio with a Papp value of $1.94\pm0.11\times10^{-6}$ cm/s and $4.86\pm0.04\times10^{-6}$cm/s for curcumin and Cur-PC NS respectively. Results of the study can be concluded that phycocyanin nanosponges can be used as a carrier for curcumin to permeate the BBB which may play a major role in the treatment of various brain disorders. Future studies are needed to substantiate the exact mechanism of permeability with clarification of efflux transporters presented in BBB.

* Corresponding author

E-mail: mdevi.sps@velsuniv.ac.in (Manjuladevi Kasirajan)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Blood- Brain barrier (BBB) is a protective and dynamic wall that restricts the entry of any harmful substance. This barrier system protects the brain from xenobiotics which is also a major obstacle to delivering drugs into the central nervous system (Pardridge 2005; Kadry et al. 2020; Neumaier et al. 2021; Sanchez-Dengra et al. 2021). Efflux transporters play a vital role in fluxing the drugs and limit the BBB penetration (Loscher and Potschka 2005; Neumaier et al. 2021; Sanchez-Dengraetal 2021). The Key barriers that interfere with the permeation of drugs are brain microvascular endothelial cells (BMECs) with tight junctional proteins & metabolizing enzymes like cytochrome P450 and transport barriers like P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and organic anion transporting polypeptide. Over the decade's, pharmaceutical scientists had to overcome the hurdles in the invention of drugs for central nervous system (CNS) disorders (Banerjee et al. 2016). With the knowledge of the complex nature of the blood-brain barrier, a drug can be designed by implementing nanotechnology to treat CNS-related disorders (Suresh et al. 2020).

The BBB permeability of a drug can be predicted using *in vitro* models. Various non-cerebral and cerebral origin *In-vitro* models are available. In recent studies, MDCK cell lines were more preferred when compared to $CaCo_2$ cell lines. The advantages of MDCK cells are a shorter duration for maturation time in culture, low risk of infection, low expression of P-glycoprotein (P-gp), and experimental reproducibility (Horio et al. 1989; Irvine et al. 1999; Polli et al. 2000; Jiang et al. 2022).

In the last two decades, various research studies have been performed to deliver curcumin through nanotechnology to resolve the failure of curcumin therapy in the concept of poor bioavailability and fast metabolism (Yallapu et al. 2013; MalekiDizaj et al. 2022). In current MDCK cell lines was used to study the permeability and integrity of tight junctions of curcumin-loaded phycocyanin nanosponges and compared with pure curcumin.

2 Materials and Methods

For the curcumin-loaded phycocyanin nanosponges, curcumin was obtained from Sigma Aldrich, Phycocyanin from TCI Chemicals Pvt. Ltd, and 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT), Hank's balanced salt solution (HBSS), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), non-essential amino acids (NEAA) solution, penicillin-streptomycin solution, and trypsin solution were purchased from Sigma-Aldrich. MDCK cell line was obtained from NCCS, Pune and Poly vinyl alcohol, double distilled water, ethanol and all other reagents and chemicals used are analytical grades.

Curcumin-loaded phycocyanin nanosponges (Cur-PC-NS) (Prathima and Sreeja 2013; Velmurugan et al. 2019) was prepared using the emulsion solvent evaporation technique. The final nanosponges were characterized as per Manjuladevi and Velmurugan (2020) and used for this study. The *In-vitro* cell line permeability study was performed using the MDCK cell line (Wang et al. 2014).

2.1 MDCK cell culture

For the transport studies, MDCK cells (Madin-Darby Canine Kidney cells) were cultivated in standard conditions with DMEM medium supplemented with 10% FBS, 1% NEAA, and 1% penicillin–streptomycin and was cultured in a humidified incubator at 37°C with 5% CO_2 and (80% confluent) were seeded at a density of 10^5 cell/mL on the upper side of 12 well plate filters (1.131 cm^2 growth area). The culture medium was replaced every three days following the two days of seeding to be ready for experimental use. The quality of the monolayers was assessed by measuring their transepithelial electrical resistance (TEER) at 37 °C using an EVOM epithelial Voltmeter with an Endohm electrode (World Precision Instruments, INC., Sarasota, FL.). The TEER shows the impedance to the passage of small ions through the physiological barrier and is recognized as one of the most accurate and sensitive measures of BBB integrity. Only monolayer's displaying TEER values above 400 Ω was used in the experiments (Wang et al. 2014).

