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ESTIMATION OF ABSORPTION OF MAGNESIUM NICOTINATE AND ITS DERIVATIVES WITH SELECTED AMINO ACIDS

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Abstract

Preparation of solid dispersions is a popular pharmaceutical technology designed to improve the solubility and absorption characteristics of drugs. Solubilizing and moisturizing of carriers show influence on therapeutic substances; although dissolution of molecular dispersion of particles of the therapeutic substance in a neutral carrier is of utmost importance.

This paper present the results of the research on influence of modification the structure of magnesium nicotinate Mg(Nic) with ligands, glycine and arginine, on the absorption process of Mg^{2+}ions *in vitro*. The absorption area was the small intestine of a rat. It was found that structural changes with an additional arginine or glycine ligand affect the absorption process of Mg^{2+}ions.

Moreover, the effect of hydrophilic carriers on the partition coefficient (log P) for the system of n-octanol and phosphate buffer was investigated for the solid dispersions containing the examined magnesium salts. Phosphatidylcholine (PC-45) and polyvinylpirrolidone (PVP K-30) were used as carriers for solid dispersions with of magnesium salts. It was confirmed that using auxiliary substances PC-45 and PVP changes significantly ($p<0.05$) P values, corresponding to increasing hydrophobic properties of solid dispersions of the examined salts.

It was found that modification of the structure of magnesium nicotinate by amino acids such as arginine or glycine positively influences the absorption process Mg^{2+} ions. The research carried out on properties of the solid dispersions containing magnesium salts and phospatidylcholine (PC-45) or magnesium salts and polyvinylpirrolidone (PVP K30) showed positive influence of these auxiliary substances.

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Key words: magnesium nicotinate, ligands: glycine, arginine, solid dispersion, partition coefficient, absorption.

OCENA WCHŁANIANIA NIKOTYNIANU MAGNEZOWEGO I JEGO POCHODNYCH Z WYBRANYMI AMINOKWASAMI

Abstrakt

Wytwarzanie stałych dyspersji jest popularną metodą technologiczną stosowaną w celu poprawy rozpuszczalności i wchłaniania leków. Właściwości solubilizujące oraz zwilżające nośników mają wpływ na proces rozpuszczania substancji leczniczych.

W pracy przedstawiono badania wpływu modyfikacji struktury nikotynianu magnezowego Mg(Nik) aminokwasami (argininą lub glicyną) na proces wchłaniania jonów Mg^{2+}*in vitro*. Powierzchnię absorpcji stanowiło jelito cienkie szczura. Stwierdzono, że dodatkowy ligand argininy lub glicyny w strukturze nikotynanu magnezu wpływa na zmianę parametrów procesu wchłaniania jonów Mg^{2+}.

Ponadto badano wpływ nośników hydrofilowych na współczynnik podziału o/w układu *n*-oktanol/bufor fosforanowy wybranych soli magnezowych stałych rozproszeń. Do sporządzenia stałych rozproszeń z badanymi solami magnezowymi zastosowano fosfatydylocholinę 45% (PC-45) i polivinylopirolidon (PVP K-30).

Stwierdzono, że zastosowanie substancji pomocniczych PC-45, PVP znacząco ($p<0.05$) wpływa na zmianę wartości log P, a zatem wzrasta hydrofobowość stałych rozproszeń badanych soli. Wykazano, że modyfikacja struktury nikotynianu magnezu argininą lub glicyną wpływa na poprawę absorpcji jonów magnezowych. Badania właściwości stałych rozproszeń zawierających sole magnezowe – fosfatydylocholinę (PC-45) lub sole magnezowe – poliwinylopirolidon (PVP K-30) wykazały pozytywny wpływ zastosowanych substancji pomocniczych.

Słowa kluczowe: nikotynian magnezu, ligandy: glicyna, arginina, stałe rozproszenia, współczynnik podziału, wchłanianie.

