Acta Poloniae Pharmaceutica – Drug Research, Vol. 65 No. 1 pp. 125–128, 2008
ISSN 0001-6837
Polish Pharmaceutical Society

CHARACTERISTICS OF CHELATION ABILITY OF CHOSEN METAL IONS BY PROTECTIVE OINTMENTS CONTAINING Na_2H_2EDTA

SŁAWOMIR SMOLIK[1*], EWA CHODUREK[2], PAWEŁ NOGAJ[3],
DOROTA DOMAL-KWIATKOWSKA[1], AGNIESZKA SZPAKOWSKA[1] and LUDMIŁA WĘGLARZ[1]

[1]Department of Biochemistry, [2]Department of Biopharmacy, [3]Department of Instrumental Analysis, Medical University of Silesia, 41-200 Sosnowiec, 1 Narcyzów Str., Poland

Abstract : Allergy contact dermatitis is a common occupational disease and the protective ointments are often used by the sensitized subjects. The efficacy of the chelation ability of the barrier creams containing Na_2H_2EDTA was evaluated.The *in vitro* test with the diffusion chamber and artificial membrane was performed. The effect of the Na_2H_2EDTA concentration (3, 5 or 10%), pH of the buffer for Na_2H_2EDTA dissolving and the vehicle of the ointment on the chelation of Ni^{2+} and Co^{2+} were assessed. The ointment with 10% Na_2H_2EDTA dissolved in the buffer of pH 7.0 or 7.4 buffer was found as optimal for the protection ability. There was no influence of the formula of the ointements on the efficiency of chelation.

Keywords : protective ointment, contact allergy dermatitis, chelation therapy

Barrier creams may contain the active ingredients which interfere with the absorption and penetration of irritant agents into the epidermis. The optimal barrier cream should be able to completely block, like a glove, the contact between an allergen and a skin (1). Though many preparations tested so far in this respect appeared to be inefficient, chelating agents have shown sufficient potential for practical use (2). Their addition into pharmaceutical formula increases the efficacy of creams by complexing metal ions, conceals it from the immune system and reduces the penetration of metal ions into the epidermis (3). Among many chelating agents, ethylenediaminetetraacetic acid (EDTA) is the most commonly used and it may be incorporated into topical preparations, or used orally (5). There are several forms of EDTA, which include mono, di, tri, or tetra substituted salts.

The aim of the study was to elaborate the most favorable chemical formula for the protective ointment in terms of $Na_2H_2EDTA{\cdot}2H_2O$ concentration, pH of the buffer component used for dissolving the chelating agent and the base for the ointment formulation. The *in vitro* test with the application of a diffusion chamber equipped with artificial membrane of chemically modified cellulose was performed to find the most satisfactory protection against chosen metal ions.

EXPERIMENTAL

The experiments were performed with the use of diffusion glass chamber consisting of a donor compartment, receptor compartment and the artificial membrane. The membrane of chemically modified cellulose (SPECTRAPOR) was mounted between donor and receptor of the chamber compartments. The whole volume of receptor compartment was filled with the tested ointment. The surface of the membrane was washed with the solution containing 10 mg of $CoCl_2{\cdot}6H_2O$ or 12 mg of $Ni(NO_3){\cdot}6H_2O$ dissolved in 20 mL of water. The peristaltic miniflow pump ensured the circulation of external solution. 100 µL aliquots were withdrawn from the receptor solution every 60 min, diluted to the volume of 3 mL and kept for further analysis. The experiments were run for 5 h.

The buffer solutions of the following pH: 7.0, 7.4, 7.8 and 8.2 were prepared according to the Polish Pharmacopoeia prescription (FPVI). The protective eucerin- or hascobase-based ointments containing 3%, 5% or 10% of $Na_2H_2EDTA{\cdot}2H_2O$ dissolved in each of the four buffers were made, and then they were emulgated in the eucerin or hascobase with the application of unguator. The concentration of chosen metal ions was determined by

* Corresponding author: e-mail: epimer@wp.pl; telephone 323641073

the atomic absorption spectrophotometry (model AAS vario 6) at the wavelength of 240.7 nm for Co^{2+} and 232.0 nm for Ni^{2+}. Each ointment sample was analyzed three times.

Statistical analysis was performed with the use of Statistica 6.0 computer program. The normal distribution of analyzed data was performed with the use of Shapiro-Wilk test. To compare the differences between specific chemical formulas of the ointments, the analysis of variance ANOVA or non-parametric alternative of this test (Kruskal-Wallis test) was applied. To compare the statistical significance between two samples, the t-Student's test or the non-parametric alternative of this test (U Mann-Whitney test) were used. A p value of < 0.05 was considered statistically significant.

