The analysis of the permeability process of calcium antagonists in developing transdermal forms with a cardiovascular effect

Tatyana Shyteyeva¹, Svitlana Gubar¹, Nataliia Smielova¹, Elena Bezchasnyuk¹, Liana Budanova²

¹ State Scientific Research Laboratory for Medicines Quality Control, National University of Pharmacy, Pushkinska Str. 53, Kharkiv, 61002, Ukraine
² Foreign Languages Department, National University of Pharmacy, Pushkinska Str. 53, Kharkiv, 61002, Ukraine

Corresponding author: Svitlana Gubar (gubarsn@ukr.net)

Received 23 June 2019 ♦ Accepted 5 November 2019 ♦ Published 27 January 2021

Citation: Shyteyeva T, Gubar S, Smielova N, Bezchasnyuk E, Budanova L (2021) The analysis of the permeability process of calcium antagonists in developing transdermal forms with a cardiovascular effect. Pharmacia 68(1): 189–193. https://doi.org/10.3897/pharma.68.e37632

Abstract

Aim. The aim of the work was to evaluate the possibility of using calcium antagonists, namely, nifedipine and amlodipine besylate, while conducting transdermal delivery, that included the analysis of in vitro permeability process as a primary preformulation stage of pharmaceutical development of a transdermal dosage form, determination of qualitative and quantitative characteristics of a permeability process and the expediency analysis of development of a therapeutic transdermal system (TTS) with a cardiovascular effect.

Materials and methods. The active pharmaceutical ingredients (API) of nifedipine and amlodipine besylate. The study has been carried out in vitro by a dialysis method using a modified diffusion device of the Valia-Chien design.

Results. Character analysis, description of the mathematical model and definition of the kinetic parameters in the process of permeability of the studied medicinal products (MP) of nifedipine and amlodipine besylate allowed to evaluate their potential for creating TTS as being positive and appropriate. The implemented methodological approaches allow to substantiate the further algorithm for the development of cardiovascular TTS with the mentioned API.

Keywords

Nifedipine, amlodipine besylate, in vitro permeability, a transdermal therapeutic system

Introduction

Cardiovascular diseases, in particular, ischemic heart disease and arterial hypertension are the main causes of disability and mortality among the population. Nowadays, a numerical increase in the incidence of this type of pathology may be present. The pharmacotherapy of the mentioned pathological states usually requires a long time, an individual approach and a complex adjustment, taking into account all the parts of the pathological process.

The first line of the medicinal products in treating hypertensive disease includes calcium antagonists, which show antianginal and antihypertensive properties. Among the groups of the calcium channel blockers the medicinal products of 1,4-dihydropyridine type- nifedipine and amlodipine are widely spread.

Nifedipine is a short-acting calcium antagonist, widely known in the world of medical practice. Its main drawback is a short-duration half-life period, which takes only 2–4 hours. For these purposes, nifedipine requires
an increase in the number of administration during the day and it is accompanied by the occurrence of side effects. After oral administration, it undergoes an intensive metabolism, which leads to a decrease in bioavailability (40–60%) (Oparin et al. 1998).

The next calcium antagonist generations of the dihydropyridine group differ from nifedipine by a longer effect. A vivid representative of the third generation is amlodipine, characterized by a sufficiently high bioavailability (64–80%) and slight fluctuations in the maximum and minimum concentration in the blood within 24 hours (Preobrazhensky et al. 2008; Preobrazhensky et al. 2011; Prikhodko et al. 2011; Bagry 2012).

The design of modern innovative dosage forms (DF) for the medicinal products of this group are based on seeking an alternative way of the delivery of active ingredients (Pastore et al. 2015). Some of the perspective DF are TTS which, thanks to the continuous controlled profile of the MP introduction through the skin, provide concentration stability and a long therapeutic level of the substance in the bloodstream, in a way that facilitates prolonging the therapeutic effect. TTS, as compared to the peroral DF, have the potential to prevent hepatic metabolism in the first pass, eliminate the risks of gastrointestinal adverse reactions development, in this way increasing their safety profile (Flowers 2008; Bala et al. 2014; Kadam et al. 2014). During the use of transdermal patches, a decrease in the dosage frequency is achieved and a high systemic bioavailability of MP is ensured. TTS are quite easy to use and allow to maintain compliance by patients. In case of the occurrence of an adverse reaction, it is possible to discontinue the use of the patch immediately, the risk of overdose is minimized.

