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The potential of Corona Charged Aerosol Detector for investigation of telmisartan - β -cyclodextrin inclusion complexes

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Summary

Cyclodextrins (CDs) are widely used in pharmaceutical analysis due to its biodegradability and eco-friendly character. The particular structure of CDs, characterized by hydrophobic cavity, enables the formation of inclusion complexes with variety of organic compounds. As structures lacking chromophores, CDs could not be detected by Photodiode Array (PDA) detector and Corona Charged Aerosol Detector (CAD) was introduced as the most appropriate detector for the formed complexes. The aim of the study was to investigate the degree of complexation between telmisartan, used as a model substance, and β -CD. Moreover, the effect of different β -CD concentrations (5-15 mM) and pH of the aqueous part of mobile phase (3-7) on the degree of complexation was also assessed. The intensity of the formed complex appeared to be the highest when 15 mM β -CD was used for the complexation. Also, it was noticed that the increase in peak areas with an increasing β -CD concentration was more evident at pH 7 in comparison with the same trend at lower pH values. The reproducibility of the measurement was confirmed by low relative standard deviation (RSD) of peak areas within five measurements. These findings support the use of HPLC-CAD methods for studying the process of inclusion complexes formation.

Keywords: β -Cyclodextrin; Inclusion complex;
Corona Charged Aerosol Detector; Telmisartan;

1. Introduction

Liquid chromatography is one of the most frequently used techniques in drug analysis, during research and development, as well as in routine control of pharmaceutical substances and pharmaceutical products (1). To achieve the desired chromatographic efficiency, it is favorable to use organic solvents, in the first place acetonitrile. Most organic solvents used in HPLC are toxic and could harmfully impact the environment and human health as well (2-4). To avoid the negative consequences of large amounts of acetonitrile used during the analysis, different “greening” strategies were applied (4). Nowadays, development of green liquid chromatography methods is a recognized trend in pharmaceutical analysis (5, 6). In that respect, CDs are applied as mobile phase additives allowing an increase of water and simultaneous decrease of organic solvent content, resulting in the consequent reduction of analyte’s retention times (7, 8). CDs are well-known cyclic oligomers derived from starch. Their particular structure, characterized by a hydrophilic outer surface and an internal relatively hydrophobic cavity, is responsible for the good water solubility, as well as ability to fully or partially encapsulate hydrophobic molecules of suitable size within their cavity (9-11).

In this study, telmisartan (Figure 1), as a representative of the group of sartans, was selected as a model substance. Physicochemical characteristics of telmisartan made it a suitable candidate to be incorporated in CD cavity. Telmisartan is amphoteric compound, weakly dissolved in water, but moderately to highly dissolved in methanol or acetonitrile (12).

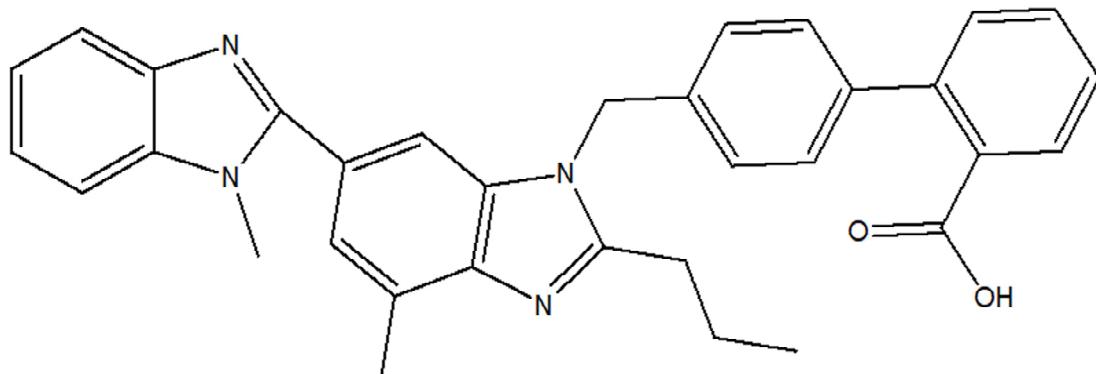


Figure 1: Chemical structure of telmisartan

The ability to form inclusion complexes with structurally diverse drugs served as a base from which numerous applications of CDs were made in the pharmaceutical industry (6, 13). CDs are used to modify the unpleasant smell or taste of the pharmaceuticals, their physicochemical instability, low solubility in water and poor bioavailability. For this reason CDs are profoundly useful in pharmaceutical industry and are incorporated in a number of pharmacopeia sources (14). Moreover, regarding the prominent solubilizing potency, as well as, unfavorable permeability through hydrophobic bio-membranes, CDs are used very successfully in formulations intended for oral, ocular, nasal and, even, parenteral route of drug application (15). Having in mind the great attention CDs have attracted, it would be of high importance to characterize the formed complexes in more detail, and define the parameters which significantly influence the complexation procedure.

Regarding the detection of the complex, there are certain limitations originating from the lack of chromophores in the structure of CDs. Several methods for evaluation of inclusion complexes can be found in literature, applying mass spectrometry (MS) or nuclear magnetic resonance (NMR) (11, 16, 17). However, these techniques are rather expensive, so would not be the first choice for routine analysis. Therefore, a strong need for detector generating sensitive and universal response, independent of spectral or physicochemical qualities of the investigated compounds, arises. Having in mind the aforementioned, the purpose of the study was to investigate the potential of CAD in determining the degree of complexation. CAD, as a detector based on aerosol charging, generates a universal response over a broad dynamic range regardless of the analyte's structure. Among all so far constructed aerosol based detectors, it stands out as an easy-to-use and highly sensitive mass dependent detector (18, 19). Thus, suitability of CAD to investigate the complexation process occurring between telmisartan and β -CD was tested for the first time. We assessed the influence of certain parameters such as β -CD concentration and pH of the sample on the intensity of the complex.

1.1 Theory

During the past years, scientists have dealt with finding the appropriate technique to characterize the complexes formed between CDs and various organic hydrophobic compounds (17). ESI-MS was recognized as soft ionization technique able to study the stoichiometry of the formed complexes (16), however the information about the degree of complexation was not provided. NMR was also successfully used for stoichiometry determination, as well as assessing the complex stability (20, 21), but the same limitation as in case of ESI-MS occurred. Furthermore, CAD, as a universal detector producing a response independent on the analyte's structure, was the detector of choice (22, 23). CAD's principle of functioning is reflected in nebulization of analytes together with mobile phase, leading to droplets formation. Analyte particles are produced while

droplets are dried to remove the mobile phase. Further, generated aerosol is reaching the reaction chamber where it is collided with a stream of positively ionized nitrogen that has primarily passed over high voltage corona wire. The positive charge is then transferred to the opposing stream of analyte particles and the transferred charge is generating a response directly proportional to the amount of the analyte investigated (23). The intensity of the signal is dependent on the amount of charge the analyte surface can receive, so the detector's response would mostly depend on the mass of investigated analyte (22). Implied phenomenon encompass that larger particles carry more charge than smaller ones. As a particle detector, it is believed that CAD produces the equal amount of particles if the same amount of different materials is entering detector under the stable conditions. However, there is variability in CAD response which originates from the influence of different factors affecting the process of particle formation. Taking into account the evaporation process, mobile phase components have to meet the certain volatility criteria, similar to MS mobile phase requirements (24). In the same course, higher content of organic solvent in the mobile phase is beneficial. Additionally, mobile phase with higher percentage of organic modifier regulates production of larger number of substance particles via lower viscosity. Furthermore, the decreased mobile phase flow rate also contributes to enhanced detector response, as in the case of HPLC-MS. Apart from being a detector of choice for analytes lacking chromophores, CAD's additional advantage is its ease of use reflected in small number of parameters to be optimized (22, 23).

2. Experimental

2.1 Chemicals and reagents

Reference standard substance of telmisartan (2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl]methyl]phenyl]benzoic acid) was purchased from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany). β -CD was obtained from Acros organics (Geel, Belgium). For mobile phase preparation, deionized water obtained from a Simplicity 185 purification system, Millipore (Billerica, MA, USA), was used. HPLC-grade acetonitrile was also purchased from Sigma Aldrich, while ammonium hydroxide and acetic acid used for pH adjustment were obtained from Centrohem d.o.o. (Stara Pazova, Serbia) and Merck (Darmstad, Germany), respectively. pH of the aqueous phase and working samples was adjusted on PHM210 Standard pH-meter (Radiometer Analytical SAS, Lyon, France) equipped with glass electrode. Before use, mobile phase and working samples were filtered through 0.45 μm nylon membranes (Agilent Technologies, Santa Clara, CA, USA).

2.2 Sample preparation

Stock solution of telmisartan was prepared by dissolving the appropriate amount of the reference standard substance in the mixture of water and acetonitrile (50:50, v/v) to obtain the concentration of 0.5 mg mL⁻¹. β-CD stock solutions were prepared by dissolving an appropriate amount of β-CD in deionized water to obtain the following concentrations: 5 mmol L⁻¹, 10 mmol L⁻¹ and 15 mmol L⁻¹. pH of the working solutions was adjusted by addition of ammonium hydroxide or acetic acid. pH was set to 3, 5 or 7 and five working solutions were prepared at each pH level. The first working solution was consisted of 100 μL of stock solution of telmisartan, 760 μL of deionized water with adjusted pH and 140 μL of acetonitrile. The second working solution consisted of 760 μL of the stock solution of β-CD in 10 mM concentration, 50 μL of deionized water with pH set to a certain level and 190 μL of acetonitrile. The remaining three working samples contained mixture of stock solution of telmisartan (100 μL), stock solution of β-CD in 5 mM, 10 mM and 15 mM concentration, respectively (760 μL) and acetonitrile (140 μL). The complexation was assisted by vortexing the samples for 1 h before the analyses.

2.3 Instrumentation

Experiments were carried out on Dionex Ultimate 3000 HPLC system, equipped with Diode Array Detector (DAD) and Corona Veo Charged Aerosol Detector (Thermo Fisher Scientific, Germerring, Germany), serially connected. Separation was achieved on HypersilGOLD-C4 (150 x 4.6mm, 5μm) column (Thermo Fisher Scientific, Germerring, Germany). The temperature of the column was set to 25 °C. Gradient elution with mobile phase consisted of mixture of water and acetonitrile was applied according to the program presented in Table I. pH of the aqueous part of the mobile phase was set to 3, 5 or 7 with addition of acetic acid or ammonium hydroxide. Detection on DAD was performed at 295 nm, while CAD signal intensity was examined by adjusting the parameters presented in Table II. Injection volume was 10 μL.

Table I Gradient elution program

Time (min)	Mobile phase flow (mL min ⁻¹)	Content of acetonitrile (%)	Content of aqueous phase (%)
0	0.7	10	90
14.0	0.7	90	10
14.1	0.7	10	90
15	0.7	10	90

Table II Parameters of CAD

Parameter	Optimized values
Evaporation temperature	50 °C
Filter constant	5 s
Gain range	10 pA
Power function	1
Data rate	10 Hz

The stoichiometry of the formed complexes was confirmed by injecting the samples into a mass spectrometer with electrospray ionization (ESI-MS). Experiments were performed on TSQ Quantum Access Max (Thermo Fisher Scientific Inc.).

2.4 Docking study

Molecular docking studies were performed applying an on-line available software Autodock v4.2. (The Scripps Research Institute, CA, U.S.A.) (25). β -CD was labeled as host compound and its x-ray crystal structure necessary for the study was retrieved from the Brookhaven protein data bank. Calculation of pKa and selection of dominant forms of telmisartan at different pH values were performed using Marvin Sketch 4.1.13 ChemAxon Ltd (Budapest, Hungary) (26). Conformations of telmisartan with minimal energy content across the examined pH range were obtained by the MOPAC/AM1 method of Chem 3D Ultra 7.0.0 (Surrey, UK) (27). They were further used as ligands to β -CD for docking study. Auto Dock Tools (ADT) was used for preparation, running and analyzing the docking simulation. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers (28). During the docking process, a maximum of 100 conformers was considered for each compound. A grid of 40 points in each direction (x, y, and z) with grid spacing of 0.375 Å was built centered on the center of ligand molecule. The maximum number of energy evaluation was set to 2 500 000.

3. Results and discussion

The stability of the complex formed between CDs and various organic hydrophobic compounds mostly depends on size complementarity between drug molecule and CD cavity. For that reason, β -CD was chosen as the appropriate one, since there is evidence that large cavity of β -CD is a better host for a phenyl group (29, 30). This group is also present in telmisartan, which theoretically gives the advantage to β -CD in comparison with α -CD possessing smaller cavity. Formed inclusion complex is additionally stabilized by numerous hydrogen bonds and dipole-dipole interactions between certain groups in the structure of telmisartan and primary and secondary

hydroxyl groups present inside and outside the β -CD cavity (31), which was confirmed with docking study explained in more detail in section 3.1.

