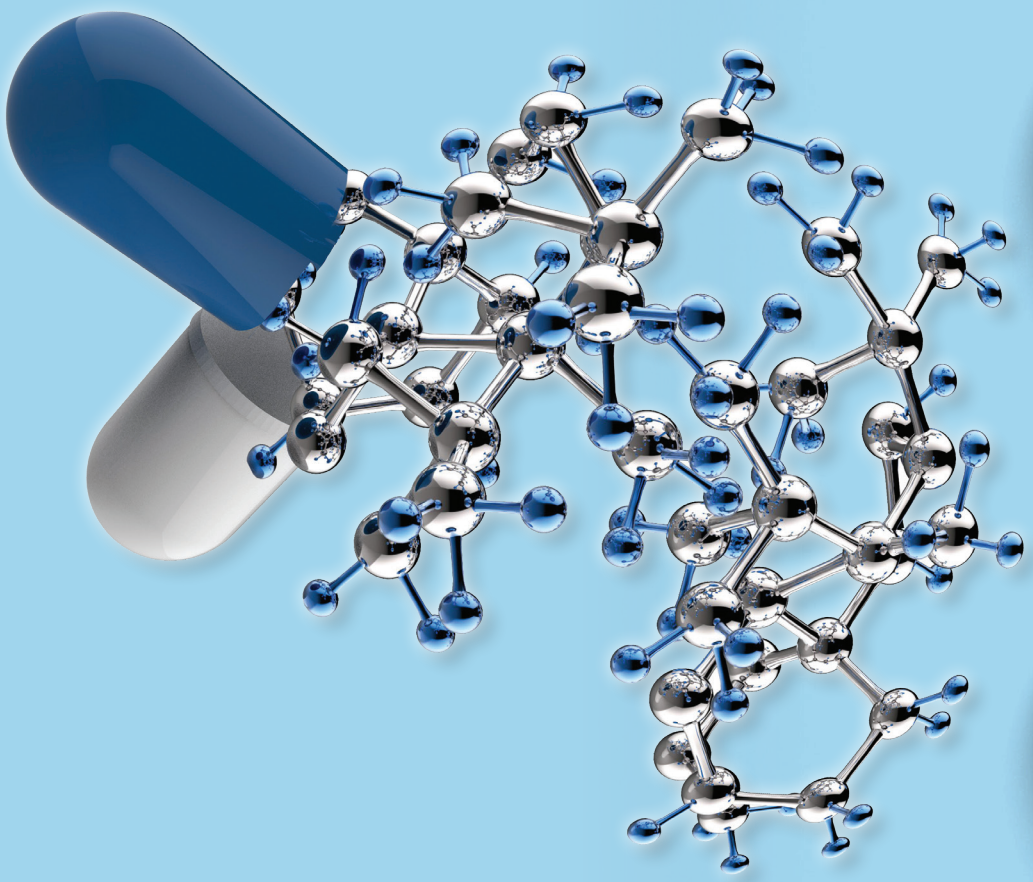
ISSN 1105-4999

ΦΑΡΜΑΚΕΥΤΙΚΗ
PHARMAKEFTIKI

ΤΡΙΜΗΝΙΑΙΑ ΕΚΔΟΣΗ ΜΕ ΘΕΜΑΤΑ ΦΑΡΜΑΚΕΥΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ
A QUARTERLY EDITION ON PHARMACEUTICAL SCIENCES' TOPICS



ΤΟΜΟΣ VOLUME 34 • ΤΕΥΧΟΣ ISSUE IV

ΟΚΤΩΒΡΙΟΣ - ΔΕΚΕΜΒΡΙΟΣ
OCTOBER - DECEMBER 2022

ΦΑΡΜΑΚΕΥΤΙΚΗ

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ΤΟΜΟΣ 34, ΤΕΥΧΟΣ IV,
ΟΚΤΩΒΡΙΟΣ - ΔΕΚΕΜΒΡΙΟΣ 2022

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VOLUME 34, ISSUE IV,
OCTOBER - DECEMBER 2022

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ΕΤΑΙΡΕΙΑΣ

PHARMAKEFTIKI

A QUARTERLY JOINT EDITION OF
THE HELLENIC SOCIETY OF
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ΦΑΡΜΑΚΕΥΤΙΚΗ, 34, IV, 2022 | 144-149 ΣΥΝΤΟΜΗ ΕΠΙΣΚΟΠΗΣΗ

PHARMAKEFTIKI, 34, IV, 2022 | 144-149 MINI REVIEW

An overview of Dysregulation of miRNAs in Th1, Th2 and Treg cells responsible for the pathogenesis of multiple sclerosis.

Dimitrios Pavlos[1]*, Thomas Mavromoustakos[1]*

[1]Department of Chemistry, National and Kapodistrian University of Athens, Zographou, 15784 Athens, Greece; dpavlos@chem.uoa.gr (D.P.); tmavrom@chem.uoa.gr (T.M.).

KEYWORDS:
Multiple sclerosis, microRNA, Dysregulation of miRNAs, Th1, Th2 and Treg cells.

ABSTRACT

An additional important factor in the pathogenesis of multiple sclerosis according to some recent studies is the role of microRNA, an unencoded RNA consisting of approximately 22 nucleotides that is primarily involved in the posttranscriptional regulation of gene expression[1,2]. This section lists the miRNAs associated with the pathogenesis of Multiple Sclerosis (MS) in Th1, Th2 and Treg cells.

ARTICLE INFO:
Received: October 22, 2022
Accepted: November 5, 2022
Published on line: January 13, 2023

*** CORRESPONDING AUTHOR:**
Dimitrios Pavlos
dpavlos@chem.uoa.gr
and
Thomas Mavromoustakps:
tmavrom@chem.uoa.gr

1. Introduction

Multiple Sclerosis (MS) is an autoimmune disease that affects the central nervous system (CNS). It is the result of myelin alteration and is found more specifically in peritoneal white matter, the spinal cord, the optic nerve, brain stem and cerebellum[1]. Multiple Sclerosis (MS) occurs mainly in young adults, preferably females, with the main symptom being clinical seizures of neurological dysfunction lasting more than 24 hours. The diagnosis of (MS) can be made with a clinical evaluation supported by relevant research. In fact, there is no predetermined diagnostic test[2].

The three types of multiple sclerosis that have been identified include Relapse-Remitting MS (RRMS), Secondary Progressive MS (SPMS) and Primary Progressive MS (PPMS). RRMS occurs in 85% of young people aged 20 years and then recurrent episodes of the disease occur with severe symptoms and remission intervals. SPMS is a continuation of RRMS and occurs between 10 and 20 years after the diagnosis of RRMS. PPMS occurs in 10% of middle-aged people 40 years of age with reduced and undefined symptoms. Although the pathogenesis of MS has not been precisely elucidated, the cause consists of both genetic predisposition (heredity) and environmental factors. The result of the application of these factors is the demyelination of the nerves and the neuronal dysfunction[3].

In patients with MS it has been observed that T-cells through a mechanism of action contribute to the

formation of inflammatory responses mainly due to the release of cytokines by immune cells[4-5]. An additional important factor in the pathogenesis of multiple sclerosis according to some recent studies is the role of microRNA, an unencoded RNA consisting of approximately 22 nucleotides that is primarily involved in the posttranscriptional regulation of gene expression[6,7].

Mir-155 RNA is a miRNA that plays a very important role in the regulation of the immune system. More specifically, it is observed that its concentration increases in peripheral blood B cells in patients with rheumatoid arthritis (RA) compared to in peripheral blood B cells in healthy people. It has also been observed that antibody production is reduced and PU.1 is restored (a protein that in humans is encoded by the SPI1 gene)[8]. In addition ***miR-29***, ***miR-15a*** and ***miR-16-1*** as specific cell regulators are involved in the pathogenesis of MS[9,10]. It is noteworthy that the changes in the concentration of several other miRNA species in body fluids such as plasma, serum, etc. in patients with MS can be important biomarkers in both the early diagnosis and treatment of this disease[11-13].

This article seeks to highlight and analyze the role of miRNAs in Th1, Th2 and Treg cells responsible for the pathogenesis of MS.

2. Immune system and biological origin of miRNAs

Examining the biogenesis of miRNAs we find transformations and stages such as: The pri-miRNA to a miRNA precursor (pre-miRNA), transportation of pre-miRNA into cytoplasm both catalyzed by specific enzymes such as Drosha and Exportin5 respectively. A generation of a dual RNA structure has also been identified with integration of miRNA into a RNA-induced silencing complex (RISC) which recognizes the strand with the weakest hydrogen bond at its 5'. [14-16]

The role of non-coding microRNAs as a class of gene regulators associated with immune function is mentioned here. For example, ***miR-155*** is the growth of inflammatory T-lymphocytes (Th17 and Th1) as well as the formation of Th17 cells in experimental autoimmune encephalomyelitis (EAE) making it a promising therapeutic target [17]. In addition, the decrease in the concentration of ***miR-326*** resulted in the formation of fewer Th-17 cells associated with EAE, while on the contrary, an increase in the concentration of ***miR-326*** led to serious disease [18,19]. In contrast, ***miR26a*** reversed the effect of ***miR-326*** on Th-17 cytokines and EAE, while the Foxp3 transcription factor associated with Treg cells was positively associated with ***miR26a***, which was found to eventually reduce Th-17 cells[20]. The function of ***miR-146a*** and ***miRNA-101*** as effective biomarkers for diagnosis and prognosis as well as their potential use as therapeutic molecules is well established[21,22].

3. Involvement of miRNAs in the pathogenesis of multiple sclerosis

It is now well established that in the various pathological processes that take place during the period of MS pathogenesis, there are lesions of Th1, Th2 and Treg cells caused by specific known reasons that are closely related to the dysregulation of the expression of miRNA due to the fact that miRNA transcription is differentiated into different subunits of immune cells.

3.1. Dysregulation of miRNAs in Th1 and Th2 cells

The ***mir-29b*** belongs to the ***miR-29*** family. It should be mentioned that it plays an important role in the development of abdominal aortic aneurysm, in the development and progression of cancerous tumors, in the pathogenesis of type 1 diabetes and is associated with irritable bowel syndrome . In this case the increased expression of ***miR-29b*** in patients with multiple sclerosis reflects the problem of regulating the balance in Th1 cells and preventing T-bet and IFN-γ (a dimerized soluble cytokine that is the only member of the type II class of interferons) transcription, thus contributing to the differentiation of Th1 cells [9].

