ISSN 1993-6842 (on-line); ISSN 0233-7657 (print)
Biopolymers and Cell. 2021. Vol. 37. N 3. P 167–176
doi: http://dx.doi.org/10.7124/bc.000A54

UDC 577.115;616.43-008.6

N-stearoylethanolamine — a new inhibitor of the hepatitis C virus reproduction

N. M. Hula[1], A. A. Chumak[1], A. G. Berdyshev[1], G. V. Kosiakova[1], T. M. Goridko[1], O. F. Meged'[1], V. S. Asmolkova[1], Yu. I. Porva[2], S. L. Rybalko[2], S. T. Dyadun[2], D. B. Starosyla[2], O. M. Deryabin[2]

[1] Palladin Institute of Biochemistry, NAS of Ukraine
9, Leontovich Str., Kyiv, Ukraine, 01601

[2] Gromashevsky Institute of Epidemiology and Infection Diseases, NAMS of Ukraine
5, Amosova Str., Kyiv, Ukraine, 03038
kievlipids@gmail.com

Aim. The study of the effect of endogenous cannabimimetic compound - N-stearoylethanolamine (NSE) on the hepatitis C virus (HCV) reproduction. **Methods.** The model of the surrogate HCV is a bovine diarrhea virus; ***cell culture model*** is cells transfected with cDNA of the human HCV and molecular docking has been used. **Results.** *In vitro* studies showed that NSE effectively inhibited the reproduction of a surrogate HCV in both MDBK cells and transfected Jurkat cells. Molecular docking suggested that NSE can bind to the active centers of both NS3 serine protease and HCV NS5B-polymerase and has an inhibitory effect on their activity. **Conclusions.** The obtained data confirm that using NSE is promising for the development of antiviral drug to suppress the HCV activity.

K e y w o r d s: N-stearoylethanolamine, antiviral activity, hepatitis C virus, molecular docking

Introduction

Hepatitis C (HCV) is a viral infection, which according to WHO affects today more than 170 million people worldwide [1].

Nowadays, new antiviral drugs for hepatitis C virus (HCV) infection, known as oral direct-acting antiviral agents (ODAs) are available on the market. They are more effective, safer and better-tolerated than existing therapies: 90 % of people are cured [2]. A significant disadvantage of ODAs is their high cost. According to the information above, there is an urgent need to develop and improve additional and alternative strategies in the hepatitis C treatment, in particular, targeting the main metabolic pathways of reproduction/replication and biological action of the HCV. Recent

research has established an essential role of lipids metabolism in the life cycle of HCV. Therefore, great attention has been paid to the interaction between HCV and cell lipid metabolism, considering the widespread development of steatosis in patients with chronic hepatitis C complications [3]. However, this issue remains poorly investigated. It has been shown that HCV replication was inhibited by statins and fatty acids [4, 5].

Additionally, it has been shown that HCVs are secreted by the cell together with low density lipoproteins [6, 7] and such products can be metabolically modulated by the addition of insulin or appropriate fatty acids. Some liver lipid modulators, originally developed for the treatment of atherosclerosis, are also potentially capable of preventing the replication of the hepatitis C virus. Target intervention in the metabolic pathway of virus reproduction may be significantly more effective than the direct action on the virus and, in turn, can prevent the formation of strains resistant to antiviral drugs [8, 9]. Despite a high potential of this approach, the primary studies conducted with the use of atorvastin and bezafibrat did not meet the expectations — no significant effect of these drugs on the titer of the virus was shown [10, 11].

We early reported that N-stearoylethanolamine (NSE), which belongs to the class of minor lipids named N-acylethanolamines, showed the antiviral action against the influenza virus (H1N1 strain) [12]. The results obtained in our *in vitro* experiments, showed that the selectivity index of NSE is 100. This finding allows us to classify NSE as an active antiviral drug. Additionally, *in vitro* evaluation of NSE cytotoxicity (by determining its effect on mitotic index of cell proliferation) did not reveal any significant effect on cell mitosis. Thus, NSE is a potential non-toxic drug that does not cause the proliferative and mutagenic activities *in vitro*. The aim of this study was to investigate antiviral activity of the endogenous cannabimimetic compound NSE towards the hepatitis C virus.