INTRODUCTION

Magnesium belongs to essential macroelements which condition proper functions of human body. It takes part in all important metabolic changes, in reactions of synthesis of energetically rich compounds mainly ATP, hydrogen and electron carriers, or in synthesis and activity of numerous enzymes. It plays an important role in oxidation-reduction processes or maintaining acid-basic balance. Magnesium has also been demonstrated to alleviate stress, allergies, anaphylactic condition or inflammation states and to participate in defence processes (Szmitz et al. 2007, Touyz 2004). Magnesium is a stabilizer of cell membranes, influencing their fluidity and permeability (Konrad et al. 2004). Based on numerous studies it has been shown that Mg chelated with amino acids and pyridoxine is well absorbed. It has been proved that amino acids such as aspartic acid, cysteine, arginine and glycine act as carriers in Mg^{2+} ion transport (Olędzka 1999, Marcoin and Szulc 2002).

Magnesium is essential in human diet and its lack can cause many diseases (GRIGORYAN, KOMPANTSEVA 2005). Oral or intravenous Mg supplementation is an effective method of combating and preventing its deficiency (SKIBNIEWSKA 2000). Market demand for preparations containing their very important bioelement is continually growing.

For supplementation the best are magnesium compounds containing organic anions, chelate compounds of moderate strength, which protect metal ions against binding in sparingly soluble compounds (FIROZ, GRABER 2001) They facilitate its transport through walls of intestines and release metal ions into serum, where they can be added to right receptors and then transmitted to cells. While working on new drugs, mechanisms and factors conditioning gastrointestinal absorption are of the utmost importance. Hydrophobicity, a property indicating ability of a drug to permeate through cell membranes, has great importance for the interaction of the drug with a receptor. Hydrophobicity of a given compound can be determined experimentally by finding a partition coefficient between the two phases (log P), a parameter determining lipophilic-hydrophilic balance, which reflects passive transport of a drug in organism.

Preparations containing nicotinic acid (pyridino-3-carboxylic acid) with magnesium are applied in disturbances of peripheral circulation treatment, hypercholesteremy, migraine, podagra. Mineral amino acids chelate (glycinate nicotinate: Cu, Zn, Cr, Ca, Mg) are applied to supplement the diet its source of bioelements found in food meant for people.

In order to obtain a drug with improved Mg^{2+} ions absorption, modification of the structure of a magnesium nicotinate molecule was attained via amino acids ligands. In the previous paper (MARCOIN and SZULC 2002), positive influence of an additional ligand of glycine to the structure of magnesium nicotinate in the process of absorption of Mg^{2+} ions in the small intestine was presented.

The subject of this paper is comparative evaluation of *in vitro* absorption of Mg^{2+} ions from magnesium nicotinate and magnesium nicotinate modified with arginine or glycine. Increasing dissolution rate of therapeutic substances in solid dispersions depends on the kind and amount of the carrier as well as the production method. Production of solid dispersions is among methods frequently used in order to improve pharmaceutical availability and, consequently, bioavailability of therapeutic substances. The most common carriers to produce solid dispersions are polyvinylpyrrolidone (PVP), polyethylenglycols (PEG), derivatives of cellulose and phospholipids. Solubilizing and moisturizing properties of carriers show influence on dissolution process of therapeutic substances; yet molecular dispersion of particles of a therapeutic substance in a neutral carrier has the greatest significance.

MATERIALS AND METHODS

The following salts were examined:

- magnesium nicotinate: Mg(Nic), $Mg(C_6H_4O_2N)_2$, mol.wt. 268.31
- magnesium aginine-nicotinate: Mg(NicArg), $Mg(C_{12}H_{18}O_4N_5)$, mol.wt. 320.43
- magnesium glycine-nicotinate: Mg(NicGly), $Mg(C_8H_8O_4N_2)$, mol.wt. 284.31.

In order to produce solid dispersions the auxiliary substances such as:

- phosphatidylcholine 45% (PC-45), (Lucas Meyer, Ltd);
- polivinylopirolidon (PVP), (Serva), were used.