To get insight into the whole complexation procedure and to determine the degree of complexation and influential parameters, it is necessary to determine the stoichiometry between drug and β -CD, and propose the most probable type of binding. Complex stoichiometry was proven to be 1:1. Molecular ions of 515.6 m/z, 1135.9 m/z and 1650.6 m/z corresponding to telmisartan, β -CD and telmisartan – β -CD complex, respectively, were detected in ESI + mode. To preclude the possibility of complexes formed in 1:2 or 2:1 ratio, broader m/z range was investigated. Across the range from 100 – 3000 m/z, no 2785.6 m/z or 2165.2 m/z corresponding to 1:2 or 2:1 complexes, could be detected. Moreover, employed ESI-MS confirmed that free drug and formed complex are eluting at the same retention times. Knowing the stoichiometry, docking study was performed to predict the complex structures.

3.1 In – silico prediction

Docking study was performed to predict the type of binding occurring between telmisartan and β -CD at different pH values. The results of the performed study gave an impression of the orientation of the ligand molecule towards the cavity of β -CD. When pH of the sample and aqueous mobile phase is set to 3, telmisartan is predominantly present in its dicationic form (81.15 %) with both amino groups protonated. Benzimidazole part of the structure of telmisartan is incorporated in β -CD cavity (Figure 2a). Benzoic acid part in its non-ionized form remains free for interactions with silanol groups of stationary phase. On the other hand, in less acidic conditions (pH 5), number of protonated amino groups is equal to the number of deprotonated carboxyl groups for 56.59 % of the molecule, so electroneutral state contributes to the overall lipophilicity of telmisartan and enables the insertion of benzimidazole part of the structure into the cavity of CD, leading to inclusion complex formation (Figure 2b). On the other hand, for 30.01 % of the molecule both amino groups of benzimidazole part of the structure are protonated at pH 5. Deprotonated carboxyl groups are available for interactions with stationary phase. Finally, at pH 7 the structure with only carboxyl group deprotonated is dominant (88.10 %). However, the pathway of the complex formation remained the same as in previous two cases. Benzimidazole part of the structure of telmisartan is entering β -CD cavity (Figure 2c). These findings indicate that the type of binding is the same at all examined pHs, leading to the conclusion that ionization of carboxylic group and amino groups of benzimidazole makes the difference. At pH 3, interactions between free benzoic acid and stationary phase outreach the interactions leading to complex formation, unlike the other investigated acidity conditions.

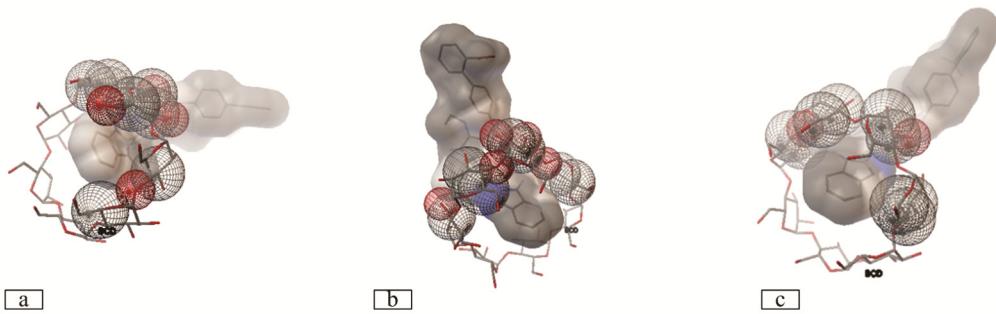


Figure 2: Docking structures

2a Telmisartan - β -CD complex formed at pH 3

2b Telmisartan - β -CD complex formed at pH 5

2c Telmisartan - β -CD complex formed at pH 7

3.2 Investigation of the influence of β -CD concentration and pH on the complexation

CAD was also employed to determine the degree of complexation and investigate its dependence on pH and β -CD concentration. The concentration of telmisartan was kept constant in all samples, while β -CD concentration was varied from 5-15 mM, as well as pH of the samples and aqueous part of the mobile phase. The influence of pH and β -CD concentration on the degree of complexation was investigated by analyzing the samples containing telmisartan only, β -CD only and their mixture. As previously mentioned, DAD was not the best choice for detection of complexes due to its inability to detect compounds without chromophores in their structure. The reason for employing DAD was to determine the retention time of free telmisartan. Furthermore, its role was to confirm that concentration of telmisartan was equal in all of the investigated samples (Figure 3).

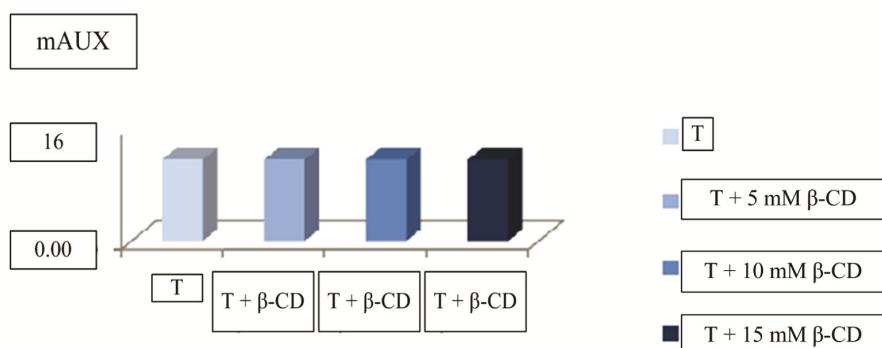


Figure 3: Peak areas originating from free and complexed telmisartan with increasing β -CD concentrations detected by DAD

On the other hand, CAD was capable to detect free β -CD as well as the complex formed between telmisartan and β -CD. When analyzing the mixture of telmisartan and β -CD with HPLC-DAD the obtained chromatogram contained only one peak. LC – ESI – MS showed that retention time of the observed peak was the same as retention time of free telmisartan leading to a conclusion that free telmisartan and formed inclusion complex are eluting at the same retention time. On the other hand, when analyzing the same mixture with HPLC-CAD, two peaks could be observed (Figure 4).

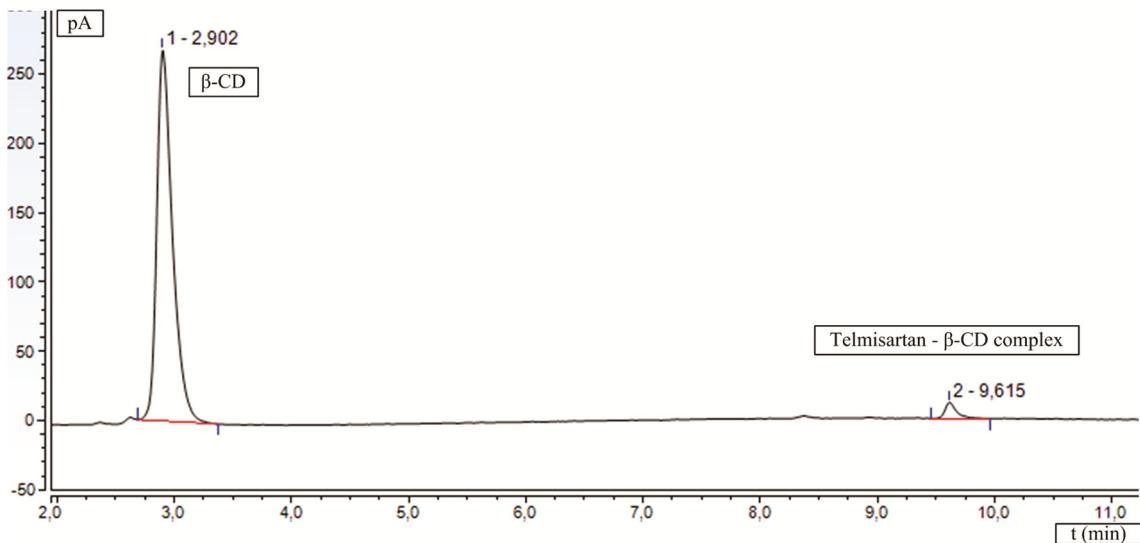


Figure 4: Chromatogram of β -CD and complex between telmisartan and β -CD obtained from HPLC-CAD

The retention time (t_r) of the first peaks is equal to retention time of free β -CD ($t_r = 2.90$ min) previously analyzed, confirming that the first peak originates from free uncomplexed β -CD. Retention time of the second peak present was the same as retention time of free telmisartan ($t_r = 9.61$ min). Consequently, the second peak represents telmisartan - β -CD complex. The influence of varying β -CD concentrations on complexation was investigated under constant concentration of telmisartan in the mixture. In the same manner the influence of different pH on the complexation procedure was also assessed. The results are presented in Table III.

Table III Signal intensities of the analyzed samples from DAD and CAD at pH = 3, pH = 5 and pH = 7

	pH = 3			pH = 5			pH = 7		
Sample	DAD	CAD		DAD	CAD		DAD	CAD	
		β -CD	T + β -CD		β -CD	T + β -CD		β -CD	T + β -CD
T	A = 21.6703 t_r = 9.48	-	A = 1.0945 t_r = 9.55	A = 16.8259 t_r = 10.48	-	A = 1.1129 t_r = 10.54	A = 18.9936 t_r = 11.38	-	A = 0.4787 t_r = 11.46
β -CD (10 mM)	-	A = 45.4792 t_r = 2.77	-	-	A = 32.3598 t_r = 2.79	-	-	A = 30.4554 t_r = 2.78	-
T + β -CD (5 mM)	A = 21.6744 t_r = 9.47	-	A = 1.1362 t_r = 9.54	A = 21.7340 t_r = 10.59	-	A = 1.4154 t_r = 10.65	A = 19.5229 t_r = 11.30	-	A = 0.9638 t_r = 11.37
T + β -CD (10 mM)	A = 21.6059 t_r = 9.47	A = 42.0940 t_r = 2.78	A = 1.1737 t_r = 9.53	A = 22.1996 t_r = 10.61	A = 30.1101 t_r = 2.79	A = 1.5213 t_r = 10.68	A = 19.2248 t_r = 11.06	A = 28.9039 t_r = 2.78	A = 1.2102 t_r = 11.13
T + β -CD (15 mM)	A = 21.5967 t_r = 9.47	-	A = 1.2197 t_r = 9.53	A = 22.7271 t_r = 10.63	-	A = 1.5286 t_r = 10.70	A = 19.8777 t_r = 10.80	-	A = 1.4993 t_r = 10.89

T – Telmisartan; A – Peak area (mAU_X for DAD, pA for CAD); t_r – retention time

When analyzing free β -CD and β -CD in mixture with telmisartan, in the same concentration, the difference in the peak area of β -CD was evident (Figure 5).

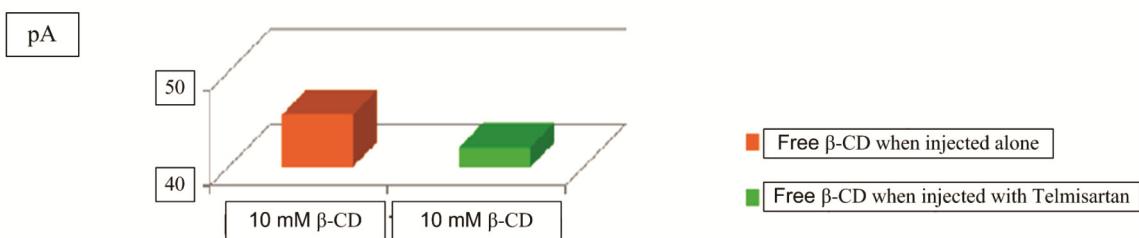


Figure 5: Difference in peak area of β -CD in the same concentration when analyzed free and in mixture with telmisartan obtained in HPLC-CAD

Moreover, peak areas of telmisartan– β -CD complex were increased with increasing β -CD concentrations from 5–15 mM. The difference in peak area of free β -CD when it was analyzed separately and in mixture with telmisartan also confirmed the hypothesis that the degree of complexation is higher with increasing concentrations of β -CD (Figure 6). The simultaneous detection on DAD showed that the signal of telmisartan was constant (Figure 3), confirming the hypothesis that the signal intensity

on CAD increases only due to the degree of complexation. Furthermore, the degree of complexation was growing with an increasing concentration of β -CD in the sample across the investigated pH range (Figure 6 and 7).