2.2 Cytotoxicity study

The cell viability of nanosponges was performed using an MTT assay. MDCK cells were seeded in 96- well plates at a density of $5X10^3$ cells/ well and cultures for 24 hr. Nanosponges were also added to the wells at various concentrations. After allowing 2 hrs co-incubation at 37°C, 100 µL of MTT (0.5 mg/mL) was added to each well and incubated for a further 4 h at 37°C. After incubation, the medium was removed, and 100 µL of dimethylsulfoxide (DMSO) was added to the residual precipitates. The absorbance was determined at 590 nm using a microplate reader. Cell viability was expressed as a percentage of the absorbance relative to that of the control. Control cells were not exposed to any materials. Experiments were performed with three replicate wells for each sample and control (Wang et al. 2014).

2.3 Monolayer integrity testing (TEER measurement)

The cell lines were used after pre-incubating at 37°C in 5% CO_2 conditions for 2 days. TEER was measured to confirm the functionality of the tight junctions. The assays were carried out using the Blood Brain Barrier cell layers with TEER values in the range between 400 and 2000 Ω cm^2. After establishing that MDCK display extremely tight barrier properties, nanosponges were suspended in a 0.2 mL assay medium or the free drug to the apical side of the Blood

Brain Barrier layers and cultured in the model. The TEER values were monitored at a fixed time (Nikandish et al. 2016).

2.4 Drug uptake and Calculation of permeability (P*app*)

At the end of the study, samples were collected from the apical and basolateral sides of the BBB model and the concentration using HPLC-UV methods was measured. To calculate the apparent permeability coefficient (transport capacity), the following formula was used

$$P_{app} = (dQ/dt) \times (1/A.Co)$$

The enhancement ratio was calculated by dividing the Papp value of curcumin-loaded phycocyanin nanosponges and pure drug, dQ/dt is the transferred drug per time; A is the surface area of the filter (1.131 cm^2); Co is the initial concentration of nanosponges in apical side (Taub et al. 2002)

2.5 Statistical analysis

The results were presented as mean and Standard deviation (SD) with n=3. Statistical significance of differences was processed by Wilcoxon matched-pairs test and student t-test (Paired and unpaired). The value of *p<0.05 & ** p<0.01 was statistically significant. All the statistical calculations were performed using the software Graph Pad prism version 9.1.2.

3 Results

3.1 Cytotoxicity study

The Cytotoxicity of Cur-PC- NS was performed using MTT assay using MDCK cell lines after 24-hrs exposure to free drug and curcumin-loaded phycocyanin nanosponges. Results of the study showed that the Cur-PC-NS at a concentration of 25µg/ml & 50µg/ml have no adverse effect on cell viability (Figure 1).

3.2 Monolayer integrity testing

Results presented in figure 2 revealed that Cur-PC-NS decreased the integrity of the Blood Brain Barrier model cell line, proving that it can open the tight junction of Blood Brain Barrier for movement of curcumin loaded phycocyanin nanosponges into the brain. The amount of curcumin and curcumin loaded phycocyanin nanosponges after crossing the monolayer is shown in figure 3.

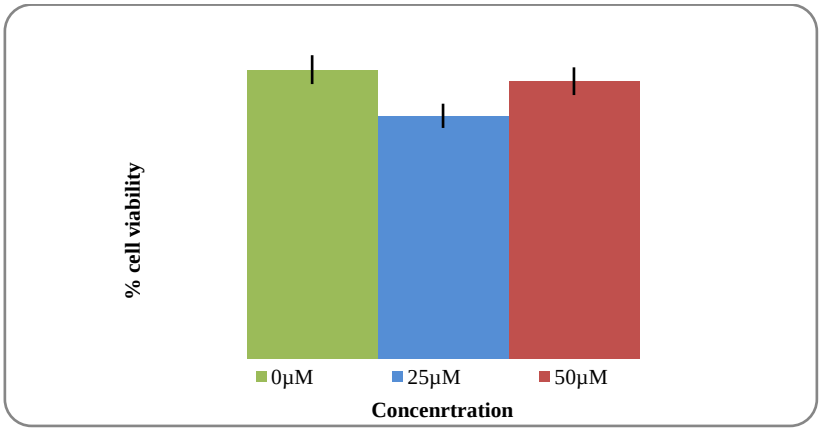

Figure 1 MDCK cell viability % of nanosponges at 24hr, the data represent mean ± SD (n=3 different monolayer)