It has also been observed that ***miR-27b*** and ***miR-***

Table 1. MiRNAs in immune cells from patients with multiple sclerosis.

miRNA	Cells/tissues expressed	Functions	Target	Expression change	References
miR-29b	$CD4^+$ T cells	Regulates Th1 cell Inhibits T-bet and IFN-γ transcription	T-bet IFN-γ	-	9
miR-27b	$TCD4^+$	Inhibits MS development	BMI1 GATA3	Increased	23
miR-27b-2	$CD4^+$ T cells	Th2 to Th1 shift	-	-	24
miR-128	$CD4^+$ T cells	Th2 to Th1 shift	-	-	24
miR-128	$TCD4^+$	Inhibits MS development	BMI1 GATA3	Increased	24
miR-340	$CD4^+$ T cells	Th2 to Th1 shift	BMI1 IL-4	Decreased	9,24
miR-142-3p	$CD4^+$ T cells and $CD8^+$ T	Suppresses function of Treg cells	Foxp3	Increased	25,26
miR-25	$CD4^+$ T cells, B cells	Promote Treg	-	-	27
miR-25	Treg	Regulates of TGF-β signaling pathway	CDKN1 A/p21	Decreased	27
miR-146a	MS lesions	Modulates of TLRs signaling	TLRs	Increased	28
miR-146a	Monocytes **DC**	Regulates of TLR signaling pathway	TRAF6 IRAK1	Increased	29
miR-146a	Murine tregs	Regulates of immune and inflammatory signaling systems	FADD AP-1	Increased	30

128 contribute to the inhibition of the expression of BMI1 (Polycomb complex protein), GATA3 and IL-4 genes and thus cause a change in the response from Th2 to Th1 which leads to pathogenesis of MS[23] while ***miR-340*** that has a similar effect to BMI1 and IL-4. ***MiR-24*** also traps IFNγ in Th1 cells and plays

the regulatory pole for the development of autoimmune diseases including MS[9,24].

3.2 Dysregulation of miRNAs in Treg cells.

The role of Treg cells in inhibiting T cell proliferation and preventing the onset of autoimmune diseases has been demonstrated. ***MiR-142-3p*** is shown to be suppressed by Forkhead box P3 (Foxp3) and thus increase the production of Cyclic adenosine monophosphate (cAMP) thereby suppressing the activity of Treg cells[25,26]. The reduced expression of ***miR-25*** based on the recording in **Table 1** which regulates of Transforming growth factor beta (TGF-β) signaling pathway is a result of the targeting against Cyclin Dependent Kinase Inhibitor 1A/ P21 (CDKN1 A/ P21)[27].

MiR-146a also has an anti-apoptotic role and reduces the expression of cell death caused by Activation- Induced Cell Death (AICD) as well as has the ability to reduce the production of interleukin 2 (IL2) which plays an essential role in T cell production[26,28-30]. It is also proven to increase its expression by fighting against TNF receptor-associated factor 6 and interleukin-1 receptor-associated kinase 1 (TRAF6IRAK1), FADD AP-1 and Toll-like receptors (TLRs) and regulates of TLR signaling pathway[28-30].

The role of ***miR-27a*** in remyelination or more specifically in the multi-step process of conversion of oligodendrocyte progenitor cells (OPCs) to new oligodendrocytes (OLs) is very important because increased levels of ***miR-27a*** inhibit OPC proliferation by disrupting the cell cycle and deregulating the Wnt-β-catenin signaling pathway and thus associated multiple MS lesions. *In vivo* administration of ***miR-27a*** has similar results[31].

4. Summary

In this article is analyzed the role of dysregulation of miRNAs in Th1, Th2 and Treg cells responsible for the pathogenesis of multiple sclerosis and records data gathered in **Table 1**. MiR-29b, miR-27b and miR-128 have been shown to be related in the response from Th2 to Th1 which leads to pathogenesis of MS. On the other hand, the disturbance of the balance of Treg cells as the result of the action of miR-142-3p, miR-25, miR-146a and miR-27a contributes in turn to the pathogenesis of multiple sclerosis. □

References

1. Katsara M., Apostolopoulos V. Editorial: Multiple Sclerosis: Pathogenesis and Therapeutics. *Med. Chem. (Shariqah (United Arab Emirates)).* 14 (2), 104–105, 2018.
2. Ford H. Clinical presentation and diagnosis of multiple sclerosis. *Clin. Med. (Lond)*. 20(4), 380–383, 2020.
3. Ghasemi N., Razavi S., Nikzad E., Multiple Sclerosis: Pathogenesis, Symptoms, Diagnoses and Cell-Based Therapy. *Cell J.* 19(1), 1–10, 2017.
4. Almuslehi M.S.M., Sen M.K., Shortland P.J., Mahns D.A., Coorssen J.R. CD8 T-cell Recruitment Into the Central Nervous System of Cuprizone-Fed Mice: Relevance to Modeling the Etiology of Multiple Sclerosis. *Front. Cell Neurosci.*. 14, 43, 2020.
5. Lee D.J., The relationship between TIGIT(+) regulatory T cells and autoimmune disease. *Int. Immunopharmacol*. 83, 106378, 2020.
6. Angelou C.C., Wells A.C., Vijayaraghavan J., Dougan C.E., Lawlor R., Iverson E., Lazarevic V., Kimura M.Y., Peyton S.R., Minter L.M., Osborne B.A., Pobezinskaya E.L., Pobezinsky L.A. Differentiation of Pathogenic Th17 Cells Is Negatively Regulated by Let-7 MicroRNAs in a Mouse Model of Multiple Sclerosis. *Front. Immunol*. 10, 3125, 2019.
7. Zhou Y., Chen M., Simpson Jr. S., Lucas R.M., Charlesworth J.C., Blackburn N., van der Mei I., Ponsonby A.L., Taylor B.V., Common genetic variation within miR146a predicts disease onset and relapse in multiple sclerosis. *Neurol. Sci.*

39 (2), 297–304, 2018.
8. Alivernini S., Kurowska-Stolarska M., Tolusso B., Benvenuto R., Elmesmari A., Canestri S., Petricca L., Mangoni A., Fedele A.L., Di Mario C., Gigante M.R., Gremese E., McInnes I.B., Ferraccioli G. MicroRNA-155 influences B-cell function through PU.1 in rheumatoid arthritis. *Nat. Commun*. 7, 12970, 2016.
9. Smith K.M., Guerau-de-Arellano M., Costinean S., Williams J.L, Bottoni A., Mavrikis Cox G., Satoskar A.R., Croce C.M., Racke M.K., Lovett-Racke A.E., Whitacre C.C. miR-29ab1 deficiency identifies a negative feedback loop controlling Th1 bias that is dysregulated in multiple sclerosis*. J. Immunol*. 189 (4), 1567–1576, 2012.
10. Lorenzi J.C., Brum D.G., Zanette D.L., de Paula Alves A., Souza F.G., Dos Barbuzano A.C., Santos A.A., Barreira W.A., da Silva W.A. miR-15a and 16–1 are downregulated in CD4+ T cells of multiple sclerosis relapsing patients. *Int. J. Neurosci*. 122 (8), 466–471, 2012.
11. Cortez M.A., Bueso-Ramos C., Ferdin J., Lopez-Berestein G., Sood A.K., Calin G.A. MicroRNAs in body fluids–the mix of hormones and biomarkers, *Nature reviews. Clin. Oncol*. 8 (8), 467–477, 2011.
12. Yang D., Wang W.Z., Zhang X.M., Yue H., Li B., Lin L., Fu J. MicroRNA expression aberration in Chinese patients with relapsing remitting multiple sclerosis*. J. Mol. Neurosci*. 52 (1), 131–137, 2014.
13. Haghikia A., Haghikia A., Hellwig K., Baraniskin A., Holzmann A., B.F. Decard B.F., Thum T., Gold R. Regulated microRNAs in the CSF of patients with multiple sclerosis: a case-control study. *Neurology* 79 (22), 2166–2170, 2012.
14. Zeng Y., Yi R., Cullen B.R. Recognition and cleavage of primary microRNA precursors by the nuclear processing enzyme Drosha. EMBO J. 24,138-148, 2005.
15. Carthew R.W., Sontheimer E.J. Origins and mechanisms of miRNAs and siRNAs. *Cell* 136, 642-655, 2009.
16. Grimson A., Farh K.K., Johnston W. K., Garrett-Engele P., Lim L.P., Bartel D.P. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol. Cell* 27:91-105, 2007.
17. O'Connell R.M., Kahn D, Gibson W.S., Round J.L., Scholz R.L., Chaudhuri A.A., Kahn M.E., Rao D.S., Baltimore D. MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. *Immunity* 33 (4), 607–619, 2010.
18. Du C., Liu C., Kang J., Zhao G., Ye Z., Huang S., Li Z., Wu Z., Pei G. MicroRNA miR326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. *Nat. Immunol*. 10 (12), 1252–1259, 2009.
19. Zhang R., Tian A., Wang J., Shen X., Qi G., Tang Y. miR26a modulates Th17/T reg balance in the EAE model of multiple sclerosis by targeting IL6. *NeuroMol*. *Med.* 17 (1), 24–34, 2015.
20. Potenza N., Mosca N., Mondola P., Damiano S,. Russo A., De Felice B. Human miR26a-5p regulates the glutamate transporter SLC1A1 (EAAT3) expression. Relevance in multiple sclerosis. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864 (1), 317–323, 2018.
21. Takahashi K., Yan I., Wen H.J., Patel T. microRNAs in liver disease: From diagnostics to therapeutics. *Clin. Biochem*. 46, 946-952, 2013.
22. Gui T., Shen K. miRNA-101: a potential target for tumor therapy. *Cancer Epidemiol*. 36, 537-540, 2012.
23. Yao J., Deng B., Zheng L., Dou L., Guo Y., Guo K. MiR-27b Is Upregulated in Cervical Carcinogenesis and Promotes Cell Growth and Invasion by Regulating CDH11 and Epithelial-Mesenchymal Transition. *Oncol. Rep. 35* (3), 1645–1651, 2016. https://doi.org/10.3892/or.2015.4500.
24. Guerau-de-Arellano M., Smith K. M., Godlewski J., Liu Y., Winger R., Lawler S. E., Whitacre C. C., Racke M. K., Lovett-Racke A. E. Micro-RNA Dysregulation in Multiple Sclerosis Favours pro-Inflammatory T-Cell-Mediated Autoimmunity. *Brain 134* (12), 3578–3589, 2011. https://doi.org/10.1093/brain/awr262.
25. Arruda L. C. M., Lorenzi J. C. C., Sousa A. P. A., Zanette D. L., Palma P. V. B., Panepucci R. A., Brum D. S., Barreira A. A., Covas D. T., Simões B. P., Silva W. A., Oliveira M. C., Malmegrim K. C. R. Au-

tologous Hematopoietic SCT Normalizes MiR-16, -155 and -142-3p Expression in Multiple Sclerosis Patients. *Bone Marrow Transplant. 50* (3), 380–389, 2015. https://doi.org/10.1038/bmt.2014.277.