RESULTS

The study showed that the protective ointment containing 10% $Na_2H_2EDTA \cdot 2H_2O$ was more effective in Ni^{2+} and Co^{2+} ions binding than the ointments with 5% and 3% of $Na_2H_2EDTA \cdot 2H_2O$ emulgated in either eucerin or hascobase (Table 1). After 5 h lasting diffusion test the ointment containing 10% $Na_2H_2EDTA \cdot 2H_2O$ and eucerin was found to chelate 25.42 ± 3.19% of Ni^{2+} and 19.93 ± 2,17% of Co^{2+}, whereas the ointments with 5% and 3% $Na_2H_2EDTA \cdot 2H_2O$ chelated 9.27 ± 1.28% of Ni^{2+} and 11.56 ± 0.98% of Co^{2+}, 6.09 ± 0.56% of $Ni^{2+}$and 6.23 ± 0.97% of Co^{2+}, respectively.

Based on these results, in the next step of this study only the ointments with 10% $Na_2H_2EDTA \cdot 2H_2O$ were analyzed. The analysis of the buffer pH influence on chelation ability revealed that the ointment prepared upon hascobase displayed the most effective capacity of Co^{2+} chelation at the pH of 7.0 and 7.4 (Figure 1). Since, there was no statistical significance in chelation ability of Co^{2+} ions between ointments with hascobase at pH = 7.0 and 7.4 both pH values were evaluated as optimal ones. For the all remaining pairs of buffer pH to be compared, differences were statistically significant ($p < 0.05$).

The protective eucerin-based ointments showed the optimal chelation ability of Co^{2+} at the pH of 7.8 and 7.4 (Figure 2). There was no statistically significant difference in chelation efficacy of Co^{2+} ions between the ointments with eucerin at pH = 7.8 and 8.2, pH = 7.4 and 8.2, as well as pH = 7.0 and 7.4. For the remaining pairs of buffer pH of analyzed ointments the differences were statistically significant ($p < 0.05$).

Furthermore, the pH of 7.0 and 7.4 was found to be optimal for chelation of Ni^{2+} ions by the analyzed ointments with eucerin (Figure 3). The chelation ability of Ni^{2+} ions was not statistically different when ointments with buffers of pH = 7.8 and 8.2, pH = 7.4 and 8.2, and pH = 7.0 and 7.4 were compared. The comparative analysis of the remaining pairs of buffer pH used, showed statistically significant differences ($p < 0.05$).

The pH values 7.0 and 7.4 appeared to be optimal for chelation of Ni^{2+} ions by the ointments with hascobase (Figure 4). No statistically significant differences were found in chelation ability of Ni^{2+} ions between the ointments of pH = 7.8 and 8.2 and pH = 7.0 and 7.4. When the remaining pairs of buffer pH were analyzed, the statistical differences were found to be significant ($p < 0.05$).

The effect of the base used (eucerin or hascobase) in the pharmaceutical formulas on the metal ions binding efficiency appeared to be statistically insignificant.

DISCUSSION AND CONCLUSION

The sensitivity resulting from the contact of the skin with metal ions is a disease commonly affecting the metal workers, the hairdressers, the cleaners and the jewellers in many Europe countries. The best way to prevent dermatitis caused by allergic reactions induced by metal ions is the avoidance by the sensitized individual a direct skin contact with relevant allergen. However, in many cases, especially in the industry, this is not possible. The alternative solution includes the protection of the skin by gloves or barrier creams. Application of the barrier creams may play an important role in the prevention of allergic contact induced dermatitis (6). Barrier creams are expected to remain effective for 5-6 h. Over the past 40 years, several substances that chemically bind metal ions have been investigated for their ability to prevent the signs of allergic contact dermatitis (7). In dermatological studies ethylenediaminetetraacetic acid as disodium salt ($Na_2H_2EDTA \cdot 2H_2O$) due to its metal ion complexing ability was most frequently used. In the present study the elaboration of a chemical formula for the protective ointment was performed with the application of a diffusion chamber equipped with artificial membrane of chemically modified cellulose. The ointment containing 10% of $Na_2H_2EDTA \cdot 2H_2O$ was found to be the most effective against Ni^{2+} and Co^{2+} aqueous solutions. These findings were consistent with the results of other authors (1, 3). The necessity of the buffer solution as a component of the protective ointment containing $Na_2H_2EDTA \cdot 2H_2O$ is well known. The reaction of binding metal ions by

Table 1. Comparison of the chelation ability of Co^{2+} and Ni^{2+} ions by the protective ointments containing 3, 5 and 10% of Na_2H_2EDTA.