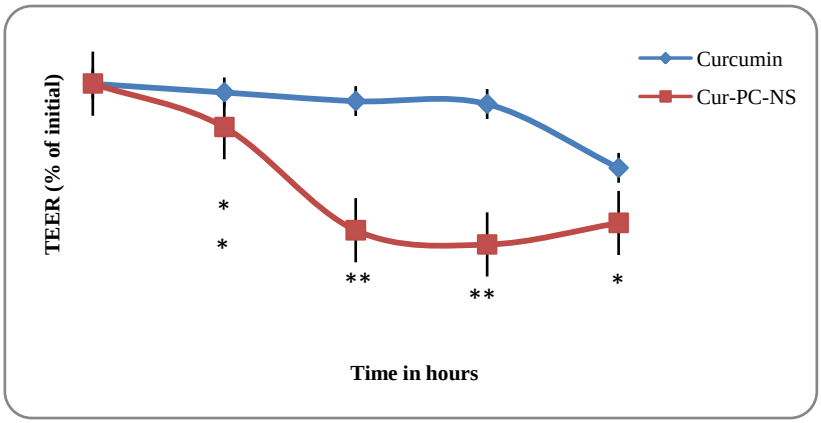

Figure 2 Effect of Cur-PC-NS and curcumin with TEER measuring. The data represent mean ± SD (n=3); **p<0.01

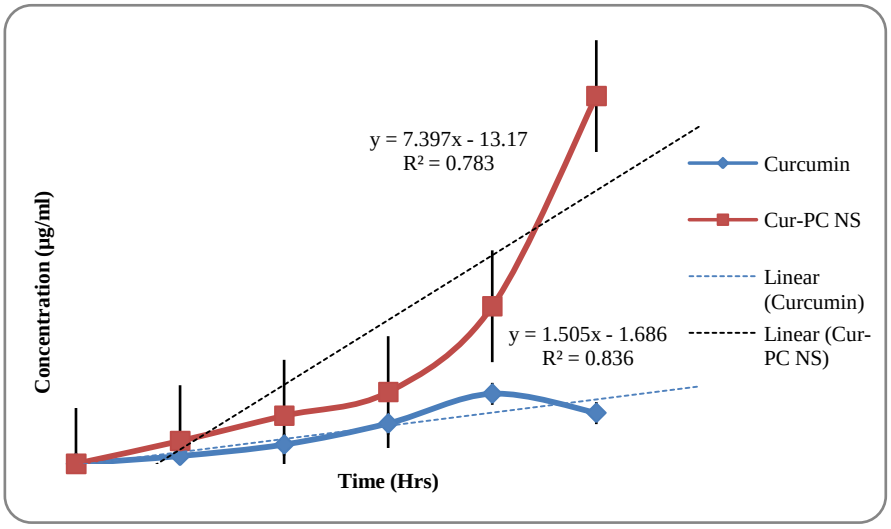

Figure 3 In-vitro cell line permeability investigation using MDCK cell line, the data's represent mean ± SD; **p<0.01

3.3 Permeability coefficient (P*app*)

The permeability coefficient was found $1.94\pm0.11\times10^{-6}$ cm/s and $4.86\pm0.04\times10^{-6}$ cm/s for curcumin and Cur-PC-NS, respectively. The enhancement ratio showed that Cur-PC-NS exhibited 2.5-fold increase in positive effect than the pure drug curcumin on MDCK cell line permeability.

4 Discussion

Curcumin loaded phycocyanin nanosponges was used to improve the BBB permeation, more entrapment, and target delivery which in turn to treat various CNS-related disorder based on earlier research (Tejashri et al. 2013; Gharakhloo et al. 2020; Suresh et al. 2020). In this study, the MDCK model was chosen, as it mimics the structure of BBB and also due to its advantage over the human colorectal adenocarcinoma cell line (Caco-2) in sense of shorter culture time (4 days vs. 21 days) drug permeability across BBB (Irvine et al. 1999; Polli et al. 2000). The rationale behind using phycocyanin as a carrier for curcumin is due to its wide range of application in the pharmaceutical field (Jiang et al. 2017), have proved to target TAM (Tumor-associated Macrophages) as photosensitizer (Wan et al. 2017).