All the chemicals were analytical reagent grade.

The synthesis of magnesium nicotinate was carried out according to the procedure described in the paper of MARCOIN and RYSZKA (1991). The magnesium nicotinate salts with amino acids (arginine or glycine) were obtained in a reaction of magnesium nicotinate and an appropriate amino acid in water solution of molar ratio 1:1. The synthesis was carried out at 60-70°C, the mixture being stirred intensely for 3 h. The products of the synthesis were isolated from the solution by water evaporation under low pressure evaporator (Unipam – 350), then crystallized from methanol and dried at room temperature. The content of magnesium was measured in an atomic absorption spectrophotometer (Carl Zeiss Jena model AAF 3) at the wavelength of 285.2 nm.

Preparation of solid dispersions. Solid dispersions were prepared in the granule form. Micronized magnesium salt was mixed with the selected carrier (PC-45 or PVP) in molar ratio (1:10) and dissolved in ethanol. After complete evaporation of ethanol, the solid dispersions were dried under vacuum and unified through a sieve (1.0 mm).

*Partition coefficient o/w (*log P*)*. For the solid dispersions the partition coefficient o/w (log P) for the system of n-octanol/phosphate buffer was determined according to the theory delineated by HANSCH et al. (1962).

Absorption process of Mg^{2+} *ions in vitro for magnesium salts.* Investigation of the absorption process of Mg^{2+} ions for magnesium salts was carried on an *in vitro* model according to the method described previously (MARCOIN and SZULC 2002), in which the absorption area was the small intestine (ileum) of a rat. The essential part of this apparatus was a glass chamber of 30 cm^3 capacity, thermostated at 37°C and filled with solution of 4 mM of the analysed magnesium salt. Aqueous 0.9% NaCl solution was pumped with a peristaltic pump through the intestine segment at a constant collected rate of 1.2 ml min^{-1}. Samples were collected every 15 min. and the magnesium content was measured by atomic absorption spectrophotometry (Spectrophotometer of AAF 3 Carl Zeis Jena) at the wavelength 285.2 nm. The study had been approved by the Bioethics Committee of the Medical University of Silesia. The results consisting of the absorption rate constant (k) and

absorption half time ($t_{50\%}$) were calculated. The measurements were repeated six times in order to minimise statistical errors. Standard deviation (SD) and variance (V) were determined.

Statistical analysis. All values were expressed as mean ± SEM. The measurements were repeated six times. Statistical significance was tested by repeated measures using ANOVA followed by Kruskal-Wallis test or esle Post Hoc multiple comparisons were done. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Modification of the magnesium nicotinate structure with ligand of arginine or glycine had positive influence on the parameters describing kinetics of Mg^{2+} ionic absorption in the small intestine of a *in vitro* rat system. The results are presented in Table 1 and Figure 1. Absorption of Mg^{2+} ions in a segment of the small intestine was carried out in agreement with the first order kinetics. The absorption of Mg^{2+} ions was the fastest for Mg(NicArg), followed by Mg(NicGly), but it was the slowest for the parent compound Mg(Nic). The amount of absorbed Mg^{2+} ions for all the measurement points showed statistically significant differences ($p<0.05$) compared with Mg(Nic) (Figure 1). Significant difference was found between Mg(Nic) and Mg(NicGly) ($p<0.05$) after 75 and 90 minutes.

Comparing the half time of absorption ($t_{50\%}$) of Mg^{2+} ions with magnesium nicotinate modified by arginine with the parent compound shows its decrease of 0.75 hour. The parameters specifying the absorption process indicate that arginine and glycine are good carriers for transporting Mg^{2+} ions. Analysis of the present results shows that structural changes with an

Table 1

Parameters describing Mg^{2+}-ions absorption from magnesium salts in a small intestine

salts	$k*10^{-3}$ (min)	$t_{50\%}$ (h)	Total amount (%) of absorbed Mg^{2+} ions within 2 h of the experiment	V (%)	(±) SD
Mg (Nic)	1.99	6.04	19.80	5.31	1.04
Mg (NicArg)	2.20*	5.29*	22.51*	3.88	0.87
Mg (NicGly)	2.07	5.58	21.40	5.48	1.15