Only the oral formulations of nifedipine and amlodipine besylate are currently presented in the pharmaceutical market and none of them are presented in transdermal forms. Nevertheless, a number of scientists have been carrying out research and development of the transdermal forms of these medicinal products for a long time (McDaid and Deasy 1996; Ahmed et al. 2010; John et al. 2013; Yasam et al. 2016). Besides, the range of TTS in the Ukrainian pharmaceutical market is limited and includes only anti-inflammatory and antipyretic medicinal products. That is why the product-line expansion of TTS and the agents that can be used for a mixed pharmacotherapy of cardiovascular diseases is a task of vital importance.

The modern approach to the problems of transdermal DF design involves a detailed and comprehensive study of the various biopharmaceutical aspects of their development. The experimental research should be directed to the determination of the target quality profile of a medicinal product. For optimal therapeutic effect with transdermal administration of the product it is necessary to take into account physico-chemical properties of the active substance, the influence of the excipients, which are part of the combinations, and the skin condition. With the aim of defining a more rational approach for developing TTS a pharmaceutical consideration of a transdermal product should be preceded by preformulation studies of the medicinal products permeability in vitro through the membrane. The main advantage of such research is a possibility of a control over the experiment conditions and, therefore, the possibility to control changes in permeability due to the influence of different factors (Yasam et al. 2016; Shyteyeva et al. 2017; Shyteyeva et al. 2018).

In this context, the aim of our work is to study in vitro the process of calcium antagonist permeability, nifedipine and amlodipine besylate, and determining their application perceptiveness in the creation of transdermal patches with cardiovascular effect.

**Materials and methods**

The API of nifedipine (Suchem Laboratories company, India) and amlodipine besylate (Hetero Drugs Limited company, India) have been chosen as the objects of the study.

Nifedipine (Fig. 1) is a yellow crystal powder, which is practically insoluble in water, poorly soluble in ethanol. $M_\text{r} = 346,34\text{Da}$. log P (octanol-water) = 2,20. Hydro solubility at 25 °C = 56,3 mg/l (Ukrainian compendium).

**Figure 1.** Nifedipine 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester.

Amlodipine (in the besylate form) (Fig. 2), is a yellow crystal powder, which is slightly soluble in water, sparingly soluble in ethanol. $M_\text{r} = 408,89\text{Da}$. log P (octanol-water) = 3. Hydro solubility – 75,3 mg/l at 25 °C (Ukrainian compendium).

**Figure 2.** Amlodipine 3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl 5-methyl ester.

The study of the permeability of the chosen API through a semipermeable membrane was carried out in vitro by a dialysis method using a modified diffusion de-
vice of the Valia-Chien design, which was earlier described in paper (Chien 1987). The initial saturated solutions of the test substances in a phosphate buffer solution were used as donor solutions. A phosphate buffer solution (pH 7.4) served as an acceptor solution. The experiment was conducted under the temperature of 37 + 0.5 °C (the temperature of human subcutaneous layers). At certain intervals, 1 hour, that were corresponding to 1, 2, 3, 4, and 5 hours from the beginning of the experiment, the entire solution was removed from the acceptor compartment, replacing the sample acceptor solution with a new one, which was taken into account in the calculations. For every test sample the absorption spectra were recorded on the spectrophotometer Specord 200. The optical density of the obtained solutions was determined by the maximum absorption at the appropriate wavelength for each product.

The qualitative characteristics of the permeability process were determined with Fick’s law, which describes diffusion processes, including the active substance transfer through the skin or membrane.

**Results and discussion**

The assessment of the permeability process of the studied substances through a semipermeable membrane was conducted according to the determined values of the flux $I$, permeability coefficient $K_p$, and the diffusion delay time $\Theta$. The experiment results have been presented in Table 1.

| API                | Number of a chosen sample, n | Sampling time, t, h | API quantity in a dialysis sample, $X_{i} \cdot 10^{-4}$, g | API concentration in a dialysis sample, $C_{i} \cdot 10^{-1}$, mg/ml | Specific flux of API, $Q_{i} \cdot 10^{-2}$, mg/cm² h |
|--------------------|-----------------------------|---------------------|---------------------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------|
| Nifedipine         |                             |                     |                                                               |                                                           |                                               |
| 1                  | 1                           | 30.771              | 113.9666                                                      | 74.1470                                                   |                                               |
| 2                  | 2                           | 28.539              | 105.7000                                                      | 142.9157                                                  |                                               |
| 3                  | 3                           | 31.751              | 117.5962                                                      | 142.9157                                                  |                                               |
| 4                  | 4                           | 28.727              | 106.3962                                                      | 219.4241                                                  |                                               |
| 5                  | 5                           | 27.725              | 102.6851                                                      | 219.4241                                                  |                                               |
| Amlodipine besylate|                             |                     |                                                               |                                                           |                                               |
| 1                  | 1                           | 45.890              | 167.0000                                                      | 108.6507                                                  |                                               |
| 2                  | 2                           | 45.839              | 169.7740                                                      | 219.1060                                                  |                                               |
| 3                  | 3                           | 46.997              | 170.7286                                                      | 330.1831                                                  |                                               |
| 4                  | 4                           | 47.584              | 176.2370                                                      | 444.8434                                                  |                                               |
| 5                  | 5                           | 48.010              | 177.8148                                                      | 560.5301                                                  |                                               |