Our results showed that the curcumin loaded phycocyanin nanosponges (Cur-PC-NS) had a positive effect and exhibited 96% of cells are viable at 50µg/ml which shows high biocompatibility of our nanosponges and no toxic effect on cells. Our findings are supported by previous research works exhibiting that curcumin has a non- cytotoxic effect on cells at lower concentrations (Zanotto-Filho et al. 2012; Gharakhloo et al. 2020; Susanna et al. 2020). The tight junction integrity of monolayer is also loosened or decreased to 45% of the initial TEER value in our results suggesting that the increased permeability of curcumin nanosponges, regain of the tight junction at 48hrs i.e., 52%, and the reversal of TEER value indicates the reconstruction of cell junctions (Yeh et al. 2011; Wang et al. 2014). Hence it suggests that curcumin loaded phycocyanin nanosponges are safe and compatible. The enhancement ratio of Cur-PC-NS permeability coefficient (Papp) is 2.5-fold when compared to curcumin alone, which is also supported by the research work of Pushpalatha et al. (2018) in curcumin nanoformulation. Thus, curcumin nanoformulation can be used for treating glioblastoma as a single therapy or along with chemotherapeutic agents due to its safety, biocompatibility, and pharmacological property (Neil and Sandeep 2016; Del Prado-Audelo et al. 2019). Further studies are needed to prove the exact mechanism of penetration, whether passive or endocytosis and transcellular transport of nanosponges and P-glycoprotein substrate mediated inhibition or efflux of the drug.

Conclusion and Future Perspective

Our results proved that the formulated nanosponges have no toxicity and decreased the TEER values thereby increasing the permeation of the drug into the blood brain barrier. Thus, it can be concluded that phycocyanin nanosponges can be used as a carrier to deliver curcumin into the brain. We have completed the animal study for our nanosponges along with biomarker estimation, which will be published in the future. However, future studies are needed to confirm the exact mechanism of permeation across the blood brain barrier in presence of various efflux transporters.

Acknowledgment

All the authors thank the management and heads and technical staff of the School of Pharmaceutical sciences, VISTAS, Pallavaram and Chennai for providing generous support and laboratory facilities.

Conflicts of Interest and Financial Disclosure

The authors declare that there is no conflict of interest and have not received any funds from any source for this research.

References

Banerjee, J., Shi, Y., & Azevedo, H. S. (2016). In vitro blood–brain barrier models for drug research: state-of-the-art and new perspectives on reconstituting these models on artificial basement membrane platforms. *Drug Discovery Today*, *21*(9), 1367-1386

Del Prado-Audelo, M. L., Caballero-Florán, I. H., Meza-Toledo, J. A., Mendoza-Muñoz, N., et al. (2019). Formulations of curcumin nanoparticles for brain diseases. *Biomolecules*, *9*(2), 56

Gharakhloo, M., Sadjadi, S., Rezaeetabar, M., Askari, F., Rahimi, A., et al. (2020). Cyclodextrin-based nanosponges for improving solubility and sustainable release of curcumin. *Biological Chemistry & Chemical Biology*, *Chemistry Select*, *5*, 1734–1738

Horio, M., Chin, K.V., Currier, S J., & Goldenberg, S., et al. (1989). Transepithelial Transport of Drugs by the Multidrug Transporter in Cultured Madin-Darby Canine Kidney Cell Epitheli. *Journal of Biological Chemistry*, *264*(25), 14880-14884

Irvine, J D., Takahashi, L., Lockhart, K., Cheong, J., et al. (1999). MDCK (Madin-Darby canine kidney) cells: A tool for membrane permeability screening. *Journal of Pharmaceutical Sciences*, *88*, 28-33

Jiang, L., Kumar, S., Nuechterlein, M., Reyes, M., et al. (2022). Application of a high-resolution in vitro human MDR1-MDCK assay and in vivo studies in preclinical species to improve prediction of CNS drug penetration. *Pharmacology Research & Perspectives*, *10*(1), e00932

Jiang, L., Wang, Y., Yin, Q., Liu, G., et al. (2017). Phycocyanin: A Potential Drug for Cancer Treatment. *Journal of Cancer*, *8*(17), 3416–3429

Kadry, H., Behnam, N., & Luca, C. (2020). A blood–brain barrier overview on structure function, impairment and biomarkers of integrity. *Fluids Barriers CNS*, *17*, 69

Löscher, W., & Potschka, H. (2005). Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. *NeuroRx: The Journal of the American Society for Experimental Neuro Therapeutics*, *2*(1), 86–98

MalekiDizaj, S., Alipour, M., DalirAbdolahinia, E., Ahmadian, E., et al. (2022). Curcumin nanoformulations: Beneficial nanomedicine against cancer. *Phytotherapy Research*, *36*(3), 1156– 1181

Manjuladevi, K., & Velmurugan, R., (2020). A perspective view on formulation and optimization of curcumin loaded phycocyanin nanosponges. *International Journal of Research in Pharmaceutical Sciences* , *11*(4), 8119-8123

Neil, V. K., & Sandeep, M., (2016). Therapeutic potential of curcumin for the treatment of brain tumors. *Oxidative Medicine and Cellular Longevity*, *1*, 1-14

Neumaier, F., Zlatopolskiy, B.D., & Neumaier, B. (2021). Drug Penetration into the Central Nervous System: Pharmacokinetic Concepts and In Vitro Model Systems. *Pharmaceutics*, *13*, 1542

Nikandish, N., Hosseinzadeh, L., HematiAzandaryani, A., & Derakhshandeh, K. (2016). The Role of Nanoparticle in Brain Permeability: An in-vitro BBB Model. *Iranian journal of Pharmaceutical Research*, *15*(2), 403–413.