26. Junker A., Krumbholz M., Eisele S., Mohan H., Augstein F., Bittner R., Lassmann H., Wekerle H., Hohlfeld R., Meinl E. MicroRNA Profiling of Multiple Sclerosis Lesions Identifies Modulators of the Regulatory Protein CD47. *Brain 132* (12), 3342–3352, 2009. https://doi.org/10.1093/brain/awp300.
27. De Santis G., Ferracin M., Biondani A., Caniatti L., Rosaria Tola M., Castellazzi M., Zagatti B., Battistini L., Borsellino G., Fainardi E., Gavioli R., Negrini M., Furlan R., Granieri E. Altered MiRNA Expression in T Regulatory Cells in Course of Multiple Sclerosis. *J. Neuroimmunol. 226* (1–2), 165–171, 2010 . https://doi.org/10.1016/j.jneuroim.2010.06.009.
28. Wu D., Cerutti C., Lopez-Ramirez M. A., Pryce G., King-Robson J., Simpson J. E., van der Pol S. M., Hirst M. C., de Vries H. E., Sharrack B., Baker D., Male D. K., Michael G. J., Romero I. A. Brain Endothelial MiR-146a Negatively Modulates T-Cell Adhesion through Repressing Multiple Targets to Inhibit NF-KB Activation. *J. Cereb. Blood Flow Metab. 35* (3), 412–423, 2015. https://doi.org/10.1038/jcbfm.2014.207.
29. Jurkin J., Schichl Y. M., Koeffel R., Bauer T., Richter S., Konradi S., Gesslbauer B., Strobl H. MiR-146a Is Differentially Expressed by Myeloid Dendritic Cell Subsets and Desensitizes Cells to TLR2-Dependent Activation. *J. Immunol. 184* (9), 4955–4965, 2010. https://doi.org/10.4049/jimmunol.0903021.
30. Li L., Chen X.-P., Li Y.-J. MicroRNA-146a and Human Disease. *Scand. J. Immunol. 71* (4), 227–231, 2010. https://doi.org/10.1111/j.1365-3083.2010.02383.x.
31. Tripathi A., Volsko C., Garcia J.P., Agirre E., Allan K.C., Tesar P.J., Trapp B.D., Castelo-Branco G., Sim F.J., Dutta R. Oligodendrocyte Intrinsic miR-27a Controls Myelination and Remyelination. *Cell Rep.* 29(4), 904-919, 2019. doi: 10.1016/j.celrep.2019.09.020.

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 34, IV, 2022 | 150-155 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 34, IV, 2022 | 150-155 RESEARCH ARTICLE

Antioxidant and Antiangiogenic Activities Assessementof *Abutilon indicum* Ethanolic Root Extract

Garlanka Venkata Naga Kiranmayi[1*], Kakaraparthy Ravishankar[2], Narayanaswamy Harikrishnan[1], Karumanchi Srikanth Kumar[3]

[1]Faculty of Pharmacy, Dr. M. G. R. Educational and Research Institute, Chennai - 95, Tamil Nadu, India.
[2]Aditya college of Pharmacy, ADB Road, Surampalem, 533 437, East Godavari District (India)
[3]Department of Pharmaceutical Chemistry, QIS College of Pharmacy, Ongole -523272, Andhra Pradesh , India

KEYWORDS:
Antioxidant, Antiangiogenic, Chick chorioallantoic membrane assay, neovascularization and Abutilon indicum

ABSTRACT

The current study aims to identify the antioxidant and antiangiogenic effects of the ethanolic root extract of Abutilon indicum. Antioxidant activity was evaluated against a range of free radicals, including nitric oxide scavenging and hydroxyl radicals tests, using ascorbic acid as a standard. The chorioallantoic membrane of the chick embryo is the most popular in vivo assay for assessing antiangiogenic effectiveness (CAM). The ethanolic root extract of Abutilon indicum has a significant antioxidant status as evidenced by its IC50 values of 21.4 ug/mL for nitric oxide radicals and 22.3 ug/mL for hydroxyl radicals. The results are on par with ascorbic acid's. The percentage of inhibition of antioxidant activity was estimated by computing IC50 values. It prevents neovascularization, which is probably connected to the dose-dependent decrease in the development of capillary networks (50 to 150 g/egg). The investigation's findings demonstrated that the chorioallantoic membrane had a significant antiangiogenic effect. The plant extract has shown effective free radical scavenging action, as indicated by their percentage inhibition. It exhibits considerable antiangiogenic properties as well, which may be the reason for its historical use as an anticancer medication.

ARTICLE INFO:
Received: September 22,2022
Revised : November, 2 2022
Accepted: November 9, 2022
Published on line: January 13, 2023

*** CORRESPONDING AUTHOR:**
G.V.N.Kiranmayi
E-mail: kiranmayi54@gmail.com
7286863529

Introduction:

The tiny herb *Abutilon indicum is indigenous to tropical and subtropical areas. The roots and leaves of this plant are used to treat fevers, and it is also a valued ornamental and medicinal plant. It has been widely distributed and several tropical islands consider it invasive.* Tumor angiogenesis is the outcome of an angiogenic imbalance in which proangiogenic factors exceed antiangiogenic factors[1,2]. Angiogenesis is the process by which new blood vessels form. All angiogenic processes in adults are harmful, with the exception of a few physiological process-

es including menstruation, wound healing, and placental development.[3-5]. It is hoped that by preventing the creation of new blood vessels, the tumor's supply of oxygen and nutrients, as well as its ability to grow and spread to other body parts, will be cut off[6-8]. The plant could be beneficial in malignancies at different degrees and with different mechanisms, according to the present research, which is still in progress[9]. The *Abutilon indicum* ethanolic root extract was evaluated for its antioxidant and antiangiogenic properties.

Materials and methods

Collection of plant

The roots of *Abutilon indicum*(L.) Sweet(Malvaceae) were collected from Peddapuram area of East Godavari dt., of Andhra Pradesh. Dr. T. RAGHURAM, a taxonomist at Maharani College in Peddapuram, performed the plant authentication and given specimen number 12564 .

Preparation of extract :

A. indicum plant roots that had just been harvested were thoroughly cleaned, dried in the shade for around 30 days, then chopped into small pieces and powdered into a coarse powder. Following a 72-hour maceration with 95 % ethanol, the dried powder material was extracted using hot percolation. Desiccators were used to dry the concentrated product after it was acquired.

Chemicals and instruments:

Ascorbic acid, Hydrogen peroxide, Methanol, Sulphanilamide, ortho phosphoric acid, naphthylethylenediaminedihydrochloride, Sodiumsalicylate, Ferrous sulphate, prednisone.

Incubator, U.V Spectroscopy, Colorimeter, Hot air oven.

Preliminary phytochemical screening :

A. indicum extract underwent preliminary phytochemical screening to see whether it contained any active chemical components, such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, fixed oils, and lipids[10].

Quantitative Phytochemical testing

Aliquots of extract was prepared by dissolving 10mg of individual extracts in 10 mL of methanol to get 1000µg/ml.

Estimation of Phenolic Contents

Using the Folin-Ciocalteu method, the phenolic content of the ethanolic root extract of *A. indicum* (1 mg/ml, aliquots) was ascertained[11]. A mixture of 3ml of Folin-Ciocalteu reagent and 0.5ml of extract was diluted 1:10 v/v before being let to stand for 5min. The mixture tube was filled with 4ml of sodium carbonate solution that was 20% w/v. The tubes were set aside for colour development for 15 minutes at 30°C. Using a spectrophotometer, the absorbance was measured at 765 nm. Gallic acid standard in methanol was used to assess the phenolic content, and the results were represented as gallic acid equivalent mg/100mg dry weight of extract.

Estimation of total Flavanoids

By using the aluminium chloride method, the total flavonoid content of the ethanolic root extract of *A. indicum* (1 mg/ml, aliquots) was identified[12]. The following ingredients were added to 0.6 ml of aliquots of extract: 1.8 ml methanol, 0.1 mL 10% aluminium chloride, 0.1 mL 1M sodium acetate, and 3 ml distilled water. The mixture was then kept at 30^0C. After 30 minutes, each absorbance was measured at 415 nm. Using standard quercetin in methanol, total flavonoid was calculated from the calibration curve, and the results were represented as quercetin equivalent mg/100 mg dry weight of extract.

Estimation of alkaloids

The method developed was used to determine the extract's alkaloid concentration[13]. After being treated in 2N hydrochloric acid, the ethanolic root extract of *A. indicum* (1 mg/mL, aliquots) was filtered. 0.1 N NaOH was added to the filtrate, 1 ml of this solution was transferred to a separating funnel, and then 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. Chloroform was used to remove the mixture after shaking it. At 470 nm, the absorption was detected. The concentration of alkaloid content in atropine equivalents was measured using the units mg/100mg dry weight of extract. Alkaloid

Table1: Quantitative phytochemical determination of Ethanolic root extract of A. indicum

S.No	Total Phenolics mg/g	Total flavanoids mg/g	Total alkaloids mg/g
Ethanolic root extract of A. *indicum*	10.04±0.19	38.06±0.28	32.12±0.38

All the values are expresses as mean ± SEM, n=3

Table 2: Effect of Ethanolic A. indicum root Extract against NO and hydroxyl radical

Tested Material	Concentration (µg/ml)	NO method		Hydroxyl method	
		%Inhibition	IC50 ug/ml)	%Inhibition	IC50 (ug/ml)
***A. indicum* Ethanolic root extract**	50	50.71±0.001	44.77	44.8±0.06	57.27
	100	62.22±0.005		62.76±0.16	
	300	75.41± 0.004		80.52± 0.04	
	500	83.74± 0.007		85.36±0.15	
Ascorbic acid	50	55.36 ±0.22	33.11	69.47±0.32	1.69
	100	60.32±0.27		82.55±0.26	
	300	70.85±0.13		83.46±0.12	
	500	80.32±0.12		87.65±0.16	

Values are indicated in terms of Mean ± SEM; n=3 in each concentration;

content was determined from the calibration curve using standard atropine calibration curve.

Evaluation of In-vitro antioxidant activity:

Hydroxyl Radical Scavenging Assay

The sample extracts were tested for their capacity to scavenge hydroxyl radicals using a modified version of the procedure described by Smirnoff and Cumbes[14]. Individual sample extracts (1 mL) were added to a reagent comprising 1 mL 1.5 mM $FeSO_4$, 0.3 mL 20 mM sodium salicylate, and 0.7 mL 6 mM H2O2 at varied concentrations (50, 100, 300, and 500 ug/ml). Later, the sample was incubated for 1 hour at a temperature of 37°C, and the reaction mixture's absorbance was measured at 562 nm.

Scavenging ability on hydroxyl radicals (%) = $[(Ao–A^1)/Ao] \times 100$

A^1 denotes the sample extract absorbance and Ao denotes the absorbance of the control reaction, which contains all reagents except the sample extract. Positive controls included ascorbic acid in the study.

NO scavenging activity

The extract's ability to scavenge NO was evaluated using the Marcocci and colleagues' approach[15]. In a nutshell, different concentrations of the test sample (100-1000 g/ml) were combined with sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) (pH 7.4), and incubated at 25°C for 150 minutes. After incubation, the Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% 1-naphthylethylenediamine dihydrochloride in water) was used to determine the amount of nitrite formed from sodium nitroprusside. At 570 nm, the absorbance was immediately measured. The positive control utilised was catechin.

NO scavenging activity (%) = $[(Ao–A^1)/Ao] \times 100$

Table 3: Antiangiogenic activity of ethanolic extract of A. *indicum root*

Tested Material	Egg 1	Egg 2	Egg 3	Average No. of Vessels	Egg 1	Egg 2	Egg 3	Average No. of Vessels
	No. of Vessel in untreated CAM				No. of Vessel in treated CAM			
Normal control (0.9% NaCl)	8	10	7	8.33	9	10	7	8.67
Positive control (Prednisone 5mg/ml)	12	9	10	10.33	3	4	3	3.33
EEAI 25µg/egg	11	9	8	9.33	9	7	6	7.33
EEAI 50µg/egg	10	10	12	10.67	7	5	5	5.67
EEAI 75 µg/egg	8	7	9	8.00	5	4	4	4.33
EEAI 150 µg/egg	8	9	9	8.67	4	3	4	3.67

A^1 denotes the sample extract absorbance and Ao denotes the absorbance of the control reaction, which contains all reagents except the sample extract. Positive controls included ascorbic acid in the study.