According to the obtained results of the API quantity, $X$ and its concentration $C$ in a dialysis sample (Table 1), it can be noted that these indices practically do not change within the duration of the experiment, that the passage of all the tested substances through the selected membrane has been carried out uniformly that corresponds to the zero-order kinetics with respect to the concentration gradient of donor and acceptor solutions.

The statistical equivalence of the obtained data was evaluated on the basis of a study of samples with experimental values, organized in ascending order. Changes in the variant $X$ of the received samples can be considered insignificant if the values of their extreme variants do not exceed the limit values of the confidence interval, calculated by the maximum permissible half-width of the confidence interval ($max \Delta_i$). The value $max \Delta_i$ was defined on the basis of the relative uncertainty of quantitative analysis of the given API ($\Delta_{w}$) (equation 1) based on the relative tolerance of API quantitative content in TTS $B = 25\%$ according to the State Pharmacopoeia of Ukraine (SPhU) requirements (State Pharmacopoeia of Ukraine).

$$\Delta_{w} = 0.32 \cdot B = 0.32 \cdot 25 = 8.0\% \quad (1)$$

The limit values of the confidence interval were determined by equations 2 and 3:

- the upper limit

$$X_{high} = \bar{X} \cdot \left(1 + \frac{max \Delta_{w}}{100}\right) \quad (2)$$

- the lower limit

$$X_{low} = \bar{X} \cdot \left(1 - \frac{max \Delta_{w}}{100}\right) \quad (3)$$

The convergence estimation results of experimental values of process parameters of the examined API permeability through a membrane have been presented in Table 2.

According to the results, presented in Table 2, it can be seen that the variant values of all the selections $X$ do not exceed the limit values of the confidence interval $X_{low}$ and $X_{high}$. Thus, all the obtained experimental values of the studied parameters are within the limits of the confidence interval.

The graphic interpretation of the in vitro permeability process of the tested substances through a semipermeable membrane and the statistical analysis parameters of the obtained results are presented in Figure 3 and in Figure 4.

It has been observed that in all experiments the obtained kinetic equations have the form of a general linear regression ($Y = A + B \cdot X$). For the obtained kinetic equa-
tions, within the time of the experiment, the correlation coefficient \( R^2 \) was not less than 0.999.

The main quantitative characteristics of the permeability process of the studied API in vitro, calculated on the basis of a statistical analysis, have been presented in Table 4.

Table 4. The kinetic parameters of the permeability process of API with cardiovascular effect in vitro through a semipermeable membrane.

| API            | API steady-state flux, \( I_s \), mg/cm²·h | Time of diffusion delay, \( \Theta \), min | Permeability coefficient, \( K_p \), cm/h | Linear correlation coefficient, \( r \) |
|----------------|------------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| Nifedipine     | 0.7083                                   | -3.06                                   | 0.075                                   | 0.9997                                 |
| Amlodipine besylate | 1.1295                               | 3.28                                     | 0.124                                   | 0.9999                                 |

According to the obtained results (Table 4), the kinetic parameters of the amlodipine besylate permeability process are 1.6 times higher than the same indices for nifedipine. Thus, the value of the steady-state fluxrate \( I_s \), obtained for nifedipine, is 0.7083 mg/cm²·h, and for amlodipine besylate is 1.1295 mg/cm²·h. In the same ratio for these substances the values of the permeability coefficient \( K_p \) were defined, that is 0.075 cm/h for nifedipine and 0.124 cm/h for amlodipine besylate. The diffusion delay time determines the duration of the non-stationary period of the process. In the frame of the experiment, the non-stationary period for the permeability process of both nifedipine and amlodipine besylate is defined within 3 minutes but at that point, the negative significance of this indicator for nifedipine indicates a lack of the membrane saturation.

Conclusions

In the result of the carried out research it was defined that nifedipine and amlodipine besylate permeability process in the simulated conditions is characterized by uniform velocity. Based on the statistical analysis, the linear dependence of this process was confirmed. The obtained quantitative values of the steady-state flux velocity and the coefficient of permeability indicate the potential of the selected substances in overcoming membrane barriers, and allow to predict a positive assessment of the acceptability of the selected API for the use in the design of TTS.
References

Ahmed MG, Kumar KG, Kumar SB (2010) Formulation and evaluation of nifedipine transdermal patches. Journal of Pharmacy Research 3(8): 1785–1787.