Materials and Methods

1. Reagents

NSE — stearic acid ethanolamide — white crystalline substance (gross formula $C_{20}H_{41}NO_2$), soluble in non-polar solvents, molecular weight 327.545, density 0.9 ± 0.1 g / cm^3, ignition temperature 247.7 ± 24.0 °C, boiling point 486.0 ± 28.0 °C at 760 mm Hg. Art. NSE is part of the cell membranes.

NSE was synthesized in the Department of Lipid Biochemistry at Palladin Institute of Biochemistry as described previously [13]. Briefly, ethanolamine and stearic acid were co-condensed in argon atmosphere and upon defined temperature regime and NSE obtained was purified by re-crystallization in ethanol. The end product was verified by Agilent 7890A/5977B GC/MS system analysis.

Different concentrations (10^{-5}–10^{-10} M) of NSE water suspension were used in this study.

2. Viruses and cells cultures

Surrogate model of the HCV

BVDV was obtained from Institute of Veterinary Medicine of The National Academy of Agrarian Sciences of Ukraine bank and maintained by passaging in the MDBK cells.

MDBK cells were propagated in DMEM, supplemented with 10 % heat-inactivated bo-

vine serum (Sigma-Aldrich, USA) at 37 °C in humidified atmosphere containing 5 % of CO_2, and passaged by trypsinization with trypsine-EDTA solution (Sigma-Aldrich, USA) twice a week.

About 10^4 MDBK cells per well were seeded in wells of 96 plates and 24 hours later infected with a 10-fold dilution series of BVDV samples. The medium was replaced after 1 hour of incubation. The infectious titer BVDV in MDBK cells was 5.0 lg $TCID_{50}$/ml.

The antiviral activity of NSE was studied in the MDBK cell culture, to which NSE in various concentrations (10^{-10}–10^{-6} M) and 100 TCD_{50} dose of BVDV were added. The cells were incubated in a thermostat until the specific cytopathogenic action of the virus, and then the infectious titer in the culture medium was determined.

Maximum nontoxic concentration (MNTC) of NSE. The MDBK cells were cultured in 96-well plates at the temperature of 37 °C in humidified 5 % CO_2 environment for 5 days. Control cells and cells treated with NSE were examined daily. Cytopathogenic effects (CPE) were estimated by cell morphology (rounding and shrinkage of cells, detachment of degenerative cells from the surface).

Minimum inhibitory concentration (MIC) of NSE. MDBK cells in 96-well plates were infected with 100 TCD_{50}/0.1 ml BVDV. Following 1-h absorption at 37 °C, virus-containing medium was washed out and the maintaining medium (RPMI-1640 with 2 % of fetal calf serum) containing NSE within the range of 10^{-10}–10^{-6} M was added. BVDV in control and NSE-treated cells were then titrated in MDBK cells by CPE. MIC was evaluated by the reduction of the infectious titer by at least 2 lg TCD_{50}.

Selectivity index (SI) of NSE. Selectivity index (SI) of NSE as anti-BVDV agent was calculated as [the] MNTC/MIC ratio.

3. Model of transfected HCV-producing Jurkat cell line

Jurkat cell line from the established cell line of human origin was obtained from Institute of Immunology RAMS (Russian Federation). These cells grew in suspension at 37 °C in the RPMI-1640 medium supplemented with 2 % [of] glutamine and 10 % of heated fetal serum, the CO_2 concentration in the incubator was 5 %. The cell density was about (3–9) $\times 10^5$ cells/ml.

HCV-producing Jurkat cell line culture model. Jurkat cells were transfected with HCV. As the HCV source we used non-diluted blood plasma samples of HCV-infected patients with different virus load; such plasma contained HCV RNA. All viral RNA preparations were isolated using "RIBO-prep" kit (Russian Federation).

Measurement of [the] HCV content in clinical samples. Quantitative estimation of the HCV mRNA levels in clinical samples was carried out using the real-time PCR with hybridization and fluorescent detection. For this aim, a reagent kit "AmpliSens HCV-Monitor-FRT" (Russia) and an apparatus "Rotor-Gene 3000/6000" ("Corbett Research", Australia) were used. The HCV complementary DNA (cDNA) was synthesized by the reverse transcription reaction using «Reverta-L» (RF) kit. 10 μl of RNA samples were added to 10 μl of prepared reaction mixture (lyophilized plant preparation, 125 μl of the RT-mix solution and 6 μl of the murine leukemia virus (MMLv)

revertase); the transcription was carried out at 37 °C during 30 min, and cDNA was obtained.