Pardridge W. M. (2005). The blood-brain barrier: bottleneck in brain drug development. *NeuroRx: The Journal of the American Society for Experimental NeuroTherapeutics*, *2*(1), 3–14

Polli, J.W., Humphreys, J.E., Wring, S.A., Burnette, T C., et al. (2000). Comparison of MDCK and bovine brain endothelial cells (BBECs) as a blood-brain barrier screen in early drug discovery. In M. Balls, A.M. van Zeller, & M Halder (Eds.) Progress in the Reduction, Refinement and Replacement of Animal Experimentation (pp 271–289), *New York: Elsevier Science*

Prathima, S., & Sreeja, K. (2013). Formulation and Evaluation of Voriconazole loaded nanosponges for oral and topical delivery. *International Journal of drug delivery and research*, *5*(1), 55-69

Pushpalatha, R., Selvamuthukumar, S., & Kilimozhi, D. (2018). Cross-linked, cyclodextrin-based nanosponges for curcumin delivery - Physicochemical characterization, drug release, stability and cytotoxicity. *Journal of Drug Delivery Science and Technology*, *45*, 45–53

Sánchez-Dengra, B., González-Álvarez, I., González-Álvarez, M., & Bermejo, M. (2021). New *In-Vitro* methodology for kinetics distribution prediction in the brain. An additional step towards an animal-free approach. *Animals*, *11*(12), 3521

Suresh, T., Fong, Y. C., & Chia, H. S. (2020). Advancements in the Blood–Brain Barrier Penetrating, Nanoplatforms for Brain Related Disease Diagnostics and Therapeutic Applications. *Polymers*, *12*, 3055

Susanna, G., Alice, C., Andrea, B., Riccardo A.. et al. (2020). Nanosponges for the protection and release of the natural phenolic antioxidants quercetin, curcumin and phenethyl caffeate. *Materials Advances*, *1*, 2501-2508

Taub, M. E., Kristensen, L., & Frokjaer, S. (2002). Optimized conditions for MDCK permeability and turbidimetric solubility studies using compounds representative of BCS classes I-IV. *European Journal of Pharmaceutical Sciences: official journal of the European Federation for Pharmaceutical Sciences, 15*(4), 331–340

Yallapu, M. M., Jaggi, M., & Chauhan, S. C. (2013). Curcumin nanomedicine: a road to cancer therapeutics. *Current Pharmaceutical Design, 19*(11), 1994–2010

Tejashri, G., Bajaj, A., & Jain, D. (2013). Cyclodextrin based nanosponges for pharmaceutical use: A review. *Acta Pharmaceutica, 63*, 335–358

Velmurugan, R., Manjuladevi, K., Keerthi, G., Yamuna, R., et al. (2019). A method to enhance blood brain barrier permeability of curcumin. *The Patent office Journal No. 46/2019*, Application No 201941045090A, 53972

Wan, D. H., Zheng, B. Y., Ke, M. R., Duan, J. Y., et al. (2017). C-Phycocyanin as a tumour-associated macrophage-targeted photosensitiser and a vehicle of phthalocyanine for enhanced photodynamic therapy. *Chemical Communications (Cambridge, England), 53*(29), 4112-4115

Wang, M., Zhang, Y., Sun, B., Sun, Y., et al. (2014). Permeability of exendin-4-loaded chitosan nanoparticles across MDCK cell monolayers and rat small intestine. *Biological & pharmaceutical bulletin, 37*(5), 740–747

Yeh, T. H., Hsu, L. W., Tseng, M. T., Lee, P L., et al. (2011). Mechanism and consequence of chitosan-mediated reversible epithelial tight junction opening. *Biomaterials, 32*, 6164–6173

Zanotto-Filho, A., Braganhol, E., Edelweiss, M. I., Behr, A G., et al. (2012). The curry spice curcumin selectively inhibits cancer cells growth in vitro and in preclinical model of Glioblastoma. *The Journal of Nutritional Biochemistry, 23*(6), 591-601