Chicken egg Chorioallantoic Membrane (CAM) Assay[16]

The evaluation of antiangiogenic activity was done using this unique in ovo angiogenesis assay. At day '0', fertilised leghorn chicken eggs were obtained from a nearby hatchery and examined for damage. They were arranged in groups of six eggs each at random. The eggs were cleaned with 70% ethanol before being incubated at 370°C with continuous humidity.

A tiny hole was bored on the third day, and 2-3 ml of albumin were removed. The specific window was later sealed using clear tape and retained once more for incubation. Once more on the seventh day, a little square window in the shell was opened, and sterile gel foam was inserted into the membrane.

Sterile normal saline was used to impregnate the vehicle control group, while the standard and test groups received their corresponding doses. The eggs were put back in the incubator, where they remained unaltered until day 14. On the fourteenth day of incubation, the eggs were taken out of the incubator, and the CAM tissues beneath each sponge in the control and treated CAM samples were also taken out. Later, after being stained with hematoxylin and eosin and put in formalin, tissues were examined under a trinocular microscope.

Results from CAM preparations were examined for each treatment group based on the number of vessel branch points in the square region corresponding to the area of each sponge.

Eggs were divided into the following groups -

- Group 1: Normal control (0.9% NaCl)
- Group 2: Positive control (Prednisone 5mg/ml)
- Group 3: EEAI25µg/egg (EECO – Ethanolic extract of *Abutilon indicum**)***
- Group 4: EEAI50µg/egg
- Group 5: EEAI75µg/egg
- Group 6: EEAI150µg/egg

Results and Discussion

Preliminary and Quantitative Phytochemical screening :

The results implies that extract contains alkaloids, flavonoids, saponins, carbohydrates, proteins and Amino acids which are the main phytochemical groups with biological activities.The results of QuantitativePhytochemical screening was tabulated **(Table 1).**

The optimal use of the natural resources is made pos-

Table 4: Anti-angiogenic effect of of ethanolic extract of A. *indicum root*

Tested Material	Egg 1	Egg 2	Egg 3	Average Percentage of vessels inhibition (%)
	Percentage of vessels inhibition (%)			
Normal control (0.9% NaCl)	-12.5	0	0	4.17±4.17
Positive control (Prednisone 5mg/ml)	75	55.55	70	66.85±5.83
EEAI 25µg/egg	18.18	22.22	25	21.8±1.98
EEAI 50µg/egg	30	50	58.33	46.11±8.41
EEAI 75 µg/egg	42.8	28.5	55.55	42.28333±7.82
EEAI 150 µg/egg	50	66.66	55.55	57.40333±4.90

Values are expressed as mean ± SEM, (n=3)

sible by phytochemical studies, which are particularly helpful in identifying therapeutically beneficial new sources of chemicals. The results indicate that the extract contains alkaloids, flavonoids, saponins, carbohydrates, proteins, and amino acids, which are the primary phytochemical groups with biological activity. Table 1 contains a summary of the quantitative phytochemical analyses' findings.

Using several antioxidant techniques, the results of antioxidant activity were expressed in terms of IC50 values. For Abutilon indicum, the computed IC50 values using the Nitric oxide and Hydroxyl radicals technique are 44.77 g/ml and 57.27 g/ml, while for ascorbic acid, they are 33.11 g/ml and 1.69 g/ml. Table 2 presents the outcomes. Based on the findings, it may be hypothesised that Abutilon indicum root extract reacts with the hydrogen donor in the antioxidant principles to convert radicals into the matching hydrazine. The Abutilon indicum's capacity to scavenge free radicals is concentration-dependent. Better protective activity is reflected by a lower IC 50 value.

The compounds found in these plants may be responsible for the angiogenic activities of *Abutilon indicum* root extract.Angiogenesis is the formation of new blood vessels from the vascular bed already present and is a characteristic of cancer. They are prospective targets for therapy in all types of cancer due to a variety of variables, including genetic instability and the contrast between tumour blood arteries and normal vessels. Cancer appears to be fueled by persistently increased angiogenesis, as is the case in many other clinical disorders, such as diabetic retinopathy, inflammation, hemangiomas, arthritis, psoriasis, and atherosclerosis.[17,18] In the healthy human body, angiogenesis is a tightly regulated process that is governed by a number of endogenous angiogenic and angiostaticfactors[19].

The work is motivated by the need for more effective natural antiangiogenic medicines to replace pharmaceutical therapies. Realizing that the purpose of this work was to assess the antiangiogenic potential of *Abutilon indicum* root extract utilising a CAM model.

The new pharmacological actions of *Abutilon indicum* root have been verified by the demonstrated suppression of angiogenesis in the chick CAM model. At all concentrations of treatment examined, Abutilon indicum root extract exhibited considerable antiangiogenic activity (Table 4); these extracts of Abutilon indicum root lessen CAM neovascularization as well as deformation of existing vasculature.

According to Folkman et al, the aim is to stop the creation of new blood vessels in order to stop the tumour from receiving oxygen and nutrients, which would otherwise cause it to expand and spread to other parts of the body[20-22]. This might be as a result of the phytochemicals found in these plants inducing apoptosis[23].

The findings of this study indicate that A. indicum root extract demonstrate robust antiangiogenic activity in a concentration-dependent manner and may have the

potential to be an effective tumour inhibitor. This activity may be caused by the presence of active ingredients such flavonoids. As a result, A. indicum is a powerful antiangiogenic substance. Nevertheless, more research is required.

Conclusion

As evidenced by their percentage inhibition, the plant extract has demonstrated effective free radical scavenging action. The plant employed in this study might serve as a source of fresh anticancer medications. □

References

1. Giordano F.J., Johnson R.S. Angiogenesis: the role of the microenvironment in flipping the switch. *Current Opin Genet. Dev*. 11,35-40 ,2001.
2. Udagawa T., Fernandez A., Achilles E.G., Folkman J., D'Amato R.J. Persistence of microscopic human cancers in mice: alterations in the angiogenic balance accompanies loss of tumor dormancy. *The FASEB J*. 16,1361-70 , 2002.
3. Carmeliet P. Angiogenesis in health and disease. *Nature Med*. 9, 653-660, 2003.
4. Brown R.A., Weiss J.B. Neovascularisation and its role in the osteoarthritic process. *Ann Rheumatic Dis*. 47, 881-885, 1988.
5. Waltman D., Gitter K., Yannuzzi L., Schatz H. Choroidal neovascularization associated with choroidal nevi. *Am J. Ophthalmol*. 85,704-710,1978.
6. Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. *Ann Surg*. 175 ,409-416,1972.
7. Folkman J., Kalluri R. Cancer without disease. *Nature*. 427,787, 2004.
8. Moses M., Langer R. Inhibitors of angiogenesis. *Biotech.* 9,630-634,1991.
9. Rao M.P. Ashwagandha (Withania somniifera)-ayurvedic bequest for the patients of cancer: an update on current research. *Int J Res Ayurveda Pharma*.1,234-38, 2010.
10. Khandelwal K. R(2004). Practicalpharmacognosy 11th *ed. Pune, NiraliPrakashan Press,*pp. 149–156.
11. Singleton V.L., Orthofer R., Lamuela-Raventós R.M.Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.*299,152-178, 1999.
12. Chang C.C., Yang M.H., Wen H.M., Chern J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods**.** *J. Food Drug Anal.* **10**,178-182,2002.
13. Fazel S., Hamidreza M., Rouhollah G., Verdian-rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Soc. Adv. Hort*. 12, 69-70,2010.
14. Smirnoff N., Cumbes Q.J. Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* 28,1057-60, 1989.
15. Marcocci L, Maguire J.J, Droy-Lafaix M.T. , Packer L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. *Biochem Biophys Res Commun*, 201 , 748-755,1994.
16. Lokman N.A., Elder A.S.F., Ricciardelli C., Oehler M.K. Chick Chorioallantoic Membrane (CAM) Assay as an In Vivo Model to Study the Effect of Newly Identified Molecules on Ovarian Cancer Invasion and Metastasis. *Int. J. Mol. Sci.* 13, 9959-9970, 2012.
17. Martínez-Poveda B., Quesada A.R., Medina M.Á. Hyperforin, a bio-active compound of St. John's Wort, is a new inhibitor of angiogenesis targeting several key steps of the process. *Int. J. Cancer* 117,775-780,2005.
18. Ribatti D. (2009) in: History of research on tumor angiogenesis. Chapter 1 pp 1-7, Springer Science and Busmess Media B.V.
19. Folkman J., Klagsbrun M. Angiogenic factors. *Science*. 235, 442-447,1987.
20. Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. *Ann Surg.* 175,409-416,1972.
21. Folkman J., Kalluri R. Cancer without disease. *Nature.* 427,787,2004 .
22. Moses M,. Langer R. Inhibitors of angiogenesis. *Biotech*. 9,630-34, 1991.
23. Kalimuthu K., Prabakaran R., Saraswathy M. Antiangiogenic activity of Boucerosia diffusa and Boucerosia truncatocoronata extracts in chick Chorioallantoic Membrane (CAM). *Int. J. Cur. Microbiol. Applied Sci*. 3,107-114,2014.

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 34, IV, 2022 | 156-165 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 34, IV, 2022 | 156-165 RESEARCH ARTICLE

Study of Commodity Aspects of Compressor Nebulizers in Ukraine

Alla Kotvitska[1], Yuliia Bezpala[2*], Inna Baranova[2], Qobiljon Khushvakhtzoda[3], Safol Musozoda[4]

[1]National University of Pharmacy, Kharkov, Ukraine.

[2]Department of Organization and Economy in Pharmacy of the National University of Pharmacy (NUPh), Kharkov, Ukraine.

[3]Tajik National University, Dushanbe, Tajikistan

[4]Faculty of Pharmacy, Tajik National University, Dushanbe, Tajikistan

KEYWORDS:
compressor nebulizer, assortment, commodity analysis

ABSTRACT

Nebulizer therapy is an effective and safe treatment for respiratory diseases. Since the delivery of the pharmacologic agents to the lungs depends on the correct use of the inhalation device, the pharmacist, like the doctor must have all the skills in choosing a nebulizer, namely to be informed about the modern range of nebulizers, know the basic technical characteristics of these devices, their configuration, features of use, caveats for their use, as well as labeling and storage. Materials and methods. The following compressor devices were used as materials: nebulizer "Turbo Flow", trademark "Dr. Frei" (Great Britain), nebulizer "C102 TOTAL (NE-C102-E)" and "A3 Complete" of the trademark "OMRON" (Japan), nebulizer "LD-221 C" of the trademark "Little Doctor" (Singapore). Results and discussion. The article presents studies devoted to the research and analysis of the main technical parameters of modern compression nebulizers (nebulizer volume, speed spraying, the ability to adjust the size of particles in the aerosol cloud). The resulting knowledge will help working in tandem doctors and pharmacists to choose the right device for the effective delivery of the drug in the human the respiratory tract to achieve maximum therapeutic effect. Conclusions. Commodity analysis of compressor nebulizers available in Ukrainian pharmacies has been conducted. Analysis concern the range of nebulizers, the basic technical characteristics of these devices, their configuration, features of use, caveats for their use and conditions of storage. A comparative analysis of nebulizer chambers from different manufacturers was carried out and their main parameters were established. Labeling was studied and drew attention was drawn to the special information symbols that relate exclusively to nebulizers.