Transfection procedure using the Turbofect. The transfection was conducted according to the standard protocol for *Turbofect* (Thermo Scientific, Lithuania). The virus detection was carried out using the PCR on the second passage (9th day of cultivation) and on the fifth one (17th day of cultivation). All the cDNA of hepatitis C patients transfected *Jurkat* cultures produced HCV on the 9th and 17th days of cultivation [14, 15].

4. Docking procedure

The structures of HCV NS3 serine protease (2A4Q, 4A92) and HCV NS5B polymerase (3PHE, 2IJN) are retrieved from the open access digital data resource Protein Data Bank. Using these structures, we can dock different compounds (Tabl. 1) into the active sites of the HCV proteases and polymerases. This docking is performed after the removal of water molecules, the addition of missing hydrogens and the removal of reference ligands from the protein structure using AutoDock 1.5.6 software tools (ADT) and docking protocol type A described in [16]. The same program determined the amino acid residues involved in binding test ligands to the active sites of NS3 and NS5 proteins. Docking simulations are performed using Autodock Vina 1.1.2 software (ADV) [17]. Both the ligands and proteins are prepared for docking using ADT. The number of points in x-, y- and z-box dimensions are set to 80 with grid spacing of 0.375Aº. Center grid box is set to the center active site of protein. The ligands (excluding NSE) are taken from the crystal structures and re-docked using the same protocol. The structure of NSE was obtained from ChemSpider database as mol file and converted to mol2 format by OpenBabel 2.4.1 software [18]. The lowest free binding energy (ΔG) of the ligand to the

Table 1. **Some characteristics of studied compound[s]**

Abbreviature of compound	Chemical name	Inhibitor for protein	Crystal structure used for docking (ID from Protein Data Bank)
NSE	N-stearoylethanolamine	NS3/4A -?	2A4Q, 4A92
		NS5B -?	3PHE, 2IJN
CMP1	(2R)-({N-[(3S)-3-({[(3S,6S)-6-cyclohexyl-5,8-dioxo-4,7-diazabicyclo[14.3.1]icosa-1(20),16,18-trien-3-yl]carbonyl}amino)-2-oxohexanoyl]glycyl}amino)(phenyl)acetic acid	NS3/4A	2A4Q
CMP2	(1'R,2R,2'S,6S,24AS)-17-fluoro-6-(1-methyl-2-oxopiperidine-3-carboxamido)-19,19-dioxido-5,21,24-trioxo-2'-vinyl-1,2,3,5,6,7,8,9, 10,11,12,13,14,20,21,23,24,24a-octadecahydrospiro[benzo[s]pyrrolo[2,1-g][1,2,5,8,18]thiatetraazacycloicosine-22,1'-cyclopro-2-carboxylatepan]-2-yl 4-fluoroisoindoline	NS3/4A	4A92
CMP3	(2r,3r)-3-{[3,5-bis(trifluoromethyl)-phenyl]amino}-2-cyano-3-thioxopropanamide	NS5B	2IJN
CMP4	4-chlorobenzyl 6-fluoro-7-(4-methylpiperazin-1-yl)-1-[4-(methylsulfonyl)benzyl]-4-oxo-1,4-dihydroquinoline-3-carboxylate	NS5B	3PHE

macromolecule was determined using ADV. Inhibition constant Ki was calculated as exp(ΔG/RT), R — the gas constant $1.98720425864083...\times10^{-3}$ kcal·K^{-1}·mol^{-1}; **T — room temperature = 298,15 °K.**

Data analysis

All experiments were performed in triplicates. The antiviral activity of the NSE was expressed as the log10 reduction of the viral titer by comparison with untreated controls (inhibition of infectious titer). The standard deviation in the reduction of virus titer was about 0.5 log10. The NSE was considered active only when the virus yield decreases ≥ 2 log10, at the effective dose ED50.