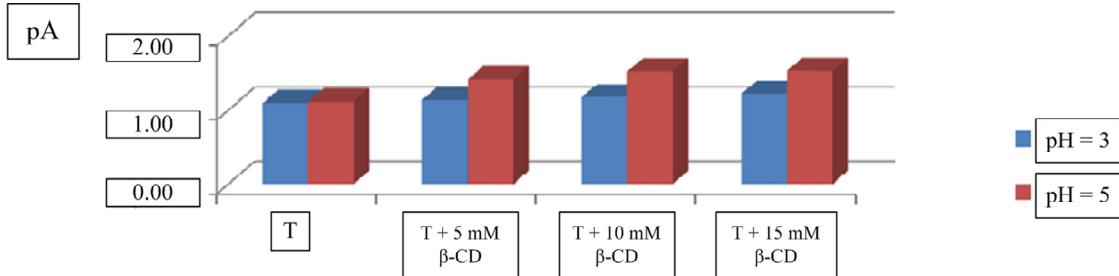


Figure 6: Comparison of degree of complexation with increasing β -CD concentrations at pH 3 and pH 5

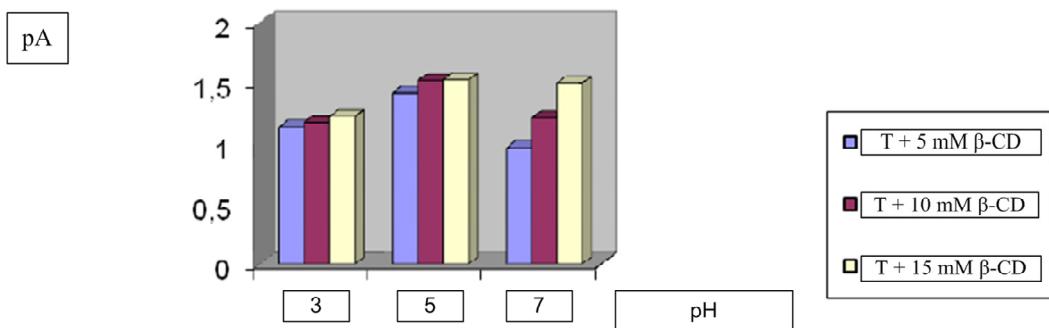


Figure 7: Comparison of degree of complexation with increasing β -CD concentrations at pH 3, pH 5 and pH 7

3.3 Reproducibility of the measurement

The reliability of the presented results was examined through the reproducibility test. Low variances between replicates confirmed reproducibility of the complexation. Furthermore, significantly lower variances between replicates compared to the variances between the samples with different concentration of complexation agent excluded the

possibility that the variances between samples with different concentration of complexation agent originate from the imprecision of the sample preparation (complexation) or instrument. To assess the reproducibility of the measurement, five identical samples were prepared and analyzed respectively. Five samples containing mixture of telmisartan with 5 mM β -CD, five samples containing telmisartan in the same concentration with 10 mM β -CD and five samples containing the same concentration of telmisartan with 15 mM β -CD were prepared and analyzed. As a measure of reproducibility, relative standard deviation (RSD) was calculated, for both HPLC-PDA and HPLC-CAD analysis. All of the obtained RSD values were less than 5% indicating good reproducibility. Results are presented in Table IV.

Table IV Assay of reproducibility of the measurement

Measurement number	T + 5 mM β -CD		T + 10 mM β -CD		T + 15 mM β -CD	
	DAD	CAD	DAD	CAD	DAD	CAD
1	20.2146	1.4491	21.6330	1.4764	16.9340	1.1187
2	20.0409	1.4381	21.7395	1.5373	16.4978	1.1876
3	19.9300	1.4224	21.6763	1.4921	16.2257	1.1111
4	19.7824	1.4567	21.5925	1.5602	15.8532	1.1474
5	19.7072	1.4410	21.5302	1.6443	15.6645	1.0357
RSD (%)	1.02	0.89	0.37	4.37	3.12	4.99

4. Conclusion

This study highlighted the potential ofto use HPLC-CAD in complexation procedure elucidation. The repeated measurements confirmed that CAD response is reproducible. Therefore, the reliability of the obtained results was also proven. An increase in β -CD concentration affected the intensity of the complex in a positive manner. Effect of pH on the complexation was also shown. At pH 7 the difference in peak areas of the complex with increasing β -CD concentrations was larger in comparison with the observed differences at pH 3 or 5. Finally, HPLC-CAD could be selected as one of the methods for studying complexes with β -CD. Moreover, due to its performances and simplicity, it could accompany the other techniques in analytical research and development.

Acknowledgment

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Uloga farmaceuta u lečenju nekih od najčešćih akutnih bolnih stanja

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Kratak sadržaj

Terapija bola izazov je i imperativ za sve zdravstvene stručnjake, uključujući i farmaceute. Najčešći razlog zbog čega se pacijenti obraćaju farmaceutu u javnoj apoteci je neko akutno bolno stanje, poput glavobolje tenzionog tipa (GTT) i dismenoreje. Kako su farmaceuti prva linija zdravstvenih radnika sa kojima se ovi pacijenti susreću, oni imaju važnu ulogu u prepoznavanju bola koji može biti simptom nekog životno ugrožavajućeg zdravstvenog problema kada pacijenta treba uputiti lekaru. Dalje, farmaceut može na osnovu verovatne dijagnoze da izvrši izbor odgovarajućeg OTC analgetika, njegove doze/farmaceutskog oblika/puta primene, preporuči nefarmakološke mere, izvrši savetovanje i edukaciju pacijenta o bolnom stanju, isprati efikasnost/podnošljivost terapije i spreči nastanak nepovoljnih ishoda terapije (npr. razvoj glavobolje usled prekomerne upotrebe analgetika). Kao lekove prvog izbora u lečenju GTT farmaceut treba da preporuči OTC nesteroidne antiinflamatorne lekove (NSAIL) ili paracetamol, dok se za lečenje dismenoreje prednost daje NSAIL-ima (ibuprofenu). Izbor leka prevashodno zavisi od prethodnog iskustva pacijenta sa lekom. U pacijenata sa GTT treba ograničiti upotrebu monokomponentnih preparata NSAIL/paracetamola na najviše 14, a kombinovanih preparata sa kofeinom/kodeinom na najviše 9 dana u mesecu, da bi se sprečio razvoj glavobolje usled prekomerne upotrebe analgetika. Samo partnerskim odnosom na relaciji pacijent-farmaceut-lekar može da se ostvari efikasna i bezbedna terapija akutnih bolnih stanja.

Ključne reči: uloga farmaceuta, OTC analgetici, glavobolja tenzionog tipa (GTT), dismenoreja, nesteroidni antiinflamatori lekovi (NSAIL)

Uvod

Lečenje bola, akutnog i hroničnog, izazov je i imperativ za sve zdravstvene radnike. Adekvatnom i blagovremenom terapijom bola značajno se poboljšava kvalitet života pacijenta, radna produktivnost i smanjuju ukupni troškovi lečenja. Međutim, podaci ukazuju da je terapija bola često suboptimalna (1,2). U cilju efikasnije kontrole bola poželjan je multidisciplinaran pristup, koji podrazumeva uključivanje i farmaceuta u zdravstvene timove koji učestvuju u lečenju različitih bolnih stanja (3).

Farmaceuti poseduju znanja i veštine od posebnog značaja za uspešnu kontrolu bola, a koje se ogledaju kroz sledeće aktivnosti:

- prepoznavanje bola koji može biti simptom nekog životno ugrožavajućeg zdravstvenog problema kada bi pacijenta trebalo odmah uputiti lekaru
- izbor optimalnog analgetika koji se izdaje bez lekarskog recepta, njegove doze/formulacije/puta primene
- edukacija pacijenta o prirodi bolnog stanja i preporučenim terapijskim opcijama
- savetovanje pacijenta u vezi sa minimiziranjem neželjenih reakcija i interakcija analgetika sa lekovima koje pacijent primenjuje za druge bolesti
- savetovanje pacijenta u vezi sa sprečavanjem prekomerne upotrebe analgetika
- praćenje efikasnosti/bezbednosti terapije bola.

Najčešći razlog zbog čega se pacijenti obraćaju farmaceutu u javnoj apoteci je upravo neko bolno stanje. Čak 8 od 10 poseta pacijenata javnim apotekama u vezi je sa bolom (4). Farmaceuti su prva linija zdravstvenih radnika sa kojima se ovi pacijenti susreću. Dugoročno posmatrano, partnerstvo između pacijenta i farmaceuta sa jedne, i lekara i farmaceuta sa druge strane, preduslov je za uspešnu terapiju bola i racionalnu primenu analgetika (2,4,5).

U ovom radu biće reči o ulozi farmaceuta u lečenju nekih od najčešćih akutnih bolnih stanja – glavobolje tenzionog tipa i dismenoreje, čija terapija se dominantno zasniva na primeni OTC analgetika (engl. over-the-counter; lekovi koji se izdaju bez lekarskog recepta, Tabela I). Otuda je farmaceut često jedini zdravstveni profesionalac koji učestvuje u lečenju ovih pacijenata i ima ključnu ulogu u obezbeđivanju efikasne i bezbedne farmakoterapije.

Tabela I OTC analgetici u Republici Srbiji (5,6)**Table I** OTC analgesics in the Republic of Serbia (5,6)

Aktivna supstanca	Doza (mg)	Farmaceutski oblik	Preparat
Monokomponentni preparati			
Ibuprofen	200	Granule za oralni rastvor	<i>Spedifen® 200</i>
	400	Granule za oralni rastvor	<i>Argifen®, Spedifen® 400</i>
	200	Film tableta	<i>BlokMax®, Brufen®, Ibuprofen</i>
	200 i 400	Šumeće granule	<i>Brufen®</i>
	200	Šumeća tableta	<i>Caffebol® Moment</i>
	200	Tableta	<i>Caffetin menstrual®</i>
	200 i 400	Kapsula, meka	<i>Dolorofen 200, Dolorofen 400, Rapidol®S</i>
	400	Film tableta	<i>BlokMax® forte, Brufen® Vivo, Ibalgin® 400, Ibumax®, Spedifen®</i>
	400	Obložena tableta	<i>Nurofen® Forte</i>
Aspirin	300	Tableta	<i>Anbol®</i>
	500	Obložena tableta	<i>Aspirin®</i>
	500	Tableta	<i>Aspirin® 500</i>
	500	Šumeća tableta	<i>Aspirin® 500</i>
	500	Granule	<i>Aspirin® Zipp</i>
Naproksen	220	Film tableta	<i>Aleve®</i>
	275	Film tableta	<i>Nalgesin® S</i>
Paracetamol	500	Šumeća tableta	<i>Efferalgan®</i>
	500	Tableta	<i>Febricet®, Paracetamol, PARACETAMOL NINI</i>
	500	Film tableta	<i>Panadol®, Panadol® Advance</i>
Kombinovani preparati			
Paracetamol + ibuprofen	500 + 200	Film tableta	<i>Avebol®</i>
	325 + 200	Tableta	<i>Metafex</i>
	500 + 150	Film tableta	<i>Rapidol® Plus</i>
Paracetamol + kofein	400 + 50	Oralni prašak	<i>Kombinovani prašak</i>
	500 + 65	Film tableta	<i>Panadol® Extra Advance</i>
Paracetamol + kofein + kodein	460 + 50 + 10	Tableta	<i>Caffebol</i>
Paracetamol + propifenazon + kofein	250 + 210 + 50	Tableta	<i>CAFFETIN sc®</i>
Paracetamol + kofein + kodein + propifenazon	250 + 50 + 10 + 210	Tableta	<i>CAFFETIN®</i>

I Uloga farmaceuta u lečenju glavobolje tenzionog tipa (GTT)

Prema Međunarodnom udruženju za glavobolju (*International Headache Society*) (8), glavobolje mogu biti *primarne* (glavobolja kao bolest po sebi) ili *sekundarne* (posledica nekog drugog oboljenja). U primarne glavobolje se ubrajaju migrena, GTT i trigemino-autonomne glavobolje (npr. klaster glavobolja) (8). Najčešći tip glavobolje (30-78% opšte populacije) je GTT. Prema učestalosti, GTT može biti *epizodična* (retka, < 1 dan/mesečno, odnosno < 12 dana sa glavoboljom godišnje; i česta, 1-14 dana/mesečno, odnosno > 12, a < 180 dana sa glavoboljom godišnje) i *hronična* (≥ 15 dana/mesečno, odnosno ≥ 180 dana sa glavoboljom godišnje) (8,9). GTT se opisuje kao bilateralni, nepulsirajući bol koji ima karakter stezanja/pritiskanja, blagog do umerenog intenziteta. Za razliku od migrene, GTT se ne pogoršava rutinskim fizičkim aktivnostima i uglavnom nije praćena mučninom i povraćanjem (8,9). Patofiziologija ove vrste glavobolje nije u potpunosti razjašnjena. Smatra se da bol potiče iz mišića glave i vrata, usled čije preopterećenosti nastaju „okidačke tačke“ (preosetljiva mesta) koja pod određenim okolnostima oslobađaju prostaglandine i druge medijatore koji dovode do senzitizacije perifernih i centralnih (nakon dugotrajne stimulacije) neurona bolnog puta (10). Senzitizacija neurona bolnog puta je u pozitivnoj korelaciji sa intenzitetom i trajanjem bola, zbog čega je *vrlo važno analgetik uzeti što pre po otpočinjanju bola i lečiti glavobolju u svakoj epizodi, čime se smanjuje mogućnost da glavobolja dobije hroničan tok* (9,10). Veliki problem u lečenju glavobolja je samomedikacija kojoj pacijenti pribegavaju, koristeći OTC analgetike (Tabela I). Samomedikacija nosi rizik od razvoja glavobolje usled prekomerne primene analgetika (tj. **glavobolje prekomerne upotrebe medikamenata, GPUM**) sa jedne strane i neželjenih efekata i zloupotrebe analgetika sa druge. Značaj farmaceuta u uspešnoj kontroli GTT i sprečavanju GPUM potvrđena je u opservacionim studijama (2,11). Ipak, postoji mišljenje da su farmaceuti iz javnih apoteka još uvek nedovoljno iskorisćen potencijal u lečenju glavobolja na nivou primarne zdravstvene zaštite (12). Pored toga, farmaceuti nisu dovoljno upoznati sa GPUM (13), te je potrebno sprovoditi kontinuirane edukacije na ovu temu među strukom, ali i šire među opštom populacijom.