Babushkin AV (2009) Optically clean connections are key to the future. S-amlodipine. Ukrainian Medical Journal 5: e75.

Bagry AE (2012) Place of amlodipine in modern cardiology practice. Health of Ukraine 2012(1): e75.

Bala P, Jathar S, Kale S, Pal K (2014) Transdermal Drug Delivery System (TDDS): A Multifaceted Approach For Drug Delivery. Journal of Pharmacy Research 8(12): 1805–1835.

Chien YW (1987) Development of transdermal drug delivery systems. Drug development and industrial pharmacy 13(4–5): 589–651. https://doi.org/10.3109/03639048709105212

Flowers FP (2008) Transdermal and topical drug delivery. Theory to Clinical Practice 38(4): 726–728. https://doi.org/10.1345/aph.1D555

John L, Kumar A, Samuel S (2013) Formulation and evaluation of Amlodipine Transdermal Patches Using Ethylcellulose. International Research Journal of Pharmacy 4(10): 84–88. https://doi.org/10.7897/2230-8407.041019

Kadam AS, Ratnaparkhi MP, Chaudhary SP (2014) Transdermal drug delivery: An overview. International Journal of Research and Development in Pharmacy and Life Sciences, June-Jule 3(4): 1042–1053.

Kadam AS, Ratnaparkhi MP, Chaudhary SP (2014) Transdermal drug delivery: An overview. International Journal of Research and Development in Pharmacy and Life Sciences 3(4): 1042–1053.

McDaid DM, Deasy PB (1996) An investigation into the transdermal delivery of nifedipine. Pharmaceutica Acta Helvetiae 71(4): 253–258. https://doi.org/10.1016/S0031-6865(96)00022-2

Oparin AG, Oparin AA, Yakovenko EL (2014) Calcium antagonists: mechanism of action and clinical features. East European Journal of Internal and Family Medicine 1: 51–56. https://doi.org/10.15407/internalmed2014.01.051

Pastore MN, Kalia VN, Horstmann M, Roberts MS (2015) Transdermal patches: history, development and pharmacology. British journal of pharmacology 172(9): 2179–2209. https://doi.org/10.1111/bph.13059

Preobrazhensky DV, Nosenko NA, Nekrasov NO, Pataraya SA, Talyzina IV (2011) Third-generation calcium antagonist amlodipine: clinical pharmacology features and spectrum of therapeutic use. Russian Medical Journal 19(4): 884–890.

Preobrazhensky DV, Vysheisky ID, Pataraya SA, Skorik AV (2008) Third-generation calcium antagonist amlodipine: clinical pharmacology features and therapeutic use. Russian Medical Journal 16(11): 1524–1531.

Prikhodko VF, Maslennikova NA, Maskulo IP, Kononenko OA (2011) Amlodipine and S-amlodipine in the treatment of arterial hypertension effectiveness, safety, improvement of prognosis. Journal "Art of Treatment" 2: 49–50.

Shiteyeva TO, Beschashnyuk EM, Gubar SM, Rusak IV (2018) Investigation of permeability in vitro of ketorolac tromethamine through a semipermeable membrane in the process of pharmaceutical development of a transdermal therapeutic system. Management, Economics and Quality Assurance in Pharmacy 1(53): 13–18.

Shyteyeva TO, Beschashnyuk EM, Gubar SM, Rusak IV (2017) A study of the permeability of NSAIDs in vitro in semipermeable membrane for the development of transdermal dosage form. The 8th International Conference on Pharmaceutical Sciences and Pharmacy Practice, Kaunas (Lithuania), December, 2017, 128–130. https://doi.org/10.24959/uekj.18.5

State Pharmacopoeia of Ukraine [in 3 vols.] (2015) The state enterprise “The Ukrainian scientific pharmacopoeial centre of the medicinal products quality” (2nd ed.). Kharkiv State Enterprise “Ukrainian Research Center Expert Pharmacopoeia Quality Medicines [in Ukrainian], 1128 pp.

Ukrainian compendium (1999‒2021) Ukrainian compendium. https://compendium.com.ua

Yasam VR, Jakki SL, Natarajan J, Venkatachalam S, Kuppusamy G, Sood S, Jain K (2016) A novel vesicular transdermal delivery of nifedipine-preparation, characterization and invitro/in-vivo evaluation. Drug delivery 23(2): 619–630. https://doi.org/10.3109/10717544.2014.931484