Results and Discussion

Considering the fact that human HCV cannot be obtained enough in its pure form to perform the experiment, the surrogate bovine diarrhea virus (BVBD) was used in this study. BVBD is morphologically close to the human HCV and does not require special safety procedures to work with [19]. Therefore, this virus was used in our screening studies to test active compound against hepatitis C [13].

The results of the infectious titer of BVDV for MDBK cells with or without NSE addition are presented in Table 2.

Table 2. **The infectious titer of BVDV in MDBK cells**

Preparation concentration of NSE, M	Virus titer, lg TCID50/ml
$1\cdot10^{-6}$	4.0
$1\cdot10^{-7}$	4.0
$1\cdot10^{-8}$	3.0
$1\cdot10^{-9}$	3.0
$1\cdot10^{-10}$	5.0
0	5.0

It is shown that NSE at concentrations of 10^{-6}–10^{-7} M inhibits BVDV reproduction by 10 times, herewith NSE at concentration of 10^{-8}–10^{-9} M — by 100 times. NSE in concentration 10^{-10} M did not affect the BVDV reproduction.

As was shown the results of morphological test the minimum of toxic concentration of NSE was 10^{-4} M and maximum nontoxic concentration of NSE was 10^{-5}M.

Maximum nontoxic concentration (MNTC), minimum inhibitory concentration (MIC) and selectivity index (SI) for NSE in MDBK cells was shown in Table 3.

Table 3. **Maximum nontoxic concentration (MNTC), minimum inhibitory concentration (MIC) and selectivity index (SI) for NSE in MDBK cells**

Indication	Values
MNTC	10^{-5} M
MIC	10^{-9} M
SI	10000

The results of selectivity index (Table 3) estimation indicate that NSE has high antiviral potential to BVDV (or HCV).

Because BVDV is morphologically close to human HCV we have supposed that the inhibitory NSE action could be extrapolated on HCV.

To obtain the HCV-producing cell cultures, HCV RNA was isolated from infected patients. Subsequently, cDNA, obtained on the HCV RNA matrix, was used for the transfection of Jurkat cells and further PCR analysis (Table 4).

Therefore, as a result of transfection procedures with Turbofect, we obtained a cell culture transfected with cDNA of HCV that shows a stable reproduction of the HCV.

Table 4. **Transfection results of Jurkat suspension cell cultures for stable production of HCV**

Passage number after transfection	HCV load (genomic equivalent for different patients)				
	1	2	3	4	5
2nd passage	2530	1321	1027	835	950
5nd passage	5253	4094	3153	4010	5094

The results of studying the NSE effect on the reproduction of HCV in transfected Jurkat cells line on the 5th day of cultivation presented in table 5 show that NSE at concentrations from 10^{-6} M to 10^{-9} M effectively inhibits the reproduction of HCV. NSE at concentration 10^{-10}M did not affect the HCV reproduction.

Table 5. **Human HCV RNA content in Jurkat cell line with or without NSE**

NSE, M	RNA content of human HCV in Jurkat line (genomic equivalent)	
	2nd passage	5th passage
$1 \cdot 10^{-6}$	0	0
$1 \cdot 10^{-7}$	0	0
$1 \cdot 10^{-8}$	0	0
$1 \cdot 10^{-9}$	0	0
$1 \cdot 10^{-10}$	252	370
0	240	383

Molecular docking is an effective tool to study the mechanisms of biologic processes, compare the effectiveness of various drugs, and predict the molecular and physicochemical properties of molecules. Molecular docking is an important method for studying protein-ligand interactions and facilitating the creation of potent drugs. A dock is a computing tool that places a small molecule (ligand) at the binding site of its macromolecular target (receptor) and evaluates its binding affinity. In molecular docking based on protein structures, thousands of possible positions of association of a ligand with its receptor are tested and evaluated using the energy counting function. The position with the lowest energy index is predicted to be "the best match", that is, the binding mode [20]. In accordance with these considerations, we used molecular docking to study the binding mode of HCV-NS3 protease and HCV-NS5B polymerase known inhibitors and NSE (see Table 1) to various viral proteins.