Lečenje GTT

Cilj lečenja napada GTT je otklanjanje glavobolje unutar 2 h od primene leka. GTT se može efikasno lečiti kombinacijom farmakoloških i nefarmakoloških mera (9).

Farmakološke mere. Generalno, lekovi prvog izbora u lečenju napada GTT su **NSAIL-i i paracetamol** (9). Lekovi koji su dostupni u obliku OTC preparata u Srbiji, a čija je efikasnost u lečenju napada GTT potvrđena u kliničkim studijama su ibuprofen, aspirin, naproxen i paracetamol. Kofein povećava efikasnost ne-opioidnih analgetika, međutim kombinacije sa kofeinom svrstavaju se u drugu liniju lečenja (u slučaju

neefikasnosti monokomponentnih analgetičkih preparata), jer imaju veći potencijal izazivanja GPUM (9).

Većina kliničkih studija koja je upoređivala efikasnost NSAIL-a i paracetamola u ublažavanju bola kod GTT ustanovila je veću efikasnost NSAIL-a. Pored toga, pokazano je da među pojedinim predstavnicima NSAIL-a nema značajne razlike u efikasnosti u lečenju napada GTT (9,14). Međutim, treba imati u vidu da postoji velika interindividualna varijabilnost među pacijentima u odgovoru na analgetike. *Dakle, svaki pacijent bi trebalo da „pronađe“ svoj analgetik.* Za brži nastup dejstva treba preporučiti formulacije koje se rastvaraju/disperguju u vodi neposredno pre primene (npr. praškovi, granule za oralni rastvor, šumeće tablete/granule) ili koje brzo oslobođaju lekovitu supstancu u gastrointestinalnom (GI) traktu (npr. meke kapsule) (Tabela I).

Kratkotrajna/povremena primena NSAIL-a je generalno bezbedna. Pregledom 41 randomizovane kliničke studije koje su ispitivale efikasnost i podnošljivost određenih NSAIL-a i paracetamola u tretmanu akutne epizodične GTT, uglavnom nije detektovana razlika u incidenciji neželjenih efekata između NSAIL-a i paracetamola (14). Ipak, dobro je poznato da NSAIL-i relativno često izazivaju GI neželjena dejstva (nakon akutne primene ona uključuju abdominalni bol, dispepsiju, mučninu i dijareju), dok paracetamol nema GI neželjenih efekata. Mogućnost ispoljavanja GI neželjenih dejstava NSAIL-a može se umanjiti primenom leka nakon obroka ili zajedno sa hranom (imati u vidu da u tom slučaju efekat leka može biti odložen).

Prilikom duže primene većih doza NSAIL-a, povećan je rizik od GI krvarenja i kardiovaskularnih neželjenih efekata. Ovakav režim primene NSAIL-a mogao bi da se javi kod hronične i česte epizodične GTT, kao i kod GPUM. Međutim, kod ovako učestalih glavobolja, potrebno je uputiti pacijenta lekaru koji će razmotriti uvođenje leka za profilaksu GTT (terapija prvog izbora je amitriptilin, a drugog mirtazapin i venlafaksin i to pre svega za pacijente koji ne tolerišu amitriptilin), odnosno sprovesti mere okončanja upotrebe analgetika u pacijenata sa GPUM, čime će se sprečiti česta/dugotrajna primena NSAIL-a i neželjena dejstva koja prate takvu primenu (9).

Prilikom izbora OTC leka za lečenje napada GTT svakako je potrebno voditi računa o kontraindikacijama koje, za NSAIL-e, uključuju preosetljivost na lek, aktivni ili rekurentni peptički ulkus/GIT krvarenje, tešku insuficijenciju srca, bubrega ili jetre i poslednji trimestar trudnoće.

Pacijente koji imaju GTT potrebno je upozoriti da većina preparata koji se koriste za ublažavanje simptoma prehlade i gripe sadrže paracetamol, i da prilikom paralelne primene monokomponentnog preparata paracetamola za ublažavanje GTT, postoji rizik od prekoračenja maksimalne dnevne doze (koja iznosi 4 g za zdrave odrasle osobe) i ozbiljnog oštećenja jetre.

Farmaceut bi trebalo da vodi računa i o interakcijama OTC analgetika sa lekovima koje pacijenti hronično primenjuju. Generalno, savetuje se izbegavanje primene NSAIL-a uz druge NSAIL-e (koji se koriste dugotrajno, za lečenje reumatskih bolesti), antiagregacione lekove (aspirin i dr.), antikoagulanse, kortikosteroide, kao i kod osoba koje redovno konzumiraju alkohol, zbog povećanja rizika od GI oštećenja. Međutim, ukoliko se primena NSAIL-a za lečenje akutnog bolnog stanja ne može izbeći, rizik se može redukovati primenom gastroprotectora (npr. inhibitora protonske pumpe) (15).

Na osnovu svega navedenog, *izbor konkretnog NSAIL-a za ublažavanje akutne GTT prevashodno zavisi od prethodnog iskustva pacijenta sa lekom koji je ispoljio zadovoljavajuću efikasnost i podnošljivost.*

Nefarmakološke mere. Važan su aspekt terapije GTT i treba ih primeniti uvek kada je moguće. Kao primarna mera navodi se prepoznavanje i izbegavanje okidača glavobolje. Najčešće prijavljivani okidači GTT su stres (mentalni ili fizički), neodgovarajući ili neredovni obroci, visok unos ili nagla obustava unosa kofeina, dehidratacija, poremećaji spavanja, nedovoljno ili previše sna, dugotrajni nefiziološki položaj tela (npr. prilikom sedenja za stolom ili računarom) i hormonske varijacije. Pacijenta treba savetovati da vodi dnevnik glavobolje na bazi koga se mogu identifikovati okidači GTT, proceniti učestalost glavobolja i uspešnost i podnošljivost primenjene terapije (9) (Tabela II).

Tabela II Primer dnevnika glavobolje (16)

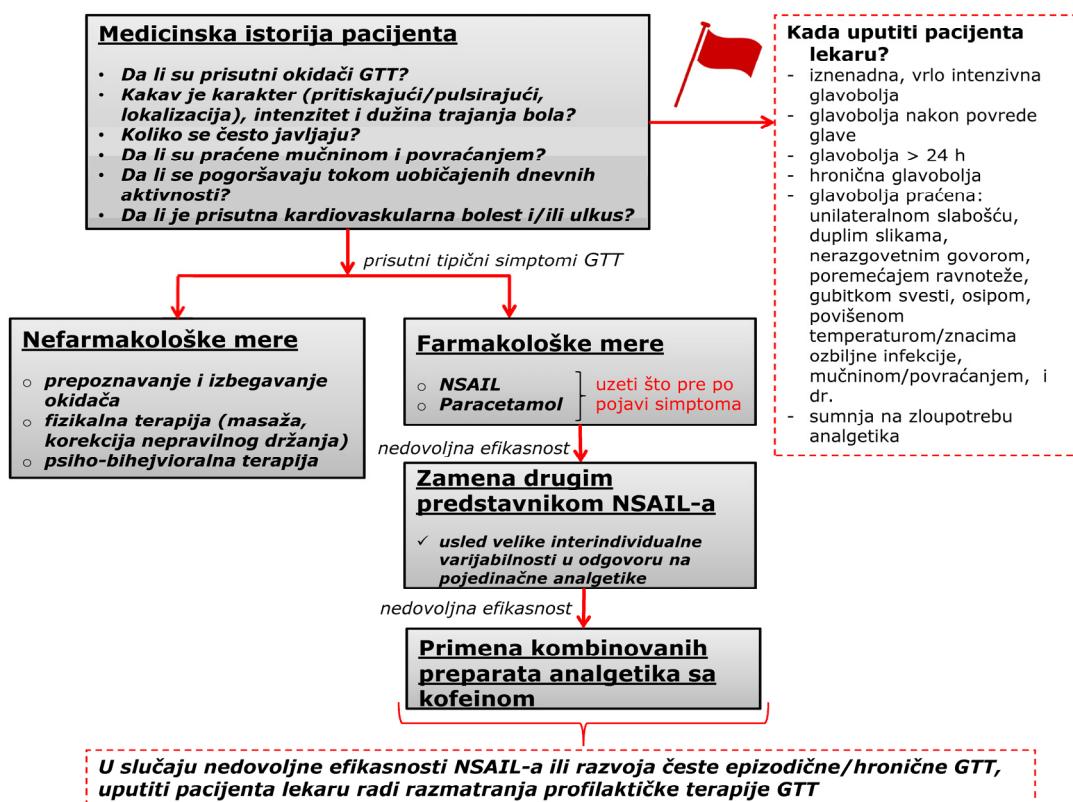
Table II Headache diary example (16)

Ime i prezime: _____									
Datum/ dan u nedelji	Opis bola (u kojoj polovini glave; pulsiranje, probadanje ili stezanje)	Intenzitet glavobolje (blaga/ umerena/ jaka)	Drugi prateći simptomi (mučnina/ povraćanje/ poremećaji vida)	Koji ste lek popili? (naziv leka, jačina)	Vreme kada je glavobolja počela	Vreme kada ste uzeli lek	Da li je lek u potpunosti otklonio bol nakon 2h?	Okidači	Dan menstrualnog ciklusa

Druge nefarmakološke mere koje mogu biti od koristi su fizikalna terapija (masaža, korigovanje lošeg držanja), psihobihevioralna terapija (relaksacija, korigovanje reakcije na stres i bol) i akupunktura (9,10).

Minimiziranje rizika od razvoja glavobolje usled prekomerne upotrebe analgetika (GPUM). GPUM je glavobolja koja se javlja 15 ili više dana/mesečno u pacijenata sa prethodno postojećim epizodičnim glavoboljama (GTT, migrenom ili kombinacijom obe), duže od 3 meseca. Da bi se spričao razvoj GPUM, monokomponentne NSAIL i paracetamol **ne** bi trebalo koristiti **više od 14 dana mesečno**, a kombinovane analgetike sa kofeinom (kao i one koji sadrže opioide) **ne više od 9 dana mesečno**. Farmaceuti imaju važnu ulogu u prevenciji i blagovremenom prepoznavanju GPUM, jer su glavni kandidati da je razviju upravo pacijenti koji samomedikacijom leče glavobolje. Najvažnija preventivna mera je racionalna primena analgetika, stoga je pacijentu važno *uvek naglasiti koliko maksimalno tableta (i ostalih formulacija) analgetika za lečenje akutne glavobolje sme da popije na dan/mesečno* (10,17).

Sumirane preporuke za farmaceute iz javnih apoteka za lečenje GTT prikazane su na Slici 1. (5,9,10,18).



Slika 1. Intervencije farmaceuta u javnoj apoteci u lečenju glavobolje tenzionog tipa (5,9,10,18).

Figure 1. Community pharmacist-led interventions in tension-type headache treatment (5,9,10,18).

II Uloga farmaceuta u lečenju dismenoreje

Dismenoreja, bolna menstruacija, je jedan od najčešćih ginekoloških problema (19,20). Može biti *primarna* (u odsustvu pelvične patologije) i *sekundarna* (prisutna pelvična patologija). Primarna dismenoreja nastaje kao posledica prekomerne produkcije prostaglandina (PG) iz endometrijuma tokom lutealne faze menstrualnog ciklusa, usled čega dolazi do povećane kontraktilnosti uterusa (i posledičnog smanjenja lokalnog protoka krvi) i hipersenzitizacije perifernih nervnih završetaka (21,22). Zbog toga su lekovi koji inhibiraju stvaranje PG (NSAIL) i oni koji inhibiraju ovulaciju (oralni kontraceptivi) efikasni u prevenciji/lečenju primarne dismenoreje. Tipično se primarna dismenoreja manifestuje kao grčevit suprapubični bol, koji se javlja neposredno pre ili na početku menstrualnog krvarenja i obično postepeno slabi u intenzitetu u narednih 12-72 h. Može biti udružen sa mučninom, povraćanjem, dijarejom, vrtoglavicom i glavoboljom. Faktori rizika za razvoj primarne dismenoreje su: godine starosti (najčešće se javlja u periodu adolescencije i kod mlađih žena), nultiparitet, rana menarha, izraženo/produženo menstrualno krvarenje i porodična istorija dismenoreje. Dobro je dokumentovano da pušenje pogoršava menstrualni bol, a jedna prospективna studija pokazala je da čak i izloženost duvanskom dimu povećava incidencu dismenoreje (20,23). Simptomi dismenoreje obično opadaju sa godinama, otpočinjanjem seksualne aktivnosti i nakon porođaja (10,20,24).