ARTICLE INFO:
Received: May 9, 2022
Revised: July 22, 2022
Accepted: September 25,2022
Published on line: January 13, 2023

*** CORRESPONDING AUTHOR:**
Yuliia Bezpala, Department of Commodity Science, National University of Pharmacy, Kharkov, Ukraine, 4, Valentinivska Street, 61121, Ukraine.
Email: yuliyabespalaya5 @ gmail.com

Introduction.

Knowledge of information about the modern assortment, technical requirements, purpose and principles of nebulizers is the main and relevant not only for pharmacists but also for consumers. The Department of Commodity Science in the National Pharmaceutical University has recently introduced the topic "Commodity analysis of nebulizers" into the educational process of the "Medical and Pharmaceutical Commodity Science" course of study.

Recently, the incidence of chronic obstructive pulmonary disease has increased worldwide, which in turn leads to the search for new effective treatments. Inhalation delivery of drugs to the respiratory tract is traditionally used in patients with broncho-pulmonary diseases. This allows direct influence over the local pathological process, reduction of the total dose of drugs and minimization of side effects[1,2].

Nebulizer therapy is one of the most efficient treatments for broncho-pulmonary diseases. However, due to the high cost and complexity of their use, nebulizers were employed only in medical institutions of the regional level. In recent years though, they have become widely used in the therapeutic department of city hospitals and even at home. Therefore, the main task is to teach students not only the theoretical part, but also to provide basic practical skills when working with the assortment of these products. To possess these skills, in our opinion, is definitely useful for the practice of future pharmacists. Therefore, a detailed and focused study of the assortment of nebulizers is one of the main stages in teaching this topic[3-5].

The goal of our work was to study the assortment of nebulizers which are presented on the Ukrainian market; the main technical parameters that should be considered when choosing a nebulizer, as well as labeling and packaging of these products.

Materials and methods.

In the course of work there were used scientific publications, as well as systematic, logical, analytical, retrospective methods. There were used current standards, namely: EN 13544-1: 2007 + A1: 2009 Respiratory therapy equipment - Part 1: Nebulizing systems and their components, DSTU EN 13544-1: 2015 Respiratory therapy equipment. Part 1. Inhalation systems and their components (EN 13544-1: 2007 + A1: 2009, IDT), DSTU EN 980: 2007 "Graphical symbols for labeling of medical devices" (EN 980: 2003, IDT). The analysis of manufacturers was carried out using the State Register of Medical Equipment and Medical Devices. The technical characteristics of nebulizers were studied with the help of Directive 93/42 / EEC "Medical Devices Directive"[6-9].

The following compressor devices were used as materials: nebulizer "Turbo Flow", trademark "Dr. Frei" (Great Britain), nebulizer "C102 TOTAL (NE-C102-E)" and "A3 Complete" of the trademark "OMRON" (Japan), nebulizer "LD-221 C" of the trademark "Little Doctor" (Singapore).

Results and discussion.

Nebulizer (Latin *nebula* means fog, cloud) - a medical device designed for inhalation with a number of drugs in the form of liquids powders by spraying them into microparticles which are able to penetrate deep into the respiratory tracts and have a therapeutic effect directly at the site of inflammation through delivery of high doses of drugs.

The term "nebulizer" (*nebula* means cloud, fog) was used for the first time about 150 years ago, when a device was invented for converting liquid into steam. The invention of such a portable device is attributed to J. Sales-Girons (France, 1858). A nebulizer was used for inhalation of resins and antiseptics in patients with tuberculosis. To ensure a continuous supply of this drug substance, the steam jet was used as an energy source. Over time, the device has been improved[3].

The main task of the nebulizer is to create an aerosol cloud with a given size of particles, in which the bulk of the drug for the treatment of the respiratory tract is concentrated.

According to the current standards of Ukraine and the EU (DSTU EN 13544-1: 2015 and EN 13544-1: 2007 + A1: 2009) the following sizes of aerosol particles are singled out and their distribution in the respiratory tracts[6,10].

- 8-10 μm- they are usually accumulated upon the oral cavity,
- 5-8 μm - upon the throat and larynx,
- 3-5 μm - upon the trachea and bronchi,
- 1-3 μm - upon bronchioles,
- 0.5-2 μm - upon the alveoli

Modern nebulizers generate particles from 1 μm to 5 μm. The size of the aerosol particles can also be determined using the indicator - " Mass Median Aerodynamic Diameter (MMAD)" and "Geometric Standard Deviation (GSD)"[5,6].

MMAD - Mass Median Aerodynamic Diameter is the aerodynamic diameter of a particle that divides (by size range) the total mass of an aerosol cloud into two equal parts. 50% of the aerosol mass is concentrated in particles smaller than MMAD, the other 50% of the aerosol mass is concentrated in particles larger than MMAD. The practical value of MMAD is determination of the respirable fraction quality[11].

Nebulizers are classified as: compressor (jet), ultrasonic and membrane nebulizers (or "mesh nebulizers")[12-15].

Using the data obtained from the State Register of Medical Equipment and Medical Devices, we analyzed the current market of nebulizers. Analyzing the obtained data, we found that at present the supply of nebulizers is carried out by a number of world-famous manufacturers. They are: OMRON HEALTHCARE Co., Ltd. (Japan); Shenzen Homed Medical Device Co. Ltd. (China); Rossmax International Ltd. (Taiwan, R.O.C.); Microlife AG (Switzerland); Vega Technologies Inc. (Taiwan); 3A HEALTH CARE S.R.L. (Italy); Vega Technologies Inc. (Taiwan) and others.

As of today, compressor nebulizers have been widely used in clinical treatment. In this nebulization system there is a device consisting of a container for the drug - nebulizer chamber, mouthpiece (mouthpiece) or mask, nasal cannula, thin silicone tubes and a source of «working» gas - compressor (device that produces an air flow) (Figure 1).

Compressor nebulizers are designed for inhalation treatment with the ability to adjust the size of the aerosol particles. Due to the adjustment of the aerosol particles size, it enters all parts of the respiratory organs (upper, middle, lower). It is successfully used for inhalation therapy for both adult patients and children of the first year of life.

This device has its advantages: high speed of spraying in comparison with other types (for example, ultrasonic); greater versatility, compactness. It also has disadvantages: the bulkiness and weight of the device (from 1.2 to 2 kg), the need to keep the nebulizer chamber only in a vertical position, which significantly complicates inhalation for children and bedridden patients.

When choosing a nebulizer, consumers pay attention to the following consumer characteristics, namely the size, weight, noise during the compressor operation. Modern manufacturers are trying to make nebulizers with low noise, especially for children. Many manufacturers have a wide range of nebulizers for children. They differ from standard nebulizers by the design of the compressor and can be in the form of toys (Figure. 2).

Also, modern manufacturers supply nebulizers with nasal aspirators - (which work on the basis of the Venturi effect) – they remove mucus from the nasal cavity to facilitate breathing or with nasal shower (nozzle for washing the nasal cavity) (Figure 3). Nasal shower is recommended to be used for:

- prevention of allergic reactions (because not only microparticles are removed but allergens as well)
- general immune system reinforcement (it occurs by the effect of improvement of cells functioning and reinforcement of capillaries)
- prevention of inflammatory diseases (because dust is removed)
- raise body tone.

Figure 1. *Complete set of the Little Doctor LD-221 C compressor inhaler, the Little Doctor trademark.*

When choosing a compressor nebulizer, it is important to pay attention to the following technical parameters:

Respirable fraction (RF) is the number of particles in an aerosol cloud, smaller than 5 μm, in terms of percentage. The higher the rate of respirable fraction - the more the drug will get into the respiratory tracks.

The residual volume (RV) of the nebulizer chamber is the amount of drug that cannot be sprayed because some of the drug remains on the walls of the nebulizer chamber. This figure should not exceed 1.0 ml. The less the RV, the more pharmacologic agent will be converted into an aerosol. The residual of the liquid remains in the so-called "dead" space of the nebulizer chamber (even if the chamber is almost completely empty). RV depends on the design of the nebulizer and does not depend on the amount of filling, however based on the value of RV, there are given recommendations as far as the amount of solution that is added to the nebulizer chamber.

Type of the nebulizer camera. The choice of design allows us to increase the ingress of the drug into the respiratory tract, providing a mode of natural respiration.

Spraying rate (productivity) is the volume of aerosol yield per time unit (ml / min.). It determines the rate of inhalation and affects the patient's adherence to nebulizer therapy. It is calculated as the difference between the filling volume and the residual volume during inhalation: Spray rate (FV - RV) / t.

On the other hand, compressor nebulizers are divided "according to the principle of the nebulizer chamber operation" into 3 types[12,13]:

• Convectional– they operate continuously, producing an aerosol on both inhalation and exhalation of the patient. Therefore, about 70% of the pharmacologic agent is lost when the patient exhales and only about 7% is deposited in the patient's respiratory tracks.

• With manual control - it is possible to adjust the pharmacologic agent flow. That is, the "inhale button", which is installed on advanced convection nebulizers, it helps to regulate the supply of the

Figure 2. *Nebulizers in the form of toys for children: 1 - "Panda" (trademark "Dr. Frei"), 2 - "Chu-Chu-Train" (trademark "Dr. Frei"), 3 - "Nemo" (trademark "Dr. Frei »), 4 - Ne-c801 kd (trademark «OMRON»).*

Figure 3. *Nebulizer with a nasal shower of the OMRON trademark (Japan).*

pharmacologic agent. At the same time the patient or the medical worker who provides him with help, can switch the valve manually which stops the aerosol supply at the moment of an exhalation.

• Respiratory-activated nebulizers – these nebulizers are more convenient to use, the aerosol supply in them is adjusted by the patient's breathing. When inhaled through a special valve in the aerosol chamber an additional air flow is supplied; when exhaling, there is no air flow, so there is no spraying of the aerosol into the environment, as it is in case with a simple convectional nebulizer.

• Dosimetric – the nebulizers like that are equipped with air flow sensors that stop the formation of aerosol on exhalation and activate on inspiration with special valves. Thus it is possible to control the dose of the pharmacologic agent entered through the respiratory tract and to achieve the maximum therapeutic effect from its use.

It is also worthy to mention that today each manufacturer has its own unique nebulizer chambers in which the consumer can independently adjust the size of the aerosol particles at home, i.e. using only one nebulizer chamber to treat different parts of the respiratory tracks. Examples are shown in Table 1.

For these devices, there are some recommendations and caveats for their use.

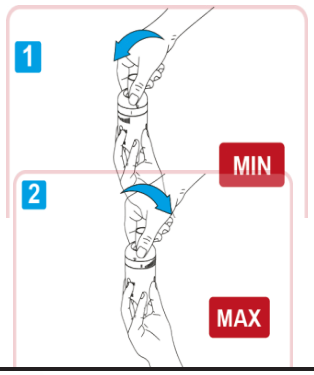
➢ The components of the nebulizer must be checked regularly and replaced in the event of any defect. Use only the components (masks, tubes, nebulizer, etc.) of the device that are supplied with the nebulizer, others may affect the effectiveness of treatment and make the device inoperable.