*$P<0.05$ vs. Mg(Nic), k – absorption rate constant; $t_{50\%}$ – absorption half -time;
V – variance; SD – standard deviation

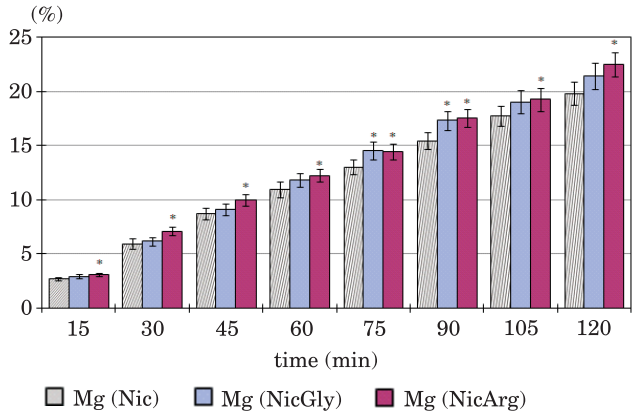

Fig. 1. The absorption rates of Mg2+ through the rat's small intestine from solutions of magnesium salts with modified structure.
Values are expressed as mean ± SD (n=6). *P<0.05 vs. Mg(Nic) at the same time point

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Parameters describing Mg^{2+}-ions absorption from magnesium salts in a small intestine

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additional arginine or glycine ligand modify absorption of Mg^{2+}. Increasing the length of a magnesium nicotinate chain by adding ligand positively influences polarity, which is the sum of various intermolecular reactions of donor – acceptor type.

In the case of magnesium nicotinate modified with glycine ligand, the factors characteristic for magnesium absorption were improved. Comparing the effects of amino acids applied to modify magnesium nicotinate, the influence of the substituent structure is evident. By introducing appropriate substituents into a particle, it is possible to influence its physicochemical properties and, eventually, its biological activity. The donor atoms such as oxygen and nitrogen found in particles of amino acids condition formation of bindings. The guanidine group may form characteristic pairs of dionic hydrogen

bonds, which are responsible for the power of bonding. It is well known that the guanidine group of arginyl radicals is important for proteins to maintain the tertiary protein structure through interior "salt bridges" with carboxyl groups and for bonding and differentiating amino substrates by enzymes, receptor sites (LEHN 1985).

Reactions with amino acids may alter molecule properties such as solubility, partition coefficient between n-octanol phase and water one as well as other characteristics which are of importance for drug absorption, distribution and excretion. Particles capable of reacting with anions by means of hydrogen bonds through electrically neutral polar centers (e.g. hydroxyl, amide groups) become a class of potential carriers making diffusion via membranes easier (MARGALIT et al. 1979).

Figure 2 contains the log P values determined for the partition coefficient of the examined dispersions containing selected magnesium salts between n-octanol phase and water phase. As the calculated values of the partition coefficient (log P) for solid dispersions without addition of auxiliary substances such as PVP and PC-45 show, modification of the parent compound with glycine ligand increases log P value by 0.149 units ($p<0.05$) while for the modification with arginine ligand the increase is 0.090 ($p<0.05$). Using auxiliary substances such as PVP, PC-45 influences significantly ($p<0.05$) change of log P values, therefore hydrophobicity of the examined solid dispersions containing magnesium salts increases. Addition of PC-45 is more effective than that of PVP for solid dispersions containing Mg(NicArg) and