Lečenje dismenoreje

Primarna dismenoreja se efikasno može kupirati odgovarajućim farmakološkim i nefarmakološkim merama.

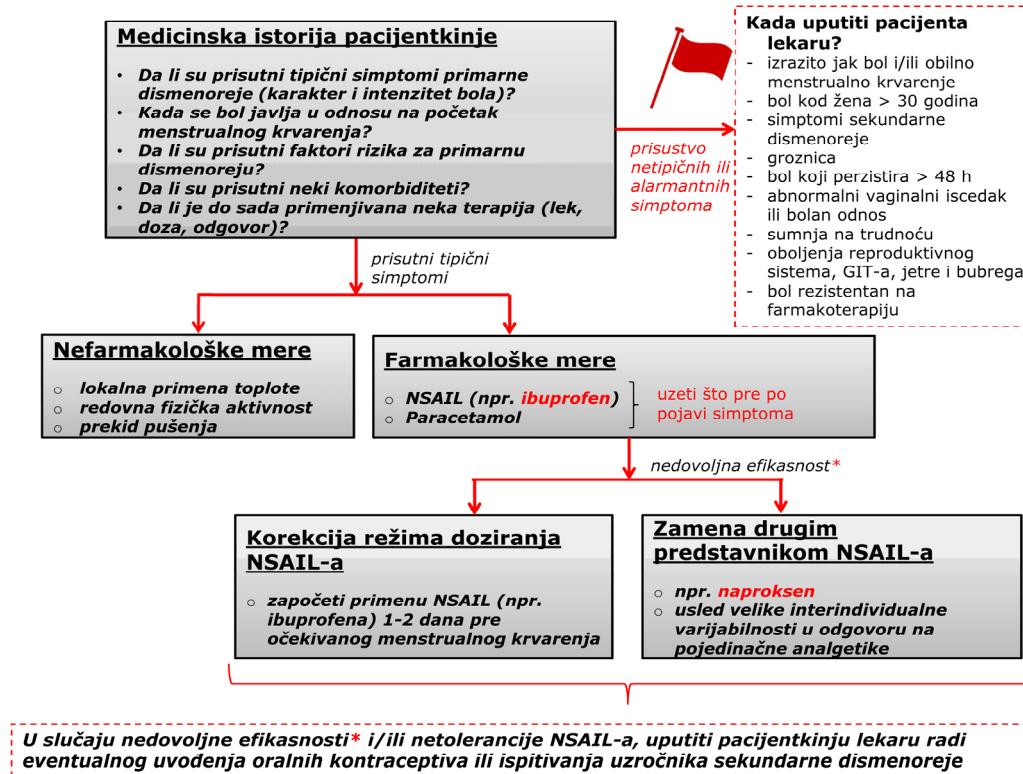
Farmakološke mere. U nedavnoj Cochrane meta-analizi preko 80 kliničkih studija pokazana je slična efikasnost i podnošljivost ispitivanih NSAIL-a (20 različitih predstavnika) u lečenju bolnih menstruacija, kao i njihova veća efikasnost u odnosu na paracetamol (19). Raniji pregled, koji je obuhvatio 56 studija, ustanovio je da su ibuprofen, naproksen i aspirin efikasni u primarnoj dismenoreji, da je paracetamol manje efikasan, a da ibuprofen ima najpovoljniji odnos korist/rizik, na bazi čega je proistekla preporuka da se **ibuprofen** razmatra kao lek prvog izbora (25). Niža efikasnost paracetamola u lečenju menstrualnih bolova u poređenju sa NSAIL-ima je očekivana, jer ovaj analgetik deluje na centralnom nivou, dok NSAIL-i inhibiraju sintezu PG i u centralnom nervnom sistemu i na periferiji. Ipak, **paracetamol** je indikovan kod menstrualnih bolova i može da se preporuči kao terapijska opcija posebno u svetu njegovog povoljnog bezbednosnog profila (20). Smatra se da primena spazmolitika (hioscin butilbromid, drotaverin) u monoterapiji ne obezbeđuje efikasno smanjenje bola i ne treba ih preporučivati (10). *Dakle, farmaceut bi trebalo da preporuči NSAIL (pre svega ibuprofen) kao terapiju prvog izbora za bolne menstruacije.* U slučaju neefikasnosti i/ili netolerancije NSAIL-a, kod pacijentkinje

može da se razmotri uvođenje **oralnih kontraceptiva** ukoliko postoji potreba za kontracepcijom, po preporuci lekara. Ukoliko izostane efikasnost i kontraceptiva, moguće je da postoji neki sekundarni uzročnik dismenoreje, kao što je endometrioza (20).

Nefarmakološke mere. Najviši nivo preporuke postoji za primenu toplove, tj. zagrevanje bolnog mesta (20). U jednoj randomizovanoj kontrolisanoj studiji zabeležena je poredbena efikasnost zagrevanja bolnog mesta flasterom koji produženo oslobađa toplotu sa oralnim ibuprofenom (26). Takođe, pacijentkinjama se može preporučiti umerena fizička aktivnost, bihevioralne tehnike opuštanja, visokofrekventna transkutana električna stimulacija nerava (TENS), akupunktura i akupresura (20). Ispitivan je i uticaj mnogih dijetetskih suplemenata na primarnu dismenoreju. Izostala je efikasnost vitamina E, a zabeležena izvesna, skromna efikasnost ribljeg ulja, vitamina B₁, valerijane i cink-sulfata (27), međutim kvalitet sprovedenih kliničkih studija nije bio zadovoljavajući, a rezultati su nedovoljno ubedljivi da bi se navedeni suplementi mogli preporučiti. Na bazi rezultata nekoliko malih randomizovanih studija koje su pokazale efekat đumbira veći od placebo i komparabilan sa ibuprofrenom i mfenaminskom kiselinom (28-30), smernice kanadskog udruženja opstetričara i ginekologa za lečenje primarne dismenoreje (20), navode đumbir (750 – 2000 mg tokom 3-4 dana ciklusa) kao opciju za ublažavanje dismenoreje za pacijentkinje koje žele da koriste komplementarnu/alternativnu terapiju (20).

Nefarmakološke mere (posebno primena toplove i fizička aktivnost) deluju komplementarno sa farmakoterapijom i mogu se savetovati pacijentkinjama.

Sumirane preporuke za farmaceute iz javnih apoteka za lečenje primarne dismenoreje prikazane su na Slici 2 (10,20,31).



*nakon najmanje 3 meseca primene (menstrualna ciklusa)

Slika 2. Intervencije farmaceuta u javnoj apoteci u lečenju primarne dismenoreje (10,20,31).

Figure 2. Community pharmacist-led interventions in primary dysmenorrhea treatment (10,20,31).

Zaključak

Za lečenje GTT i dismenoreje većina pacijenata primenjuje OTC analgetike, često bez konsultacije sa zdravstvenim radnicima, zbog čega su izloženi povećanom riziku od neželjenih ishoda (npr. neefikasnosti terapije, neželjena dejstva/interakcije, predoziranje), a pacijenti sa glavoboljom i od razvoja GPUM. Kako su farmaciuti prva linija zdravstvenih radnika sa kojima se ovi pacijenti susreću, oni imaju ključnu ulogu u pravilnom izboru i racionalnoj primeni analgetika, kao i prepoznavanju bola koji može biti simptom nekog životno ugrožavajućeg zdravstvenog problema kada pacijenta treba uputiti lekaru. Farmaceut može na osnovu verovatne dijagnoze, da izvrši izbor odgovarajućeg OTC analgetika, odgovarajuće doze/farmaceutskog oblika/puta primene, da preporuči odgovarajuće nefarmakološke mere, izvrši savetovanje/edukaciju pacijenta i isprati efikasnost/podnošljivost terapije. Zato je važno partnerstvo pacijent-farmaceut-lekar. Farmaceut ima znanja i kompetencije neophodne za uspešnu i bezbednu terapiju bola.

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The role of pharmacists in the management of certain common acute pain states

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Summary

Pain management is a challenge and imperative for all health professionals, including pharmacists. The most of visits to community pharmacies involve acute pain issues, such as tension-type headache (TTH) and dysmenorrhea. Pharmacists are the first-line health care providers faced by those pain sufferers therefore they are in a unique position to recognize pain as a symptom of possibly life threatening underlying health condition, when the patient should be referred to a physician. Based on the probable diagnosis, the pharmacist is able to select the appropriate OTC analgesic, its dosage/pharmaceutical form/route of administration, recommend non-pharmacological measures, advise and educate the patient regarding its pain condition, evaluate treatment efficacy/tolerability and prevent the adverse treatment outcomes (e.g. medication overuse headache development). Pharmacist should recommend OTC nonsteroidal anti-inflammatory drugs (NSAIDs) or paracetamol as drugs of first choice for the treatment of TTH, and NSAIDs, preferably ibuprofen, for the management of dysmenorrhea. To select the appropriate analgesic, the pharmacist should consider the prior patient experience with analgesics. To prevent medication overuse headache development, simple analgesics should not be used more than 14 days/month and combination analgesics with caffeine/codeine no more than 9 days/month, in patients suffering from TTH. Patient-pharmacist-physician partnership is necessary for effective and safe acute pain treatment.

Key words: pharmacist's role, OTC analgesics, tension-type headache (TTH), dysmenorrhea, nonsteroidal anti-inflammatory drugs (NSAIDs)

In silico metodologija u toksikologiji: softveri za predviđanje toksičnosti

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Kratak sadržaj

Već više od sto godina jedan od glavnih ciljeva toksikologa je povezivanje hemijske strukture supstanci sa njihovom toksičnošću. Progres u razvoju kompjuterskih tehnologija omogućio je kreiranje softverskih modela za ovu namenu. Međutim, primena *in silico* metodologije u toksikologiji je veoma složena. Kako se toksični efekat može ostvariti preko više mehanizama na različitim tkivima i organima, veoma je kompleksno kreirati softvere kojima se može dovoljno pouzdano predvideti toksičnost ispitivane supstance. Upotreba *in silico* metoda našla je primenu u proceni toksičnosti onih supstanci za koje ne postoji dovoljno podataka dobijenih tradicionalnim testovima toksičnosti (metaboliti, nečistoće u lekovima, itd.). Takođe, *in silico* metode se mogu koristiti u procesu sinteze hemikalija, sa ciljem dobijanja bezbednijih hemikalija, dajući uvid u strukturne karakteristike odgovorne za toksičnost. *In silico* metode za predviđanje toksičnosti trenutno se najčešće koriste uz standardna toksikološka ispitivanja, dok je tendencija da u budućnosti zamene deo *in vitro* i *in vivo* testova toksičnosti. U ovom radu biće prikazane mogućnosti i ograničenja najčešće korišćenih softvera u *in silico* toksikologiji (OECD QSAR Toolbox, Derek Nexus, ToxTree i MultiCASE).

Ključne reči: toksičnost, *in silico* metode, softveri

Uvod

Trenutno se na tržištu nalazi više od 30 miliona hemikalija, koje je čovek sintetisao ili izolovao iz prirodnih izvora. Iako mnoge od njih doprinose poboljšanju savremenog načina života, sve hemikalije, pod određenim uslovima izloženosti, u određenoj dozi mogu izazvati pojavu toksičnog efekta, te je njihova bezbedna upotreba od suštinskog značaja (1). Definisanje hemijskih svojstava i mogućih interakcija hemikalija sa biološkim sistemima predstavlja jedan od ključnih koraka u proceni njihove bezbednosti, koja se tradicionalno vrši na osnovu standardnih toksikoloških testova na eksperimentalnim životinjama (*in vivo*). Ova ispitivanja obuhvataju procenu akutne toksičnosti, iritacije kože/oka, senzibilizacije, toksičnosti pri ponovljenom doziranju, imunotoksičnosti, neurotoksičnosti, mutagenosti, karcinogenosti, reproduktivne toksičnosti, itd. (2,3). Međutim, većina ovih testova dugo traje, zahtevaju razmatranje etičnosti testiranja na eksperimentalnim životinjama i izdvajanje velikih finansijskih sredstava. Shodno zahtevima da se smanji broj eksperimentalnih životinja, a saglasno dokumentima o dobrobiti životinja, teži se ka kreiranju alternativnih *in vitro* i *in silico* metoda (2,3,4). Već više od jednog veka jedan od glavnih ciljeva toksikologa je povezivanje hemijske strukture supstanci sa njihovom toksičnošću. Progres u razvoju kompjuterskih tehnologija omogućio je kreiranje softverskih modela za ovu namenu (5). Modeli koji povezuju hemijsku strukturu supstanci sa njihovom biološkom aktivnošću nazvani su „Kvantitativni ili kvalitativni odnos između strukture i aktivnosti“ (engl. *Quantitative or Qualitative Structure–Activity Relationships - (Q)SARs*) (3,6). Primena *in silico* metodologije u toksikologiji je veoma složena i zahteva poznavanje većeg broja parametara koji se mogu koristiti kao molekularni deskriptori. Kako se toksični efekat ostvaruje preko više mehanizama na različitim tkivima i organima, veoma je komplikovano kreirati softvere kojima se može predvideti toksičnost ispitivane supstance (3,7). I pored intenzivnog rada na ovom polju još uvek ne postoji idealan softver koji bi mogao da se primeni na sve vrste supstanci, te je potrebno pažljivo odabратi jedan ili više softverskih paketa s ciljem dobijanja što preciznijeg predviđanja. Softveri ne samo da predviđaju vrednosti toksikoloških parametara, već sadrže i bazu poznatih podataka o ispitivanoj supstanci.