It is known that the major amino acid residues of NS3 protease, likely involved in the interaction ligands, are Gln41, Ser42, Phe43, His57, Arg109, Val132, Leu135, Lys136, Gly137, Ser138, Ser139, Phe154, Arg155, Ala156, Ala157 and Cys159 [21]. The HCV-NS5B polymerase active site region constitutes [the] residues Ser96, Ala97, Ile160, Phe162, Arg168, Gly557, Asp559 [22].

From Fig. 1 and Table 6 it may be suggested that NSE can bind to the active centres of both the NS3 serine protease and hepatitis C virus NS5B-polymerase and has an inhibitory effect on their activity.

Nonstructural protein 5B (NS5B), the RNA-dependent RNA polymerase of Hepatitis C Virus (HCV), plays a key role in viral amplification and is an attractive and most explored target for discovery of new therapeutic agents against Hepatitis C.

Docking of NS3 and NS5B with NSE showed that NSE has a rather high binding affinity (inhibitory constant Ki $\approx 10^{-5}$M) for the active sites of NS3 and NS5B. Based on this result, it can be assumed that NSE is able to inhibit the hepatitis C virus replication.

Thus, it can be assumed that the observed effect of inhibiting the reproduction of the hepatitis C virus in Jurcat cells (Tabl. 5) is due