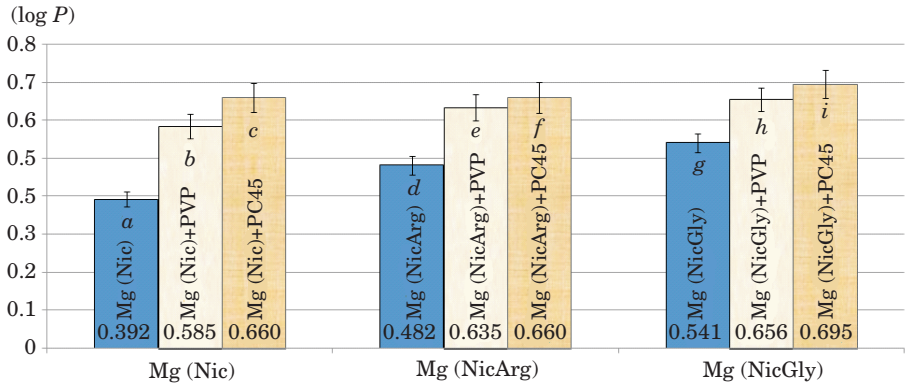

Fig. 2. The values partition coefficient (log P) for solid dispersions containing magnesium salts. Values are expressed as mean ± SD (n=6).

d,g bars : **P*<0.05 vs. *a* bar; *b,c* bars: **P*<0.05 vs. *a* bar; *e,f* bars: **P*<0.05 vs. *d* bar; *h,i* bars: **P*<0.05 vs. *g* bar.

Magnesium salts: Mg(Nic), Mg(NicArg), Mg(NicGly). Solid dispersions: Mg(Nic)+PVP, Mg(Nic)+PC45, Mg(NicArg)+PVP, Mg(NicArg)+PC45, Mg(NicGly)+PVP, Mg(NicGly)+PC45

Mg(NicGly). The highest log P value (0.695) was obtained in the case of Mg(NicGly); for Mg(NicArg) the log P value equalled 0.660. The improvement of hydrophobic properties of the magnesium salts contained in the solid dispersions via addition of PVP and PC45 carriers depends on their physicochemical properties.

Phosphatidylcholine conditions the hydrophobic balance and creates hydrogen bonds with a drug. Phosphatidylcholine was applied as a carrier in dispersion systems for indomethacin, phenobarbital, benzodiazepine derivatives (Law et al. 1992). Complex studies of solid dispersions proved that the applied carriers can improve the rate of dissolution and, consequently, pharmaceutical availability of drugs. Positive results were obtained by Marsac et al. (2008) who examined solid dispersions containing nifedipine and felodipine in the presence of PVP. Physical stability of the examined dispersions was linked with their amorphic properties as well as low hygroscopic properties.

Patel and Patel (2007) obtained improvement of lovastatin solubility for PVP polymer matrix dispersion. They identified decrease of the crystalline and increase of the amorphous fraction of the drug by means of X-ray, DSC,FT-IR analysis methods. Dhumal et al.(2007), in examinations of stability of solid oral forms of a drug containing celecoxib, used PVP and carrageenan. They did not find recrystallization of amorphous drugs since preparation, during processing and further storage.

Positive influence of water soluble polymers (PVP) on hydroxypropyl-beta-cyclodextrin complexation of rofecoxib was described by Singh and Aboul-Enein (2007). Considerable improvement of pharmaceutical availability of valdecoxib was attained by application of solid dispersions with PVP in tablets (Aftab and Pralhad 2006). In order to improve solubility of tenoxicam and flurinazine El- Gazayerly et al. (2000) and Marin et al. (2002) used PVP as a carrier for these substances in solid dispersions.

CONCLUSION

Modification of magnesium nicotinate structure with arginine or glycine ligand influences both solubility and ability of Mg^{2+} ions to penetrate through the small intestine of a rat. Additional ligands of arginine or glycine in the magnesium nicotinate structure are said to be good carriers of Mg^{2+} ions. The use of the auxiliary substances such as PVP, PC-45 in solid dispersions causes a decrease of dissolution and absorption process, which is evidenced by log P values. Addition of PC-45 to solid dispersion is more advantageous than PVP.

REFERENCES

AFTAB M., PRALHAD T. 2006. *Enhancement of dissolution profile by solid dispersion (kneading) technique* . AAPS Pharm. Sc. Tech., 7(3): 68 E1-E6.