Uzimajući u obzir sve gore navedeno, cilj ovog rada je da dâ uvid u *in silico* metode koje se mogu koristiti u toksikologiji i da prikaže mogućnosti i ograničenja najčešće korišćenih softvera za procenu toksičnosti.

Primena *in silico* metoda u toksikologiji

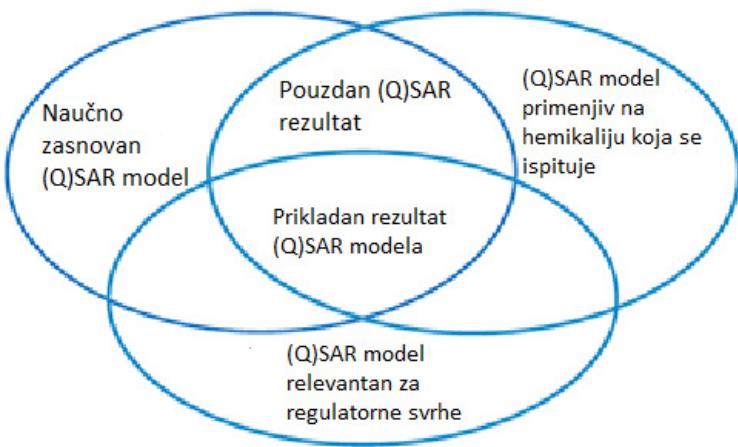
In silico metode se mogu primeniti s ciljem relativno brzog dobijanja podataka o toksičnosti supstanci koje nije moguće u potpunosti ispitati standardnim toksikološkim testovima (4,8) ili pak za supstance za koje postoje ispitivanja standardnim metodama kako bi se testirala validnost modela. Jedan od primera za primenu *in silico* metoda u

toksikologiji je biotransformacija hemikalija, s obzirom na to da nastali metaboliti mogu biti toksičniji od polazne hemikalije ili ih je teško identifikovati, izolovati i sintetisati u svrhe testiranja (4). Zatim, u toku procesa sinteze lekovite supstance može doći do formiranja nečistoća (intermedijeri i sporedni produkti) čiji toksikološki profil je potrebno ispitati, kao i proceniti rizik po zdravlje ljudi (9). Osim u toku sinteze, ove nečistoće mogu nastati i za vreme proizvodnje i skladištenja leka, kao i interakcijama - između aktivnih supstanci, leka sa ekscipijensima, ili leka sa ambalažom (10). Prvi korak u proceni rizika od ovih supstanci predstavlja ispitivanje njihove mutagenosti, pri čemu je jedna od preporuka upotreba najmanje dve komplementarne *in silico* metode (11). Još jedna značajna uloga *in silico* metoda je da pruži uvid u strukturne karakteristike supstance, koje su odgovorne za toksičnost, i na taj način omogući dizajn i sintezu bezbednijih hemikalija. Takođe, *in silico* metode se mogu koristiti za rangiranje nedovoljno ispitanih supstanci prema prioritetu za dalja toksikološka ispitivanja, radi dobijanja svih neophodnih podataka o toksičnosti supstance (4).

Kriterijumi za odabir adekvatnog (Q)SAR modela

Prilikom *in silico* procene toksičnosti određene supstance potrebno je poznavati njenu hemijsku strukturu i odabrati odgovarajući (Q)SAR model za dobijanje relevantnih podataka (Slika 1). Uslovi koji moraju biti ispunjeni da bi rezultati dobijeni pomoću ovih modela bili primenjivi navedeni su u Aneksu XI REACH (engl. *Registration, Evaluation, Authorisation and Restriction of Chemicals*) regulative Evropske ujde, koja govori o registraciji, evaluaciji, autorizaciji i ograničenjima hemikalija:

- rezultati moraju biti izvedeni iz naučno zasnovanih (Q)SAR modela,
- supstanca se mora nalaziti u domenu primenljivosti (Q)SAR modela,
- rezultati moraju biti adekvatni u svrhu klasifikacije i obeležavanja hemikalija i/ili procene rizika,
- mora biti obezbeđena adekvatna i pouzdana dokumentacija primenjene metode (12).



Slika 1. Šema načina utvrđivanja prikladnog (Q)SAR modela (12)

Figure 1. Scheme of determining a suitable (Q)SAR model (12)

Naučno zasnovani (Q)SAR modeli koji se mogu koristiti za ispitivanje različitih toksikoloških svojstava navedeni su u praktičnom vodiču „Kako koristiti i prijaviti (Q)SAR modele“ koji je izdala Evropska agencija za hemikalije (engl. *European Chemicals Agency: ECHA*) 2016. godine (12). Odgovor na pitanje da li određena supstanca može biti testirana izabranim modelom najčešće se može dobiti uvidom u njegovu specifikaciju, prema navodima proizvođača, za oblast određenog toksičnog efekta.

Pouzdanost podataka

Za procenu pouzdanosti rezultata testova toksičnosti može poslužiti *Klimish* skala, prethodno korišćena za podatke dobijene *in vivo* i *in vitro* studijama, a kasnije prilagođena tako da može da bude primenjena i na one dobijene *in silico* metodama. Prvobitna Klimish skala svrstavala je dobijene podatke u jednu od četiri kategorije: 1- pouzdani, 2- ograničeno pouzdani, 3- nepouzdani, 4- nesvrstani (13). Sa porastom upotrebe *in silico* metoda, broj kategorija inicijalne skale povećao se na pet (RS1-RS5), pri čemu pouzdanost rezultata raste od RS1 ka RS5. Kategorije RS1 i RS2 analogne su kategorijama 1 i 2 prvobitne *Klimish* skale, i u njih se mogu svrstati samo podaci dobijeni *in vitro* i *in vivo* testovima. U kategoriju RS3 svrstavaju se podaci dobijeni *in silico* metodom *Read Across* u kombinaciji sa ekspertskim mišljenjem. Ukoliko se uz pomoć više *in silico* metoda dobiju podaci koji se podudaraju, svrstavaju se u kategoriju RS4, dok se podaci dobijeni korišćenjem samo jedne *in silico* metode svrstavaju u

kategoriju RS5. Upotreba podataka svrstanih u RS1-RS4 preporučena je u proceni rizika po zdravlje ljudi (4,11).

***In silico* softveri**

U daljem tekstu dat je pregled odabralih, najčešće korišćenih, softvera u *in silico* toksikologiji, dok su u Tabeli I prikazani ulazni i izlazni podaci za date softvere.

Tabela I Ulagani i izlazni podaci za najčešće korišćene softvere u oblasti toksikologije

Table I Input and output data for most commonly used software in toxicology

Softver	Ulagani podaci	Izlazni podaci
QSAR Toolbox		Reprodukтивна токсићност, Hepatотоксићност, Nefrotоксићност, Neuroтоксићност, Imunотоксићност, Genотоксићност и mutagenost, Karcinogenost, Kramerova klasa, itd.
ToxTree	Strukturna formula, hemski naziv, EC broj*, CAS broj**, itd.	Iritacija/korozija kože i oka, Senzibilizacija kože, Hronična oralna токсићност, Genотоксићност и mutagenost, Karcinogenost, Kramerova klasa, itd.
Derek Nexus		Reprodukтивна токсићност, Hepatотоксићност, Nefrotоксићност, Neuroтоксићност, Imunотоксићност, Genотоксићност и mutagenost, Karcinogenost, itd.
CASE Ultra (MultiCASE)		Akutna oralna токсићност, Hronična oralna токсићност, Reprodukтивна токсићност, Toksiћност на endokrini sistem, Hepatотоксићност, Citotоксићност, Karcinogenost, itd.

*EC broj – broj hemijske supstance koji dodeljuje Evropska komisija

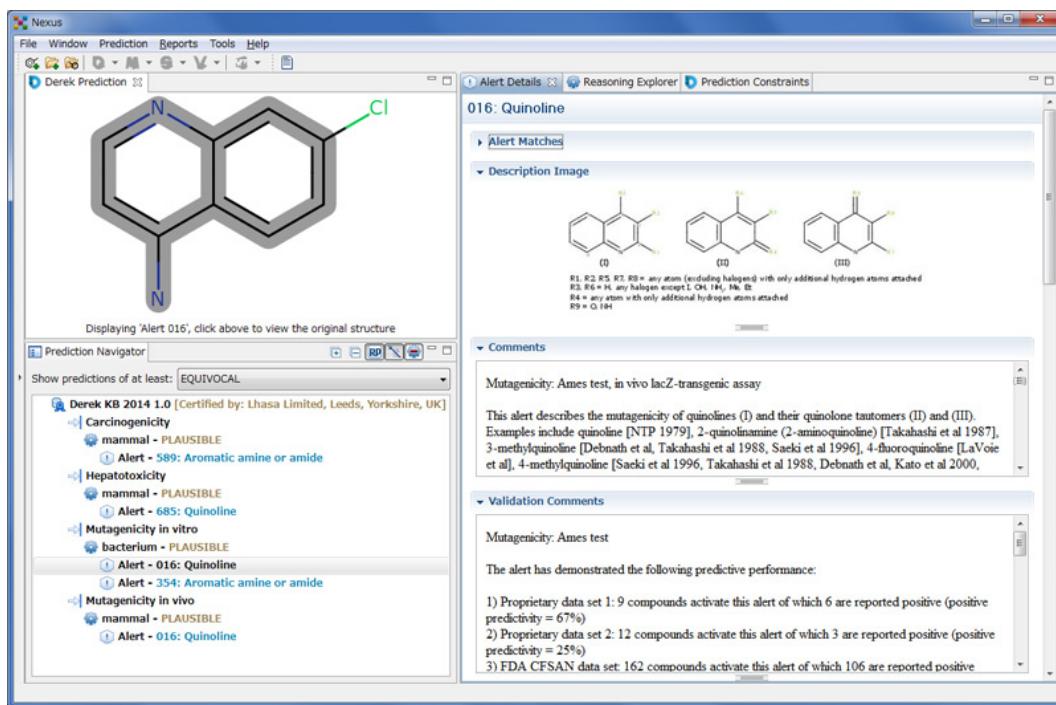
**CAS broj – jedinstveni identifikacioni broj hemijske supstance koji dodeljuje CAS (*Chemical Abstract Service*)

QSAR Toolbox: Softver OECD QSAR Toolbox (<http://www.qsartoolbox.org/>) razvijen je pod pokroviteljstvom Organizacije za ekonomsku kooperaciju i razvoj (engl. *Organization for Economic Co-operation and Development*: OECD), a njegova upotreba je podržana od strane ECHA. Namenjen je za upotrebu od strane regulatornih tela, industrije i univerziteta i predstavlja softver sa jednom od najvećih baza podataka (6,12). Nakon unosa podataka o ispitivanoj supstanci, ovaj softver formira kategorije na osnovu hemijskih karakteristika i/ili mehanizama toksičnosti (6). Kategorije hemikalija predstavljaju grupe supstanci sličnih fizičko-hemijskih i toksikoloških svojstava, za koje se očekuje da se u organizmu slično ponašaju, najčešće kao rezultat strukturne sličnosti. Podaci koji nedostaju unutar kategorija popunjavaju se upotrebom modela : preuzimanje podataka od strukturno sličnih hemikalija (*read across*), analiza trenda (*trend analysis*) i (Q)SAR.

Model *read across* se koristi za dobijanje kvalitativnih i kvantitativnih podataka, kada za ispitivanu supstancu postoji mali broj strukturnih analoga (<10). Kada za ispitivanu supstancu postoji veći broj strukturnih analoga (≥ 10), za dobijanje kvantitativnih podataka koristi se model *trend analysis*. U slučaju kada nema odgovarajućih strukturnih analoga za ispitivanu supstancu, primenjuju se (Q)SAR modeli (6). Program QSAR Toolbox najčešće se koristi za predviđanje iritacije/korozije kože i oka, senzibilizacije kože i mutagenosti (12). Ovaj softver pruža relativno brz način da se dođe do podataka, pri čemu je dostupan veliki broj uputstava za korišćenje (14). Sve više je prihvaćen od strane regulatornih tela i podržan od strane industrije, a dobijeni podaci mogu da se upotrebe kao osnova za dalja istraživanja (*in vitro, in vivo*). Instalacija i korišćenje QSAR Toolbox softvera su besplatni. I pored brojnih prednosti, ovaj softver pokazuje i određene nedostatke. Jedan od njih je to što je proces predviđanja pod uticajem subjektivnog pristupa korisnika programa, te se može desiti da dva korisnika izvedu različite zaključke za istu supstancu. Pouzdano predviđanje uslovljeno je velikim brojem faktora (definisanje kategorije, broj analoga unutar kategorije i dr.), i dobija se samo ukoliko postoje kvalitetni podaci za veći broj analoga.