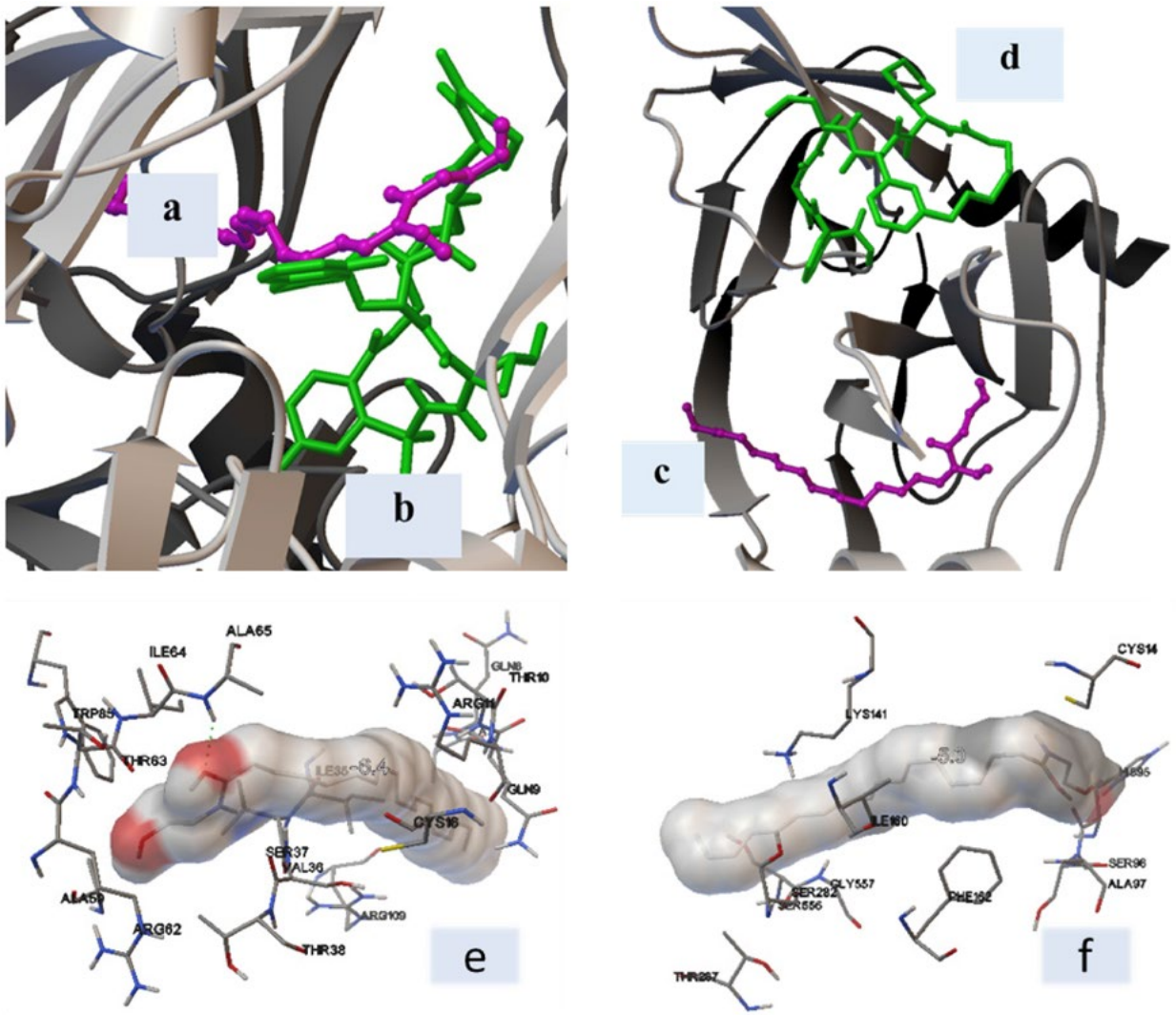

Fig. 1. Sample of docking diagram showing the binding with active site of HCV serine protease NS3/4A (4A92) of NSE (a, e), CMP2 (b) and binding with active site of HCV RNA-depended RNA polymerase NS5B (2IJN) of NSE (c, f) and CMP3 (d).

to the binding of NSE to the active center of the viral proteins (NS3 and NS5B) responsible for the replication of the virus.

Conclusions

The present study has shown the strong antiviral activity of NSE against the human hepatitis C virus, which allows suggesting NSE as a novel active compound for the treatment of human hepatitis C.

Compliance with ethical standards

This research was fully financed by National Academy of Science of Ukraine.

Table 6. **The lowest free binding energy of the studied ligand and its interaction with amino acid residues for active site**

HCV target proteins	Protein Databank ID	Compound	Estimate[d] minimal free binding energy, kcal/mol	Ki, M	Common amino acids residues interacting across compounds
NS3/4A serine protease	2A4Q	NSE	-6.4	$2{,}03 \cdot 10^{-5}$	GLN8, GLN9, THR10, ARG11, CYS16, ILE35, VAL36, SER37, THR38, ARG62, THR63, ILE64, ILE64, ALA65*, TRP85, **ARG109**
		CMP1	-8.2	$9{,}74 \cdot 10^{-7}$	**GLN41**, THR42, **HIS57**, **LYS136**, **GLY137**, **SER138**, **SER139***, **ALA156***, **ALA157**
	4A92	NSE	-5.8	$5{,}6 \cdot 10^{-5}$	**ASP81**, ARG123, **ARG155**, **ALA156**, **ASP168**, THR433, THR435, ARG481, **MET485**, PHE486, **VAL524**, CYS525, **GLN526**
		CMP2	-17.0	$3{,}45 \cdot 10^{-13}$	GLN41, PHE43, HIS57, HIS57, GLY58, ASP79, GLN80, **ASP81**, ASP81, VAL132, PHE134, LEU135, **LYS136**, **GLY137**, **SER138**, **SER139**, **PHE154**, **ARG155**, ALA156, **ALA157**, VAL158, **MET485**, **VAL524**, **GLN526**, ASP527, HIS528
NS5B RNA-dependent RNA polymerase	2IJN	NSE	-5.0	$2{,}16 \cdot 10^{-4}$	CYS14, HIS95, **SER96**, **ALA97**, LYS141, **ILE160**, **PHE162**, SER282, THR287, SER556, **GLY557**
		CMP3	-7.8	$1{,}91 \cdot 10^{-6}$	**ILE160**, **PHE162**, **ARG168**, GLN446, **GLY557**, GLY558, **ASP559**, ILE560
	3PHE	NSE	-5.8	$5{,}6 \cdot 10^{-5}$	**ALA97**, LYS141, ARG158, **ILE160**, **PHE162**, SER282, ASN291, SER556, **GLY557**
		CMP4	-9.0	$2{,}53 \cdot 10^{-7}$	PRO93, HIS95, **ALA97**, **ILE160**, **PHE162**, GLN440, **GLY557**, **ASP559**

Notes: 1. ***** — hydrogen bounds.

2. Underlined bold amino acid residues are in the active center of the HCV target protein.

All the authors declare that there are no conflicts of interest related to this work.