➢ It is forbidden to use decoctions of medicinal herbs, essential oils, mineral waters for

Table1. Characteristics of the nebulizer chambers from modern manufactures

№		**Manufacturer and nebulizer name**	**Characteristics of the nebulizer chamber**
1	 	Compressor inhaler Little Doctor LD-221 C, trademark "Little Doctor"	It consists of 3 sprayers: **Sprayer A (yellow)** is used to treat the lower respiratory tract The particle size is 3.5 μm, The spray rate is 0.3 ml / min. **Sprayer B (blue)** is a universal sprayer The particle size is 4.0 μm, The spray rate is 0.4 ml / min. **Sprayer C (red)** is used for the upper respiratory tract The particle size is 5.0 μm, Spraying speed - 0.5 ml / min.
2	 	Compressor inhaler Omron A3 Complete (NE-C300-E), trademark "OMRON"	The chamber volume is 2-12 ml, there are three positions for adjusting the particle size: **Position 1**: the particle size is from + 7.5 μm, It is used to treat diseases of the nasopharynx, larynx (rhinitis, sinusitis, laryngitis, etc.) Spraying speed - 0.7 ml / min. **Position 2**: the particle size is 4.5-7.5 μm, It is used to treat diseases of the trachea and middle bronchi (tracheitis, tracheobronchitis, etc.) Spraying speed - 0.5 ml / min **Position 3**: the particle size is 2-4.5 μm, It is used to treat diseases of the bronchi, lungs (asthma, bronchitis, pneumonia, etc.) Spray rate - 0.3 ml / min

Table1. Characteristics of the nebulizer chambers from modern manufactures

3		Compressor inhaler Turbo Flow, trademark "Dr. Frei »	Chamber volume 2-12 ml. This particle size control technology allows you to generate an aerosol with different particle sizes for efficient treatment of the upper and lower respiratory tracts. **MIN** – to start in this position it is necessary to return the chamber lid to the mark **MIN I**. In this position the size of the aerosol particles will be 3
			µm, i.e. for the treatment of the lower respiratory tract (e.g. bronchitis, bronchial asthma) **MAX** - to start in this position it is necessary to return the chamber lid to the **MAX II** mark. In this position, the particle size of the aerosol will be 6 µm, ie for the treatment of the upper respiratory tract (eg, pharyngitis, respiratory viral infections, etc.). The spray rate is 0.2-0.4 ml / min

inhalation with this type of nebulizer

➢ Do not allow liquid to enter the compressor and cover the compressor during operation.

➢ Each patient should have an individual nebulizer chamber and mouthpiece / mask, which is associated with a risk of microbial contamination with pathogens that are not destroyed by conventional disinfectant solutions when using other people's nebulizer chambers, mouthpieces and masks. Therefore, it is recommended to use disposable nebulizer sets or to have separate ones for each person when providing treatment in hospital or using it at home. Patients receiving long-term nebulizer therapy should have their nebulizer chamber and mouthpiece / mask replaced every 3 months.

➢ After inhalation, all parts of the nebulizer should be thoroughly rinsed with clean water, dried (frequent rinsing of the nebulizer is necessary to prevent crystallization of pharmacologic agents and bacterial contamination of the device). They can also be sterilized by boiling for 5-10 minutes in water or immersing them in disinfectants. If several people use the nebulizer, the instructions for clening and disinfecting the nebulizer should be followed with the utmost care after each use.

➢ The service life of the nebulizer chamber from different manufacturers is from 3 months to 3 years..

➢ The air filter should be checked no more often than once a month and replaced in a timely

№	Information sign	Meaning
1		double insulation
2		special disposal is required in accordance with national legislation
3	IP21	protection degree of the device case
4		BF type product
5		alternating voltage
6		mode off
7		mode on

manner as it becomes dirty.

➢ Monitor the operation of the compressor (over time, the nebulizer may wear out, that can cause a decrease of the air flow rate and increase the particle size).

➢ It is recommended to store the nebulizer in disassembled form in containers or primary packaging (bag, case) [16-18].

When these products are supplied to pharmacies, the pharmacists should pay attention to their packaging and labeling. Nebulizers come in the factory packaging (cardboard pack or box). A bag (case) can be used as the primary packaging for nebulizers. All nebulizer components are packed in separate airtight plastic bags.

Labeling is also one of the main criteria. Labeling is a set of text, symbols and images that are on the secondary packaging and the product itself, and serves to identify the product or its individual properties and convey to the consumer some information about the product.

Marking of nebulizers is carried out according to the requirements of GOST 20790-93 «Devices, machinery and the medical equipment, the Law of Ukraine» About protection of the consumers' rights», article 15. On primary and / or secondary packaging and / or the device the following information is put:

- product name;
- name or designation of the type (tsort, model) of the product;
- name of the manufacturing enterprise, address, trademark;
- series;
- lot
- date of production;
- designation of standards for the product,
- other data depending on product requirements (service symbols, rated system voltage, power consumption at rated operating mode, etc.);
- bar code;
- compliance marks;
- operational information signs;
- information on storage conditions.

The application of operational and informational signs on the packaging is carried out in accordance with DSTU EN 980: 2007 «Graphic symbols for marking of medical devices» (EN 980: 2003,

IDT). Technical characteristics for nebulizers are prescribed in Directive 93/42 / EEC «Medical Devices Directive».

Since most nebulizers are electrical medical devices, they contain additional information and operational marks in accordance with DSTU 3798-98 «Eclectic medical devices. Part 1. General safety requirements «(Table 2).

Conclusions:

A commodity analysis has been conducted for compressor nebulizers, which are presented in Ukrainian pharmacies. It was found that the leading positions on the Ukrainian market are occupied by imported producers. The range of nebulizers has been analysed to know the basic technical characteristics of these devices (nebulizer volume, speed spraying, the ability to adjust the size of particles in the aerosol cloud), their configuration, features of use, caveats for their use and conditions of storage of devices. A comparative analysis of nebulizer chambers from different manufacturers was carried out and their main parameters were established. Labeling has been studied and attention drawn to the special information symbols that relate exclusively to nebulizers.

In our opinion, the above information will be helpful to practicing physicians, pharmacists and consumers when choosing a compressor nebulizer. □

ORCID iDs

Alla Kotvitska https://orcid.org/0000-0002-6650-1583

Yuliia Bezpala https://orcid.org/0000-0002-0077-8934

Inna I. Baranova https://orcid.org/0000-0003-2827-265X

Qobiljon Khushvakhtzoda https://orcid.org/0000-0002-7065-0071

Safol M. Musozoda https://orcid.org/0000-0001-8512-8019

References

1. Sauteur P.M.M. Challenges and Progress Toward Determining Pneumonia Etiology. *Clin. Infect. Dis.* 71(3),514-516, 2020.
2. Leung A., Wong A., Hon Kam Community-Acquired Pneumonia in Children. Recent Pat. Inflamm. *Allergy Drug Discov.* 12(2),136-144, 2018.
3. Sorino C., Negri S., Spanevello A., Visca D. , Scichilone N. Inhalation therapy devices for the treatment of obstructive lung diseases: the history of inhalers towards the ideal inhaler. *Eur. J. Intern. Med.* 75,15-18, 2020.
4. Barjaktarevic I. , Milstone A. Nebulized Therapies in COPD: Past, Present, and the Future. *Int. J. Chron. Obstruct. Pulmon. Dis.* 15,1665-1677, 2020.
5. Tashkin P.D. A review of nebulized drug delivery in COPD. *Int. J. Chron. Obstruct.* Pulmon. Dis. 11, 2585-2596, 2016.
6. European Standard (2009) European Standard P 13544-1:2007+A1: 2009 Respiratory therapy equipment - Part 1: Nebulizing systems and their components. 43 pp.
7. National Standard (2007) National Standard P 980:2007 «Graphical symbols for use in the labeling of medical devices» (EN 980:2003, IDT). [Effective date 2008-02-01]. State Consumer Standard of Ukraine, Ukraine, 17 pp.
8. State Register of Medical Equipment and Medical Devices. https://www.dls.gov.ua/print-article/?pa=335
9. Medical Devices Directive 93/42/EEC - European Commission https://ec.europa.eu
10. Andrew R.M., Warren H.F. Nebulizers for drug delivery to the lungs. *Expert Opin. Drug Deliv.*12(6), 889-900, 2015.
11. Brun Le P.P., Boer de A.H., Heijerman H.G., Frijlink H.W. A review of the technical aspects of drug nebulization. *Pharm. World Sci*. 22(3),75-81, 2000.

12. Levchenko M., Bezpala Yu. Comparison of main consumer aspects steam inhalers and nebulizer. Topical issues of new drugs development: Abstracts of XXV International Scientific and Practical Conference of Young Scientists and Student, 18-20 April 2018. NUPh, 2018; Kharkiv, 466-466.
13. Bezpala Yu., Baranova I., Shmatenko O. (2019). The main consumer characteristics of ultrasonic nebulizers. In: The 10th International Pharmaceutical Conference Science and Practice 2019", 15 November. Kaunas, Lithuania, 940 -940.
14. Ullmann N. , Marco Di. A., Columbu F. , Negro V., Chiarini Testa M.B., Panetta V., Tripodi S., Potapova E., Allegorico A., Matricardi P.M., Cutrera R. A. Novel, Portable MESH Nebulizer-An Alternative to Metered Dose Inhaler: Efficacy and Usability in Preschool Wheezers. Front. Pediatr. 8, 598690, 2020.
15. Katial R.K., Reisner C., Buchmeier A., Bartelson B.B., Nelson H.S. Comparison of three commercial ultrasonic nebulizers. *Ann. Allergy, Asthma Immunol.* 84(2), 255-261, 2000.
16. Lauren A., Carson J., Caughan Mc J., Moore E.J., Millar B.C. Thinking inside the box: nebulizer care, safe storage, and risk of infection in cystic fibrosis. *J. Bras. Pneumol.* 46(2), e20190226, 2020
17. Ngai K., Persico A., Brodeur M. Students' perspectives on nebulized drugs and nebulizer maintenance. *Consult. Pharm.* 32(9), 519-524, 2017.
18. Joseph L.R. The inhalation of drugs: advantages and problems. *Respir. Care.* 50(3), 367-382, 2005.

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 34, IV, 2022 | 166-173 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 34, IV, 2022 | 166-173 RESEARCH ARTICLE

In vitro Study of the Permeability of Enalapril Maleate through a Semipermeable Membrane in the Process of Pharmaceutical Development of a Transdermal Therapeutic System

Tetyana Shyteyeva*, Elena Bezchasnyuk, Oleg Kryskiv

National University of Pharmacy, Kharkiv, Ukraine

KEYWORDS:
in vitro permeability, enalapril maleate, transdermal therapeutic system

ABSTRACT

At the stage of preformulation studies of the pharmaceutical development of the transdermal therapeutic system (TTS) with antihypertensive action, the in vitro permeability process of enalapril maleate was studied. Testing was performed by dialysis through a semipermeable membrane using side-by-side diffusion chambers. The effect of the initial concentration of enalapril maleate on the steady-state flux rate Is was investigated. Four donor concentrations 10 mg/mL, 20 mg/mL, 30 mg/mL and 40 mg/mL of enalapril maleate were tested. Conducted analysis of the nature, description of the mathematical model and determination of the main kinetic parameters of the permeability process of the studied enalapril maleate allow assessing its potential for creating TTS and substantiate a further algorithm for the development of TTS antihypertensive action according to API data. Review articles, research papers, case reports and letters to the editor may be submitted for publication.