Derek Nexus: Derek Nexus (engl. *Deductive Estimation of Risk from Existing Knowledge*) je komercijalan softver razvijen od strane Schering Agrochemicals i Lhasa Ltd. kompanije. Ovaj softver koristi poznate podatke o odnosu između strukture i aktivnosti molekula kako bi napravio kvalitativna predviđanja o toksičnosti ispitivane supstance. Veoma je jednostavan za upotrebu i odmah po unosu hemijske strukture ispitivane supstance pruža informacije o njenom nazivu, hemijskoj formuli, nomenklaturi, karakteristikama i vrsti toksičnosti, pri čemu omogućava i pregled literature u kojoj se može proveriti tačnost njegovog predviđanja. Takođe, pruža informacije o tome koje vrste hemijskih modifikacija se mogu izvršiti na ispitivanoj supstanci da bi se umanjio toksični efekat (npr. građenje soli ili estara) (7). Ovaj program najčešće se koristi za predviđanje toksičnosti nakon ponovljenog doziranja

(nefrotoksičnost, hepatotoksičnost), mutagenosti, reproduktivne toksičnosti, irritacije/korozije kože i oka, kao i senzibilizacije kože (7,12). Program je jednostavan za korišćenje, a uz rezultate njegovog predviđanja dobija se i slikovito i detaljno objašnjenje (Slika 2). Preporučuje se za upotrebu različitim korisnicima, od proizvođača hemikalija, pa sve do regulatornih tela. Što se nedostataku tiče, u programu ne postoje linkovi ka javno dostupnim bazama toksikoloških podataka, koji bi omogućili proveru pouzdanosti dobijenih rezultata (7).



Slika 2. Primer predviđanja dobijenog pomoću Derek Nexus softvera
Figure 2. Example of prediction obtained by using Derek Nexus software

ToxTree: Softver ToxTree je razvijen od strane *Ideaconsult Ltd.* (Sofija, Bugarska) pod pokroviteljstvom Evropske komisije *Joint Research Centre*. U pitanju je besplatan Java softver koji se koristi za kategorizaciju hemikalija, predviđanje toksičnih efekata i mehanizma toksičnosti (2,15,16). Softver ToxTree zasnovan je na Kramerovom stablu odlučivanja, šematskog prikaza pomoću koga se izvodi zaključak u smislu klasifikovanja supstanci u jednu od tri klase (I – niska toksičnost, II – srednja toksičnost and III – visoka toksičnost) (15). Prva verzija Kramerovog stabla odlučivanja sastojala se od 33 pitanja na koja se može odgovoriti sa „da“ ili „ne“. Odgovorom na svako pitanje prelazi se na sledeće sve dok se ne uspostavi finalna Kramerova

klasifikacija za ispitivanu hemikaliju (17). Svaka Kramerova klasa povezana je sa specifičnim nivoom izloženosti ljudi ispod kog se smatra da je rizik od date hemikalije zanemarljiv za ljudsko zdravlje (17,18). Supstance iz klase I jednostavne su strukture, efikasno se metabolišu i imaju nizak toksični potencijal. Za supstance klase II ne postoje dokazi o toksičnosti na osnovu hemijske strukture, dok supstance klase III sadrže strukturne elemente koji ukazuju na značajnu toksičnost (15). Softver ToxTree najčešće se koristi za predviđanje iritacije/korozije kože i oka, senzibilizacije kože i mutagenosti (12). ToxTree predstavlja jednostavnu aplikaciju sa ograničenim mogućnostima i modeli na kojima se zasniva su često inkorporirani u većim, komercijalno dostupnim softverima.

CASE Ultra (MultiCASE): Komercijalni program CASE (engl. *Computer Automated Structure Evaluation*) razvijen je od strane firme *MultiCASE Inc.* Specifičnost ovog programa ogleda se u tome da se predviđanje vrši klasifikacijom ispitivane supstance pomoću CASE jedinica, u rasponu od 10 do 99. Supstance sa vrednošću od 10 do 19 su biološki inaktivne, supstance sa vrednošću od 20 do 29 su na granici između aktivnih i inaktivnih, dok su supstance kojima je dodeljena vrednost od 30 do 99 biološki aktivne (19). Nakon sagledavanja ispitivane supstance u celosti, ovaj softver je podeli na fragmente. Fragmenti nazvani „biofore” pokazuju aktivnost, za razliku od „biofoba”, koje su inaktivne. Svakom fragmentu dodeljuje se odgovarajuća CASE jedinica, nakon čega se statističkom obradom predviđa toksičnost ispitivane supstance (7). Ovaj program je našao primenu za predviđanje akutne toksičnosti, toksičnosti nakon ponovljenog doziranja, mutagenosti, reproduktivne toksičnosti, iritacije/korozije kože i oka, kao i senzibilizacije kože (12). Koristeći veliki broj deskriptora pri predviđanju, CASE softver je u mogućnosti da odredi predviđanje za ispitivanu supstancu o čijem biološkom i toksikološkom dejstvu korisnik nema predznanja. Međutim, da bi rezultati predviđanja bili pouzdani, neophodni su kvalitetni podaci generisani od strane programa. Dobijeni rezultati često su teški za interpretaciju, pogotovo u slučaju supstanci velike molekulske mase, kod kojih program uočava sitne fragmente koji nisu od velikog značaja (7).

Zaključak

Primena *in silico* metoda u toksikologiji je u stalnom porastu. *In silico* metode doprinose smanjenju upotrebe eksperimentalnih životinja u proceni toksičnosti, ukupnih troškova toksikoloških ispitivanja i omogućavaju dobijanje rezultata u relativno kraćem vremenskom periodu. Mnogi *in silico* softveri za predviđanje toksičnosti, kako komercijalni, tako i besplatni, ažuriraju se u skladu sa potrebama potrošača, veoma su kompleksni i sadrže čitave baze toksikoloških podataka.

Ipak, trebalo bi imati u vidu da ne postoji univerzalan *in silico* softver za predviđanje toksičnosti, već je svaki od njih razvijen za specifičnu namenu. Zato je,

prilikom odabira softvera, veoma važno poznavati njegove prednosti i ograničenja, domen primenjivosti i pravilnu interpretaciju dobijenih rezultata.

Podaci dobijeni *in silico* metodama za predviđanje toksičnosti trenutno se u toksikološkim ispitivanjima najčešće koriste kao potpora drugim relevantnim naučnim dokazima. Ove metode su veoma korisne za rangiranje nedovoljno ispitanih supstanci prema prioritetu za dodatna testiranja, dok je tendencija da u budućnosti zamene deo *in vitro* i *n vivo* testova toksičnosti. Očekuje se da će se sve veći značaj pridavati njihovoj upotrebi u toksikološkoj evaluaciji nečistoća u lekovima, industrijskih hemikalija, pesticida, materijala koji dolaze u kontakt sa hranom, kao i aditiva.

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***In silico* methodology in toxicology – software for toxicity predictions**

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Summary

For over a century, one of the main toxicologists' goals was finding the relationship between the chemical structure and toxicity. The development of computer technologies has lead to the production of software models that could be applied for this purpose. However, use of *in silico* methodology in toxicology is very complex. Having in mind that toxic effect occurs through multiple mechanisms on different tissues and organs, creating software that can make reliable toxicity predictions is very complicated. *In silico* methods found the application in toxicity assessment of substances for which data obtained from traditional toxicity tests is lacking (metabolites, drug impurities, etc.). Also, *in silico* methods can be used in the process of chemical synthesis, with the aim of designing safer chemicals. Currently, *in silico* methods for toxicity predictions are most commonly used along with standard toxicological tests, with a tendency to replace *in vitro* and *in vivo* toxicity tests in the future. This paper will present the strengths and limitations of commonly used software in *in silico* toxicology (OECD QSAR Toolbox, Derek Nexus, ToxTree i MultiCASE).

Key words: toxicity, *in silico* methods, software

Providing clinical evidence under the MDR 2017/745 – new challenges for manufacturers in medical device industry

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Summary

Providing necessary clinical evidence throughout the systems for Clinical Evaluation and Post-Market Surveillance in accordance with new MDR 2017/745 requirements imposes new challenges for manufacturers in medical device industry.

Manufacturers should make clinical evaluation a continuous process that summarizes findings and results from several mutually connected processes: PMS, vigilance, CIs. Gathered clinical evidence should also be used as input for SSCP, PSUR, CEAR.

CIs should be designed, authorised, conducted, recorded and reported in accordance with provisions of Articles 62-81 and Annex XV.

PMS and PMCF activities should provide appropriate evidence of planning, implementation of protocol and reporting; target residual risks and confirm safety and performance over device's lifetime; include activities that address specific questions/residual risks.

All activities are aimed towards several goals: improving quality, safety and reliability of devices and strengthening transparency of information for consumers.

Key words: Medical Device Regulation 2017/745 (MDR), Clinical Evaluation, Post-Market Surveillance (PMS), Clinical Investigation (CI), Post-Market Clinical Follow-up (PMCF).

Abbreviations: MDR: Medical Device Regulation, PMS: Post-Market Surveillance, PMCF: Post-Market Clinical Follow-up, CI: Clinical Investigation, MDD/AIMDD: Medical Device Directive/ Active Implantable Medical Device Directive, CER: Clinical Evaluation Report, SSCP: Summary of Safety and Clinical Performance, PSUR: Periodic Safety Update Report, CEAR: Clinical Evaluation Assessment Report.

Introduction

One of the key objectives of the new MDR is to ensure consistently high level of health and safety protection for EU citizens using these products. Making clinical investigation and evaluation requirements more stringent is aimed at improving health and safety through transparency and traceability (1). As expected, the roles of clinical evaluation and clinical investigation become far more prominent under the MDR. Inclusion of MEDDEV 2.7/1 and parts of ISO 14155 into the MDR is to be applauded. Data concerning clinical investigations must be introduced in EUDAMED, as well. The electronic system must also be used for PMCF studies. The design, implementation, and requirements for documentation of a PMCF study have to meet many requirements applicable to clinical investigations (2). All these current requirements represent new challenges for the manufacturers and create future aims for the medical device industry: improving the quality, safety and reliability of medical devices and strengthening the transparency of information for consumers (3).

Clinical evaluation

In the new MDR 2017/745 great emphasis is placed on clinical evaluation and clinical evaluation related processes as key elements in demonstrating conformity with the relevant safety and performance requirements. Fortunately the manufacturers are not entirely unfamiliar with these processes. Apart from the general principles described in MDD/AIMDD, guidance for performing clinical evaluation could be found in documents such as MEDDEV 2.7.1., ISO 14155:2011 or Declaration of Helsinki. In fact, the main provisions of all these documents are incorporated in MDR (4).

According to MDR, demonstrating conformity with the relevant general safety and performance requirements should be based on clinical evidence provided in accordance with provisions prescribed mainly in Article 61 and Annex XIV.

A clinical evaluation should follow a defined and methodologically sound procedure based on the following points:

- (a) a critical evaluation of the relevant, currently available **scientific literature** relating to the safety, performance, design characteristics and intended purpose of the device,

- (b) a critical evaluation of the results of all available **clinical investigations**, and
- (c) a consideration of currently available **alternative treatment options** for that purpose, if any.

The clinical evaluation and its documentation should be updated throughout the life cycle of the device concerned with clinical data obtained from the implementation of the manufacturer's PMCF plan in accordance with Part B of Annex XIV and the PMS plan referred to in Article 84. All conclusions should be summarized in the CER (5).

Challenges for manufacturers

New requirements for the clinical evalaution become mandatory with their inclusion in the Regulation and therefore it is of great importance for the manufacturers to implement and upgrade it in such a way that it's inputs and outputs can be used for as many other related activities.

Manufacturers should make clinical evaluation a continuous process which should summarize the findings and results from several mutually connected processes such as PMS, vigilance, performing of CIs etc., and their output documents, as presented in Figure 1 (6, 7).