DHUMAL R.S., SHIMPI S.L., PARADKAR A.R. 2007. *Development of spray-dried co-precipitate of amorphous celecoxib containing storage and compression stabilizers*. Acta Pharm., 57(3): 287-300.

EL-GAZAYERLY O.N. 2000. *Characterization and evaluation tenoxicam coprecipitates.* Drug Dev. Ind. Pharm., 26: 925-930.

FIROZ M., GRABER M. 2001. *Bioavailability of us commercial magnesium preparations.* Mag Res., 14(4): 257-262.

GRIGORYAN I.V., KOMPANTSEVA YE.V. 2005. *Developing the conditions for preparing organic magnesium salt and investigating its composition.* J. Neurosurgical Problems, 4.

HANSCH C., MALONEY P.P., FUJITA T., MUIR R.M. 1962. *Nature (London)* 194, 178.

KONRAD M., SCHLIGMANN K.P., GUDERMANN T. 2004. *Insights into the molecular nature of magnesium homeostasis.* Am. J. Physiol. Renal. Physiol., 286: F599- F605.

LAW S.L., LO W.Y., LIN F.M., CHAING C.H. 1992. *Dissolution and absorption of nifedipine in polyethylene glycol solid dispersion containing phospatidylcholine.* Int. J. Phar., 84: 161-168.

LEHN J.M. 1985. *Chemia supramolekularna [Supramolecular Chemistry].* PAN, Warszawa.

MARCOIN W., RYSZKA F. 1991. *Wybrane związki magnezoorganiczne o spodziewanym działaniu farmakologicznym [Some magnesium organic compounds expected to produce pharmacological effects].* Ann. Acad. Med. Siles., 23: 45-53.

MARCOIN W., SZULC B. 2002. *Influence of aminoacid anions on the absorption process of* Mg^{2+}*ions in vitro*. Sci. Pharm., 70: 29-37.

MARGALIT R., EISENMAN G., GROSS E., MEIENHOFER J. 1979. (EDS) *Peptides: Structure and biological function. Proc. of the* 6^{th} American Peptide Symposium, Pierce Chemical Company, *pp.* 665-679.

MARIN M.T., MARGARIT M.V., SALCEDO G.E. 2002. *Characterization and solubility study of solid dispersions of flunarizine and polyvinylpyrrolidone.* Farmaco., Sep., 57(9): 723-727.

MARSAC P.J., KONNO H., RUMONDOR A.C., TAYLOR L.S. 2008. *Recrystallization of nifedipine and felodipine from amorphous molecular level solid dispersions containing poly(vinylpyrrolidone) and sorbed water.* Pharm. Res., Mar; 25(3): 647-656.

OLĘDZKA R. 1999. *Wchłanianie magnezu [Magnesium absorption].* Biul. Magnezol., 4(1): 229-235.

PATEL R.P., PATEL M.M. 2007. *Physicochemical characterization and dissolution study of solid dispersions of Lovastatin with polyethylene glycol 4000 and polyvinylpyrrolidone K30*. Pharm-Dev-Technol., 12(1): 21-33.

SINGH I., ABOUL-ENEIN H.Y. 2007. *Influence of water soluble polymers on hydroxypropyl-beta-cyclodextrin complexation of rofecoxib.* Pharmazie., 62(4): 284-286.

SKIBNIEWSKA K.A. 2000. *Jakość współczesnego pożywienia w świetle badań CRP pobieranych metodą podwójnej porcji [Quality of contemporary food as demonstrated by CRP research using the double ration method].* Kongres 2000, 26-28 kwietnia Warszawa, 106

SZMITZ C., DEASON F., PERRAUD A.L. 2007. *Molecular components of verterbrate* Mg^{2+}*homeostasis regulation.* Mag. Res., 20(1): 6-18.

TOUYZ R.M. 2004. *Magnesium in clinical medicine.* Front. Biosci., 9: 1278-93.