ARTICLE INFO:
Received: March 23, 2022
Revised: June 14, 2022
Accepted: September 29, 2022
Published on line: January 13, 2023

*** CORRESPONDING AUTHOR:**
Tetyana Shyteyeva,
shyteyeva@gmail.com

1. Introduction

The creation of innovative drugs in the form of transdermal therapeutic systems (TTS) is one of the most promising scientific directions of modern pharmaceutical technology. Pharmaceutical development of such drugs involves a thorough study of all biopharmaceutical aspects. The initial stage of development of TTS is the choice of drug substance, assessment of the acceptability of its introduction in this dosage form. In order to determine a more rational approach to the creation of TTSs, the pharmaceutical development of a transdermal drug should be preceded by in vitro preformulation studies of the permeability of the active pharmaceutical ingredient (API) across the membrane. The main advantage of these studies is the establishment at the initial stage of the factors influencing this process, the ability to control the conditions of

the experiment, and, therefore, the ability to control changes in permeability based on certain kinetic parameters[1-3].

Hypertension, is one of the most common causes of disability and mortality. Today there is a numerical increase in the incidence of this type of pathology. Pharmacotherapy of these pathological conditions is usually long-term and requires an individual approach and comprehensive correction, taking all parts of the pathological process into account[4].

The search for APIs promising to be used in transdermal dosage forms continues intensively. Among the group of antihypertensive drugs, angiotensin-converting enzyme (ACE) inhibitors occupy one of the main places. Enalapril maleate has been widely used in clinical practice for decades. Among all ACE inhibitors, it has the widest list of indications for use, including hypertension, chronic heart failure (CHF), coronary heart disease. Enalapril is the gold standard among ACE inhibitors for its ability to control blood pressure. Enalapril maleate has a dose-dependent hypotensive effect, which is observed within 24-36 hours after a single oral administration. The maximum reduction in blood pressure is achieved after 6-8 hours[5, 6].

In the last scientific publications, much attention is paid to the development of transdermal delivery of the enalapril maleate. The use of TTS provides stability of concentration and long-term therapeutic level of the substance in the bloodstream, which contributes to the therapeutic effect prolongation. TTS, in comparison with oral dosage forms, eliminates the risk of gastrointestinal side effects, increasing safety profile. When using transdermal patches, a reduction in dosing frequency is achieved and high systemic bioavailability of a drug is ensured. TTSs are quite easy to use and can significantly increase compliance with patients[7-9].

To achieve the optimal therapeutic effect with transdermal administration of the drug, it is necessary to take into account both physico-chemical properties of the active substance and external factors, in particular the effect of concentration, composition of the diffusion medium and others. In order to determine a more rational approach to the creation of TTS, in vitro preformulation studies of API permeability through membranes should precede the pharmaceutical development of a transdermal preparation. The main advantage of these studies is the ability to control the conditions of the experiment and, consequently, the ability to control changes in permeability due to the influence of various factors.

In this regard, the aim of our work was to conduct preformulation studies of the pharmaceutical development of transdermal dosage form of TTS antihypertensive action with enalapril maleate. During the study, the nature and kinetic parameters of the in vitro process of permeability of enalapril maleate through a semipermeable membrane, as well as the influence of the initial concentration of the selected API on this process were determined.

2. Materials and methods

The target of the study was the enalapril maleate API (Zhejiand Huahai Pharmaceutical Co., Ltd., CHINA).

Studies of the permeability of enalapril maleate through a semipermeable membrane were performed in vitro by dialysis using a modified diffusion device of Valia-Chien design [10]. Different concentrations of enalapril maleate were used as donor solutions: 10 mg/mL, 20 mg/mL, 30 mg/mL and 40 mg/mL. Phosphate buffer solution (pH 7.4) was used as the diffusion medium. The experiment was performed at a temperature of (37+0.5) *ºC.* At defined intervals, with an interval of 1 hr, which corresponded to 1, 2, 3, 4, and 5 hr from the beginning of the experiment, all the solution from the acceptor chamber was removed, replacing the sample of acceptor solution with a new one, which was taken into account. The content of enalapril maleate in the dialysate sample was determined spectrophotometrically.

3. Results and discussion

The results obtained (Table 1, Figure 1) show that the amount of enalapril maleate passing through the semipermeable membrane is proportional to its in-

Table 1: Quantitative parameters of enalapril maleate permeability through a semipermeable membrane in vitro

The concentration of API in the donor chamber, C_s, mg/mL	Number of a chosen sample, n	Sampling time, t, hr	Quantity of drug in a dialysis sample, $X_i \times 10^{-3}$, g	Specific flux of drug, Q(t), mg/cm^2
10	1	1	12.4910	3.0097
	2	2	11.2952	5.7312
	3	3	11.3724	8.4715
	4	4	11.2642	11.1975
	5	5	10.9343	13.8325
20	1	1	30.4608	7.3401
	2	2	26.8024	13.7986
	3	3	28.4838	20.6625
	4	4	26.0577	26.9414
	5	5	25.5105	33.0883
30	1	1	47.4724	11.4389
	2	2	38.4113	20.6944
	3	3	37.1823	29.6538
	4	4	36.0529	38.3413
	5	5	36.3799	47.1075
40	1	1	121.8189	29.3539
	2	2	52.5146	42.0081
	3	3	51.7826	54.4860
	4	4	51.2530	66.8364
	5	5	50.5682	79.0215

Table 2: Kinetic parameters of the dependence of the enalapril maleate permeability and diffusion delay time on its initial concentration through the semipermeable membrane in vitro

The initial concentration of API in the donor solution, C_s, mg/mL	Steady-state flux of drug, I_s, mg/cm^{2}hr	Diffusion delay time, Θ, min	Permeability coefficient, K_p, cm/hr	Linear correlation coefficient, r^2 *Y=A+B×X*
10	2.7112	-7.2	0.34	0.9999
20	6.4639	-9.0	0.43	0.9996
30	8.8984	-18.6	0.39	0.9998
40	12.4160	-82.8	0.44	0.9999

Table 3: The results of the study of the convergence of the experimental values of the kinetic parameters of the permeability of enalapril maleate depending on its initial concentration

	Initial concentration in donor solution, C_s, mg/mL			
	10	**20**	**30**	**40**
Investigation of convergence of values of quantity of drug in a dialysate sample, $X_i \cdot 10^{-3}$, g				
Variants of samples, x_i	10.9343	25.5105	36.0529	50.5682
	11.2642	26.0577	36.3799	51.2530
	11.2952	26.8024	37.1823	51.7826
	11.3724	28.4838	38.4113	52.5146
	11.2165	26.7136	37.0066	51.5296
X_{low}	10.3192	24.5765	34.0461	47.4072
X_{high}	12.1138	28.8507	39.9671	55.6520
Investigation of the convergence of the values of the concentration of drug in the dialysate sample, C_t, mg/mL				
Variants of samples, x_i	0.4050	0.9448	1.3353	1.8729
	0.4183	0.9651	1.3474	1.8983
	0.4190	0.9927	1.3771	1.9179
	0.4212	1.0550	1.4226	1.9450
	0.4159	0.9894	1.3706	1.9085
X_{low}	0.3826	0.9102	1.2609	1.7558
X_{high}	0.4492	1.0686	1.4802	2.0612
Investigation of convergence of values of change of specific flux of drug, ΔQ(t), mg/cm²				
Variants of samples, x_i	2.6349	6.1469	8.6874	12.1851
	2.7215	6.2790	8.7662	12.3504
	2.7260	6.4585	8.9596	12.4779
	2.7403	6.8639	9.2557	12.6542
	2.7057	6.4371	8.9172	12.4169
X_{low}	2.4892	5.9221	8.2038	11.4235
X_{high}	2.9222	6.9521	9.6306	13.4103

itial concentration in the donor solution according to Fick's law.

According to the obtained data on the amount of enalapril maleate in dialysate Xi, there was an increase in the indicators g, and the specific flux of the drug through the membrane Q(t), mg/cm^2, with increasing initial drug concentration from 10 mg/mL to 40 mg/L.

Graphical interpretation of the in vitro permeability of enalapril maleate for each concentration is presented in Figures 2-5. In all experiments, the obtained kinetic equations have the form of a general

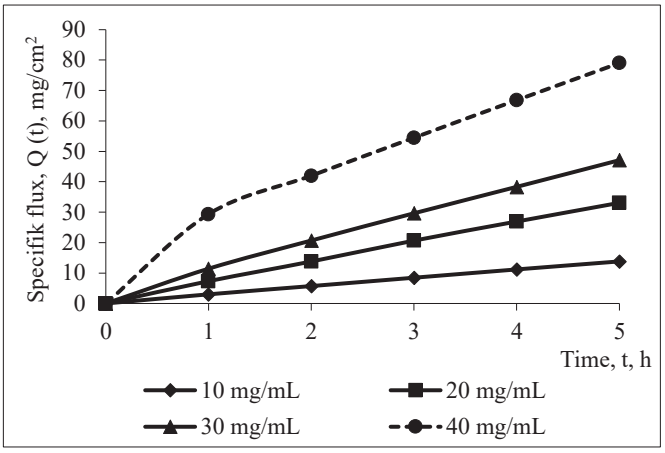

Figure 1. *Dependence of the permeability of maleate enalapril on concentration.*

linear regression *Y=A+B×X*.

The linear dependence of enalapril maleate on the membrane over time is confirmed by linear regression parameters. The correlation coefficient for the obtained kinetic equations, within the experiment time, was not less than 0.999.

The coefficient of permeability of the studied API through the membrane, Kp, cm/h, was calculated taking into account the gradient of concentrations of donor and acceptor solutions, based on Fick's law:

$Kp=Is \times \Delta Cs$ (Equation 1)

where *Is* – the value of the steady-state flux of drug through the unit area of the membrane per unit time, mg/(cm^2·hr);

Δ*Cs* – concentration gradient of of drug between the outer and inner surfaces of the membrane (or stratum corneum), mg/mL.

The main quantitative characteristics of the dependence of the enalapril maleate permeability on its initial concentration through the semipermeable membrane in vitro, calculated from the results of statistical analysis, are shown in Table 2.

According to the results (Table 2), it was found that with increase of the initial concentration of enalapril maleate from 10 mg/mL to 40 mg/mL, the steady-state flux of drug *Is* increases 4.6 times. The obtained values of the steady-state flux of enalapril maleate for all concentrations indicate the high potential of this substance in overcoming membrane barriers, and predict, including good permeability and human skin. But to determine the optimal concentration for the next stages of creating TTS, it is necessary to take into account all the factors of the permeability process together.

The diffusion delay time *(Θ)* determines the duration of the non-stationary period of the process. A negative value of this indicator indicates a lack of membrane saturation. During the experiment, it was found that the diffusion delay time for the concentration of enalapril maleate in the donor solution of 10 mg/mL and 20 mg/mL varies slightly from 7.2 to 9 min. At a concentration of enalapril maleate in the donor solution of 30 mg/mL, the diffusion delay time increases more than twice to 18.6 min. At a concentration of enalapril maleate in the donor solution of 40 mg/mL, the duration of the non-stationary period increases significantly, the diffusion delay time being 1 hr 23 min. The permeability coefficient *K*p characterizes the properties of the membrane and varies between 0.34 and 0.44 cm/hr.