REFERENCES

1. *Lauer GM, Walker BD.* Hepatitis C virus infection. *N. Engl. J. Med.* 2001; **345**(1):41–52.
2. *Danny JE, Delphi GMC, Tara LP, Laurien AR, Jayasree KI.* Access to hepatitis C medicines. *Bulletin of the World Health Organization.* 2015; **93**:799–805.
3. *Serfaty L, Andreani T, Giral P, Carbonell N, Chazouillères O, Poupon R.* Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. *J. Hepatol.* 2001; **34**(3):428–434.
4. *Kapadia SB, Chisari FV.* Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. *Proc. Natl. Acad. Sci USA.* 2005; **102**(7):2561–2566.
5. *Kim SS, Peng LF, Lin W, Choe WH, Sakamoto N, Kato N, Ikeda M, Schreiber SL, Chung RT.* A cell-based, high-throughput screen for small molecule

regulators of hepatitis C virus replication. *Gastroenterology.* 2007; **132**(1):311–320.
6. *Nahmias Y, Goldwasser J, Casali M, van Poll D, Wakita T, Chung RT, Yarmush ML.* Apolipoprotein B-dependent hepatitis C virus secretion is inhibited by the grapefruit flavonoid naringenin. *Hepatology.* 2008; **47**(5):1437–1445.
7. *Gastaminza P, Cheng G, Wieland S, Zhong J, Liao W, Chisari FV.* Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. *J. Virol.* 2008; **82**(5):2120–2129.
8. *Manns MP, Foster GR, Rockstroh JK, Zeuzem S, Zoulim F, Houghton M.* The way forward in HCV treatment - finding the right path. *Nat. Rev. Drug Discov.* 2007; **6**(12):991–1000.
9. *Kuntzen T, Timm J, Berical A, Lennon N, Berlin AM, Young SK, Lee B, Heckerman D, Carlson J, Reyor LL, Kleyman M, McMahon CM, Birch C, Schulze Zur Wiesch J, Ledlie T, Koehrsen M, Kodira C, Roberts AD, Lauer G.M, Rosen HR, Bihl F, Cerny A, Spengler U, Liu Z, Kim AY, Xing Y, Schneidewind A, Madey MA, Fleckenstein JF, Park VM, Galagan JE, Nusbaum C, Walker BD, Lake-Bakaar GV, Daar ES, Jacobson IM, Gomperts ED, Edlin BR, Donfield SM, Chung RT, Talal AH, Marion T, Birren BW, Henn MR, Allen TM. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naïve patients. Hepatology.* 2008; **48**(6):1769–1778.
10. *O'Leary JG, Chan JL, McMahon CM, Chung RT.* Atorvastatin does not exhibit antiviral activity against HCV at conventional doses: a pilot clinical trial. *Hepatology.* 2007; **45**(4):895–898.
11. *Fujita N, Kaito M, Kai M, Sugimoto R, Tanaka H, Horiike S, Konishi M, Iwasa M, Watanabe S, Adachi Y.* Effects of bezafibrate in patients with chronic hepatitis C virus infection: combination with interferon and ribavirin. *J. Viral Hepat.* 2006; **13**(7):441–448.
12. *Gula NM, Chumak AA, Rybalko SL, Dyadyn ST, Asmolkova VS, Berdyshev AG, Kosyakova GV, Starosila DB, Benkovsky LK, Bashta YuM.* Antiinfluenza effect of N-stearoylethanolamine. *J. Nat. Acad. Med. Sci.* of Ukraine. 2014; **20**(4):393–401.
13. *Hula NM., Chumak AA., Mehed OF.* Immunosuppressive characteristics of N-stearoylethanolamine a stable compound with cannabimimetic activity. *Ukr. Biokhim. Zh.* 2008; **80** (1): 57–67 (Article in Ukrainian).
14. *Rybalko SL, Porva Yu, Alekseenko IP, Diadiun ST, Zavelevych MP, Borovykov VM, Popova LA, Fedorchenko DB.* Replication of hepatitis C virus in cell culture. *Biopolymers and Cell.* 2009; **25**(1):44–49.
15. *Porva Yu, Rybalko SL, Palchykovs`ka LI, Atamanyuk VP.* Study of antiviral activity of a new plant origin preparation neoflazidum on a model of the hepatitis C virus. *Biopolymers and Cell.* 2015; **31**(6):465–472.
16. *Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ.* Computational protein–ligand docking and virtual drug screening with the AutoDock suite. *Nature protocols.* 2016; **11**(5):905.
17. *Trott O, Olson AJ.* AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry.* 2010; **31**(2):455–61.
18. *O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR.* Open Babel: An open chemical toolbox. *J Cheminform.* 2011; **3**:33.
19. *Durantel D, Alotte C, Zoulim F.* Glucosidase inhibitors as antiviral agents for hepatitis B and C. *Curr. Opin. Investig. Drugs.* 2007; **8**(2):125–129.
20. *Huang SY, Zou X.* Advances and challenges in proteinligand docking. *Int J Mol Sci.* 2010; **11**:3016–3034.
21. *Wei Y, Yang J, Kishore Sakharkar M, Wang X, Liu Q, Du J, Zhang JJ.* Evaluating the inhibitory effect of eight compounds from Daphne papyracea against the NS3/4A protease of hepatitis C virus. *Nat Prod Res.* 2018; **17:1–4.**
22. *Ahmed-Belkacem A, Guichou J-F, BrilletR.* Inhibition of RNA binding to hepatitis C virus RNA-dependent RNA polymerase: a new mechanism for antiviral intervention. *Nucl Acids Res.* 2014;**42**(14): 9399–409.