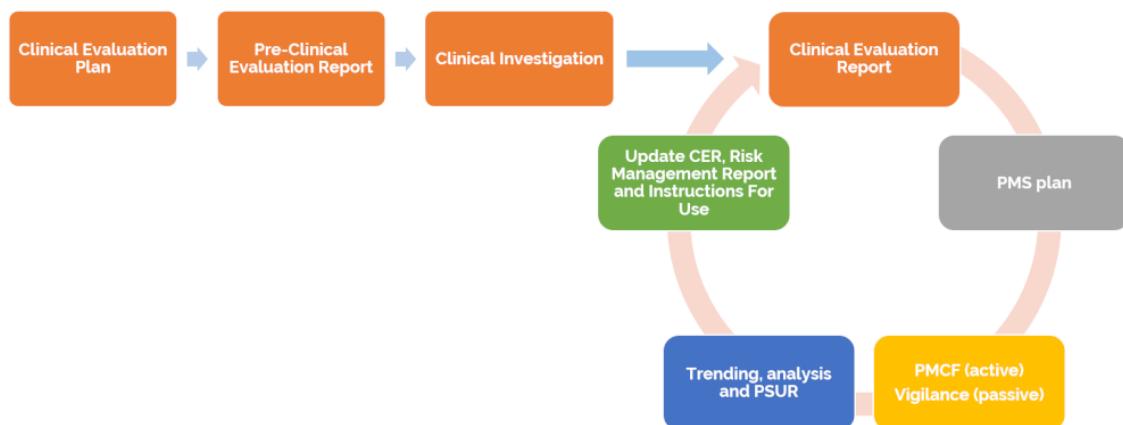


Figure 1. Processes connected with Clinical Evaluation

Slika 1. Procesi povezani sa kliničkom evaluacijom

Gathered clinical evidence should as well be used as an input for assembling SSCP, PSUR, as well as CEAR that will be prepared by Notified Body, as presented in Figure 2 (8).

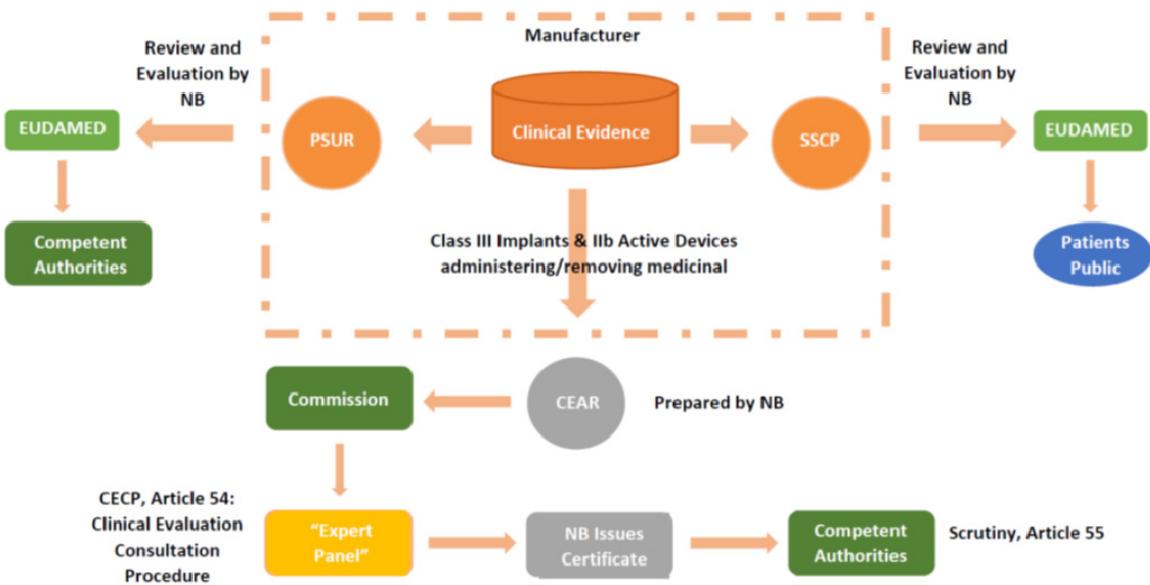


Figure 2. Clinical evidence as input for Clinical Evaluation/PSUR/SSCP/CEAR

Slika 2. Klinički dokazi kao ulazni elementi za kliničku evaluaciju/PSUR/SSCP/CEAR

The demonstration of equivalence between devices now presents a great challenge that should also be based on the clinical evaluation and can be achieved by demonstrating that each device with which equivalence is claimed must fulfil all **technical, biological and clinical characteristics**, as demonstrated in Figure 3 (8).

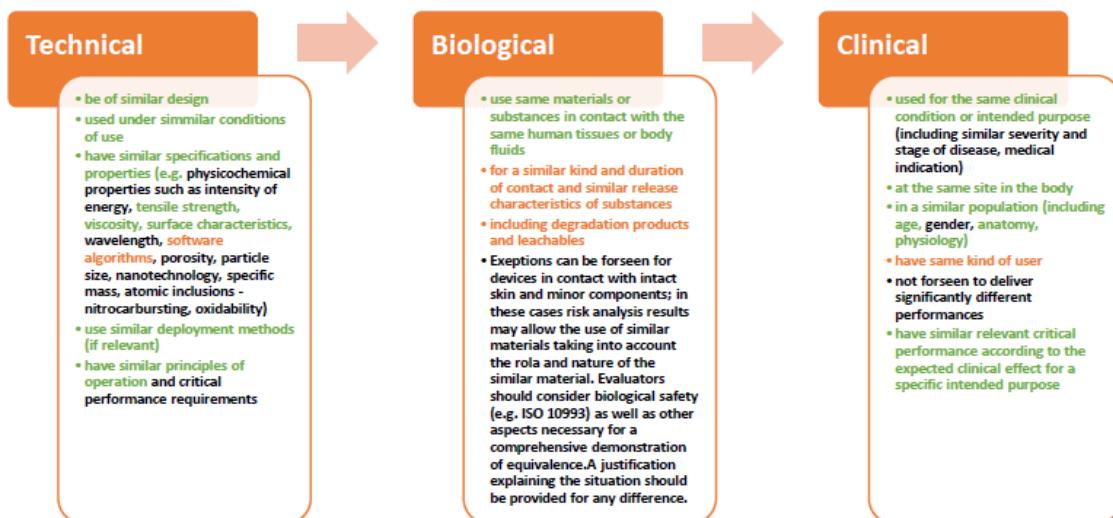


Figure 3. Technical, biological and clinical characteristics for demonstration of equivalence
Slika 3. Tehničke, biološke i kliničke karakteristike za dokazivanje ekvivalencije

Clinical Investigations

CIs shall be designed, authorized, conducted, recorded and reported in accordance with the provisions of Articles 62 to 80 of the MDR 2017/745, the acts adopted pursuant to Article 81, and Annex XV, where carried out as part of the clinical evaluation for conformity assessment purposes, for one or more of the following purposes:

- a) to establish and verify that, under normal conditions of use, a device is designed, manufactured and packed in such a way that it is suitable for one or more of the specific purposes in accordance with the definition for medical device, and achieves the performance intended as specified by its manufacturer;
- b) to establish and verify the clinical benefits of a device as specified by its manufacturer;
- c) to establish and verify the clinical safety of the device and to determine any undesirable side-effects, under normal conditions of use of the device, and assess whether they constitute acceptable risks when weighed against the benefits to be achieved by the device (5).

Manufacturers' responsibility concerning Clinical Investigations

Each manufacturer should first define the legal requirements for performing CIs and make an assessment of the necessity for gathering more clinical evidence for their devices. In Article 61, Reg. 2017/745 is stated that CIs are mandatory for all implantable and class III devices. CIs are not mandatory if a new implantable or class III device is equivalent to a device already marketed by the same manufacturer and the clinical evaluation of the latter is sufficient to demonstrate the conformity of the new one. CIs are not mandatory for class III devices already marketed under MDD and for implantable devices like sutures, staples, tooth crowns (full list presented in paragraph 6 of the Article 61), that have sufficient clinical data to demonstrate their conformity. It is important to note that, in the absence of clinical data, a low-risk MD requires a CI. Finally, according to the characteristics of the device, and including low-risk device, post-marketing CIs are requested to confirm, integrate or update existing clinical data, especially to address the safety concerns of the device within the frame of a PMCF (5, 9).

Next step would be to start planning and performing the CI. In this process, manufacturers have a good deal of responsibilities, such as:

- Design of the study
 - Clinical Investigation plan
 - Informed consent and ethical consideration
 - Clear measurable objectives
 - Criteria to stop the trial
 - Statistical power
 - Patient population
- Designation of principal investigator
- Insurance
- Application with ethical committee
- The non-EU manufacturer/sponsor may choose to appoint the Authorized Representative to complete the authorization of clinical investigation to EUDAMED.
- Incident Reporting
 - In the event of an incident during the clinical investigation, the reporting as such will need to be determined and performed by the appointed Authorized Representative through the EUDAMED database.

- Documentation available
 - The clinical investigation data (reports) should be incorporated in the technical documentation that has to be kept available by the manufacturer of his appointed European Authorized Representative established within the Union.
- Sponsor (authorized representative) shall notify the competent authorities of the ending of the clinical investigation, and submit a report (5, 10).

Post-Market Surveillance and Post-Market Clinical Follow-up

PMS and PMCF activities are interconnected with the Clinical Evaluation. PMS system is described in the Article 83, with defined activities which will result in creating the 3 most significant documents in the process:

- PMS plan (Article 84, Section 1.1 of Annex III),
- PMS report applicable for Class I devices (Article 85) and
- PSUR (Article 85).

Detailed description of the PMCF is defined in Annex IX Part B with main focus on defining the activities (PMCF plan) for proactive collecting and evaluating clinical data with the aim of:

- a) confirming the safety and performance of the device throughout its expected lifetime,
- b) identifying previously unknown side-effects and monitoring the identified side-effects and contraindications,
- c) identifying and analyzing emergent risks on the basis of factual evidence,
- d) ensuring the continued acceptability of the benefit-risk ratio, and
- e) identifying possible systematic misuse or off-label use of the device, with a view to verifying that the intended purpose is correct (5, 11).

Role of the manufacturers

The appropriate reinforcement of PMS procedures and data entails:

- Better control of distributors to ensure all PMS data is received from the distributor directly.
 - Under the MDR, distributors are economic operators with responsibility for monitoring the devices they put onto the market.

- The legal manufacturer must ensure appropriate PMS and vigilance actions are undertaken by the distributor under full control and surveillance from the legal manufacturer.
- Ensuring complaints are logged from all sources more effectively and are investigated in a timely and thorough manner.
- More detailed analysis of complaints with a thorough assessment of their impact on the overall clinical safety and performance.
- Trending of PMS data to better understand and identify emerging device concerns.
- Ensuring the PMS data feeds into risk management and is incorporated into the updated CER.

PMCF activities should:

- Provide appropriate evidence of planning, implementation of protocol and reporting with reasonable timelines.
- Target residual risks and confirmation of safety and performance over a device's lifetime, and should not be trying to answer questions that should have been addressed prior to CE marking.
- Include all targeted structured post-market activities that address specific questions/residual risks.

What PMCF is not....

- An expedient route to gain early CE certification and then gather critical clinical evidence later on.
- A post-market study with primary objectives to demonstrate safety and performance requirements which should have been addressed pre-CE marking.
- A token gesture to regulators to follow-up a few patients with an adequate survey that does not provide any meaningful information (12).

Conclusion

New requirements of the MDR regarding Clinical Evaluation and PMS are aimed towards imposing manufacturers a continuous providing of clinical evidence for the medical devices throughout their lifecycle. This could be achieved by following the provisions related to preparing detailed plans for Clinical Evaluation and PMS activities, clearly defining the process of PMCF, and designing CIs in a manner that will

ensure and continuously improve health and safety of the users. Electronic databases of EUDAMED will play a great role in providing the necessary transparency and traceability of all manufacturers' activities on this matter by making them publically available.

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Obezbeđivanje kliničkih dokaza u skladu sa MDR 2017/745 – novi izazovi za proizvođače u industriji medicinskih sredstava

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Kratak sadržaj

Obezbeđivanje potrebnih kliničkih dokaza kroz sisteme kliničke evaluacije i post-marketinškog nadzora u skladu sa zahtevima regulative MDR 2017/745 nameće nove izazove za proizvođače medicinskih sredstava.

Proizvođači treba da obezbede da klinička evaluacija postane kontinuirani proces koji sumira nalaze i rezultate nekoliko međusobno povezanih procesa: PMS, vigilanca, CIs. Objedinjeni klinički dokazi bi trebalo da budu iskorišćeni kao ulazni elementi za SSCP, PSUR, CEAR.

CIs bi trebalo da budu dizajnirana, odobrena, sprovedena, zabeležena i prikazana u skladu sa zahtevima propisanim u članovima 62-81 i Aneksa XV.

Aktivnosti u vezi PMS i PMCF bi trebalo da obezbede odgovarajuće dokaze o planiranju, sprovođenju protokola i izveštavanja; ciljni rezidualni rizik i potvrdu sigurnosti i performansi za vreme životnog ciklusa medicinskog sredstva; da uključuju aktivnosti koje se odnose na specifična pitanja/rezidualni rizik.

Sve aktivnosti su usmerene u postizanju nekoliko ciljeva: poboljšanje kvaliteta, sigurnosti i pouzdanosti sredstava i jačanje transparentnosti informacija za korisnike.

Ključne reči: Regulativa o medicinskim sredstvima 2017/745, klinička evaluacija, praćenje medicinskog sredstva posle stavljanja na tržište, kliničko ispitivanje, postmarketinško kliničko ispitivanje.