Taking into account all the results of the experiments, we consider the best initial concentration of enalapril maleate 30 mg/mL, which provides the required level of the steady-state flux of drug in a short non-stationary period.

Statistical equivalence of the obtained data was assessed based on a study of samples of experimental values (starting from the second hour), arranged

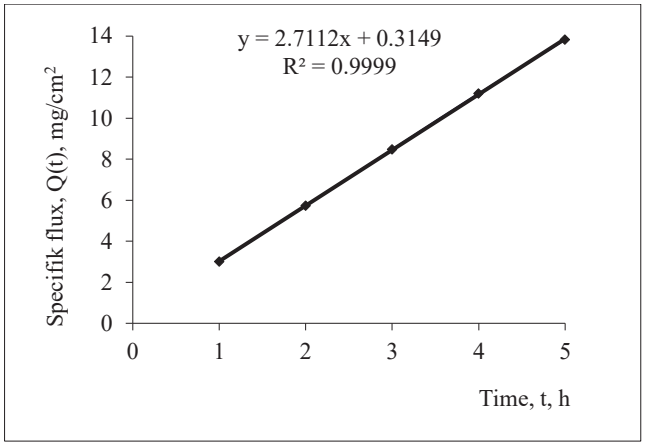

Figure 2. *Kinetics of the in vitro membrane permeability process of enalapril maleate (initial concentration 10 mg/ml).*

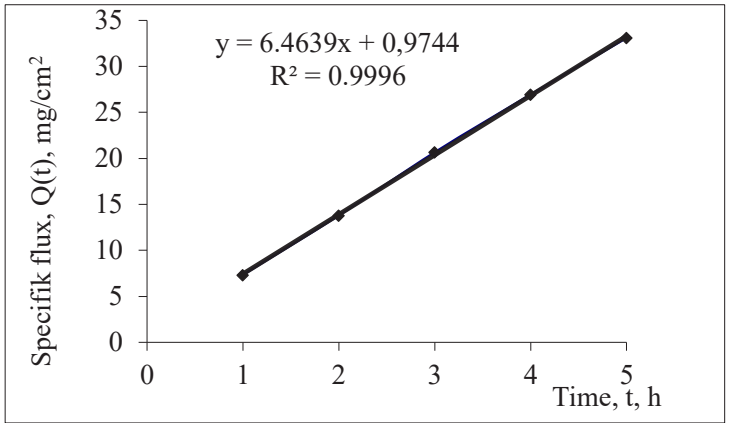

Figure 3. *Kinetics of the in vitro membrane permeability process of enalapril maleate (initial concentration 20 mg/ml).*

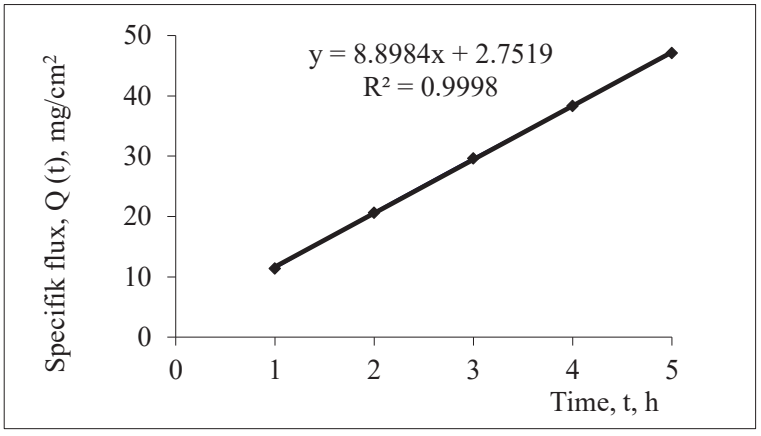

Figure 4. *Kinetics of the in vitro membrane permeability process of enalapril maleate (initial concentration 30 mg/ml).*

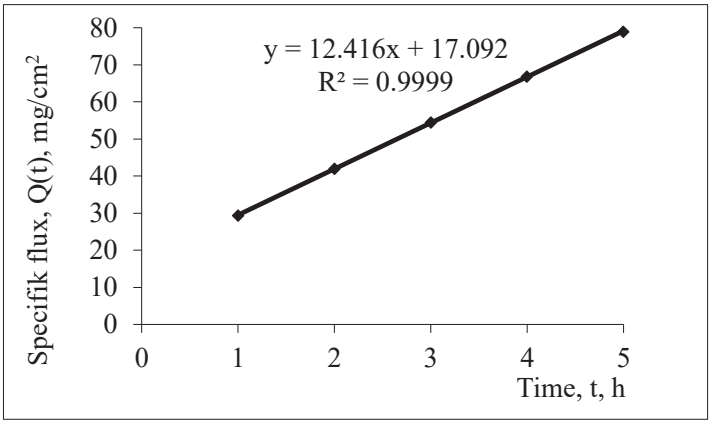

Figure 5. *Kinetics of the in vitro membrane permeability process of enalapril maleate (initial concentration 40 mg/ml).*

in ascending order. Changing the variants of the obtained samples can be considered insignificant if the values of their extreme variants do not exceed the confidence interval limit values calculated from the value of the half-width of maximum allowable confidence interval *(*max∆X*).* The value of max∆X was determined on the basis of the maximum allowable uncertainty of the method of analysis max*∆As*, based on the tolerances of the content of the substance for transdermal drugs 75% – 125%, in accordance with the requirements of Ph. Eur. Section 2.9.6[11]. According to the regulations of the State Pharmacopoeia of Ukraine (SPU)[12] for methods of quantification, the maximum uncertainty of max*∆As* in percent, expressed as a one-way relative confidence interval for a level of reliable probability of 95%, should correspond to inequality 2:

$$\max\Delta_{As} \le 0.32 \times B \qquad [2]$$

where *B* is the half-width of the regulated concentration limits.

According to these conditions, all the results of the experiment should not exceed a certain value of $\max\Delta_{As}$ 8%.

The results of the study of the convergence of experimental values of kinetic parameters of permeability of enalapril maleate depending on its initial concentration are shown in Table 3.

see Table 3.

According to the results given in Table 3, it can be seen that the variant values of all samples do not exceed the confidence interval limits X_{low} and X_{high}. Therefore, all the obtained experimental values of the studied parameters are within the confidence interval and change slightly. Based on this, it can be argued that the permeability of enalapril maleate through the membrane in vitro within the studied concentrations from the second hour is carried out at a constant rate corresponding to zero-order kinetics.

4. Conclusions

The studies carried out to determine the nature and quantitative characteristics of the enalapril maleate permeability showed, first of all, the ability of the selected substance molecules to overcome membrane barriers and make it possible to give a positive assessment of the acceptability of this API for use in transdermal form and the creation of TTS.

As a result of the experiment, the main quantitative parameters of the process of permeability of enalapril maleate through a semipermeable membrane in vitro (stationary flow of the drug *Is*, diffusion delay time Θ, permeability coefficient *K*p) were determined. With increasing initial concentration of the substance in the donor chamber within con-

centrations of 10 mg/mL – 40 mg/mL, there is an increase in all studied parameters of the experiment. The optimal initial concentration of enalapril maleate 30 mg/mL was determined for further stages of transdermal drug development.

The development of TTS for enalapril maleate is promising and relevant. Carrying out the next stages of the pharmaceutical development of TTS with enalapril maleate will make it possible to introduce a new transdermal therapeutic system with antihypertensive action into medical practice in the future. □

References

1. Jeong, W.Y., Kwon, M., Choi, H.E., Ki Su K. Recent advances in transdermal drug delivery systems: a review. *Biomater. Res.* 25, 24, 2021.
2. Vargason A.M., Anselmo A.C., Mitragotri S. The evolution of commercial drug delivery technologies. *Nat. Biomed. Eng.* 5, 951–967, 2021.
3. Peña-Juárez M.C., Guadarrama-Escobar O.R., Escobar-Chávez J.J. Transdermal delivery Systems for Biomolecules. *J. Pharm. Innov.* 6, 1–14, 2021.
4. Klimov A. V., Denisov E. N., Ivanova O. V. Arterial hypertension and its prevalence among the population. *Young scientist*. 50 (236), 86-90, 2018.
5. Molchanov D. Enalapril in the treatment of arterial hypertension 30 years later: a comparison or choice drug? *Health of Ukraine: medical newspaper*. 17, 28-29, 2008.
6. Sirenko Yu. N. Enalapril in cardiology and therapy: the standard of efficacy and safety among ACE inhibitors. *News of medicine and pharmacy*. 13-14, 376-377, 2011.
7. Fouad H., Majella E. L. Transdermal delivery of Angiotensin Converting Enzyme inhibitors. *Eur J. Pharm. Biopharm*. 88 (1), 1-7, 2014.
8. Rastogi V., Pragya, Upadhyay P. A Brief View on Antihypertensive Drugs Delivery through Transdermal Patches. *Int. J. Pharm. Sci. Res*. 3(7), 1955-1970, 2012.
9. Aqil M., Bhavna, Chowdhary I., Sultana Y., Talegaonkar S., Ahmad F.J., Ali M.M. Transdermal therapeutic system of enalapril maleate using piperidine as penetration enhancer. *Curr. Drug Deliv*. 5(2), 148-52, 2008.
10. Yie W. Chien. Development of Transdermal Drug Delivery Systems. *Drug Development and Industrial Pharmacy*. 13(485), 589-651, 1987.
11. Council of Europe. European Pharmacopoeia, 9th edition, Strasbourg: Council of Europe, 2016.
12. The State Pharmacopeia of Ukraine. 2-nd edition. Kharkiv: State Enterprise «Ukrainian Scientific Pharmacopeial Center for the Quality of Medicinal Products», 1, 1128, 2015.

ΕΚΔΗΛΩΣΕΙΣ - MEETINGS

• DECEMBER 15-17, 2022 | IISTANBUL, TURKEY
8th International Bahçeşehir University (BAU) Drug Design Congress
https://www.baudrugdesign2022.com

• MARCH 9-11, 2023 | PATRAS, GREECE
19th Hellenic Symposium on Medicinal Chemistry (HSMC-19)
https://helmedchem2023.gr

• MAY 31 – JUNE 2, 2023 | LISBON, PORTUGAL
EUFEPS Annual Meeting 2023 "Lisbon 2023, at the edge of Europe and Science".
https://annualmeeting.eufeps.org/programme.html

• JUNE 11-14, 2023 | IOANNINA, GREECE
16th International Symposium on Applied Bioinorganic Chemistry
https://isabc2023.com/registration/

• JUNE 13-16, 2023 | SNEKKERSTEN, DENMARK
3rd RSC Anglo-Nordic Medicinal Chemistry Symposium
https://www.rscbmcs.org/events/anmc/

• 16-18 JULY 2023 | THESSALONIKI, GREECE
Meeting of the Paul Ehrlich Euro-PhD Network
www.medchem.2023.com

• 17-20 SEPTEMBER 2023 | CHANIA, CRETE, GREECE
13th International Conference on "Instrumental Methods of Analysis" (IMA 2023),
Website: www.diazoma.net