N-стеароїлетаноламін — новий інгібітор репродукції вірусу гепатиту С

Н. М. Гула, А. А. Чумак, А. Г. Бердишев, Г. В. Косякова, Т. М. Горідько, О. Ф. Мегедь, В. С. Асмолкова, Ю. І. Порва, С. Л. Рибалко, С. Т. Дядюн, Д. Б. Старосила, О. М. Дерябін

Мета. Дослідження противірусної активності ендогенного канабіміметика N-стеароїлетаноламіну (NSE) відносно репродукції вірусу гепатиту С (ВГС). **Методи.** Модель сурогатного ВГС — вірус бичачої діареї, клітинна модель — культура Jurkat, що трансфікована кДНК ВГС людини, метод молекулярного докінгу. **Результати.** У дослідах *in vitro* встановлено, що NSE ефективно пригнічував репродукцію сурогатного ВГС в клітинах MDBK та в трансфікованих кДНК ВГС людини клітинах Jurkat. Проведений молекулярний докінг свідчить, що NSE може зв'язуватися з активним центром як NS3 серинової протеази, так і NS5B-полімерази ВГС і в такий спосіб інгібувати їх активність. **Висновки.** Отримані дані свідчать про перспективність використання NSE для розробки противірусного лікарського засобу, що пригнічує активність ВГС людини.

Ключові слова: N-стеароїлетаноламін, антивірусна активність, вірус гепатиту С, молекулярний докінг

N-стеароилэтаноламин — новый ингибитор репродукции вируса гепатита С

Н. М. Гулая, А. А. Чумак, А. Г. Бердышев, Г. В. Косякова, Т. М. Горидько, Е. Ф. Мегедь, В. С. Асмолкова, Ю. И. Порва, С. Л. Рыбалко, С. Т. Дядюн, Д. Б. Старосила, О. Н. Дерябин

Цель. Исследование влияния эндогенного канабимиметика N-стеароилэтаноламина (NSE) на репродукцию вируса гепатита С (ВГС). **Методы.** Модель суррогатного ВГС — вируса бычьей диареи, клеточная модель — клетки Jurkat, трансфицированные кДНК ВГС человека и метод докинга (МД). **Результаты.** В опытах *in vitro* установлено, что NSE эффективно подавлял репродукцию суррогатного ВГС в клетках MDBK и в трансфицированных клетках Jurkat. МД показано, что NSE может связываться с активными центрами NS3 сериновой протеазы и NS5B-полимеразы ВГС и подавлять их активность. **Выводы.** Полученные данные свидетельствуют о перспективности использования NSE для разработки противовирусного лекарственного средства для подавления активности ВГС.

Ключевые слова: N-стеароилэтаноламин, антивирусная активность, вирус гепатита С, молекулярный докинг

Received 02.08.2020