EDTA / ointment base	3% (Co^{2+})	3% (Ni^{2+})	5% (Co^{2+})	5% (Ni^{2+})	10% (Co^{2+})	10% (Ni^{2+})
3% Eucerin	-	-	0,025900	0,006164	0,00105	0,003582
3% Hascobase	-	-	0,011250	0,005294	0,00359	0,002582
5% Eucerin	0,02590	0,006164	-	-	0,00692	0,017459
5% Hascobase	0,011250	0,005294	-	-	0,00489	0,014582
10% Eucerin	0,00105	0,003582	0,00692	0,017459		-
10% Hascobase	0,00359	0,002582	0,00489	0,014582		-

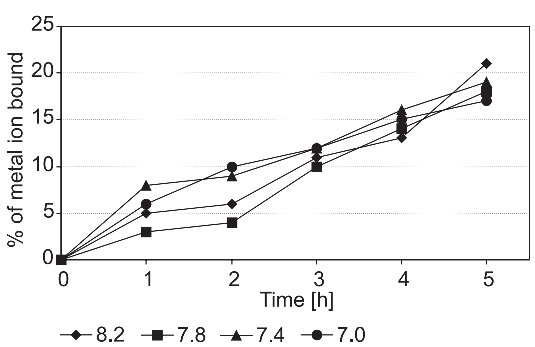

Figure 1. The effect of buffer pH on the chelation ability of Co^{2+} by the protective ointment with hascobase.

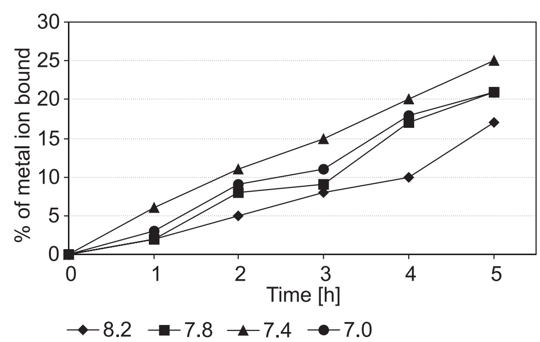

Figure 3. The effect of buffer pH on the chelation ability of Ni^{2+} by the protective ointment with eucerin.

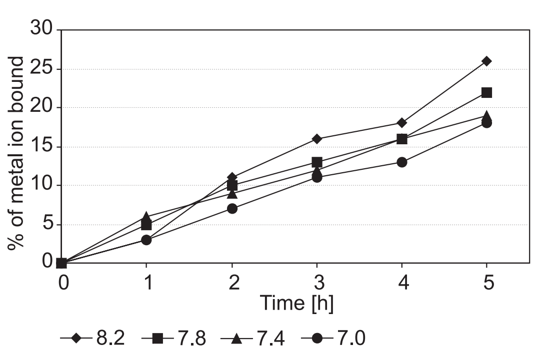

Figure 2. The effect of buffer pH on the chelation ability of Co^{2+} by the protective ointment with eucerin.

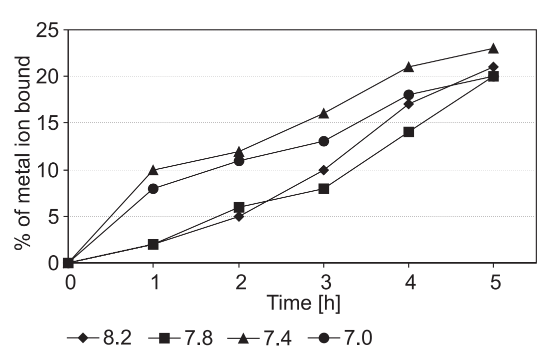

Figure 4. The effect of buffer pH on the chelation ability of Ni^{2+} by the protective ointment with hascobase.

Na_2H_2EDTA is reversible and is linked with hydrogen ion liberation according to the reaction: $M(H_2O)_x^{n+} + H_2EDTA^{2-} \leftrightarrow MEDTA^{n-4} + 2\ H_3O^+ + (x-2)\ H_2O$. The presence of the buffer ensures the binding of H_3O^+ and displacement of the reaction equilibrium to the right direction (3, 8).

In the present study the greatest capacity for Co^{2+} detoxification has been seen with the protective ointment containing hascobase and the buffer of pH = 7.0 or pH = 7.4. The remaining buffers revealed a lesser degree of Co^{2+} binding after the first and fifth hour of the test. The same pH values of buffer (7.0 and 7.4) were optimal for Ni^{2+} binding by the ointments emulgated in either eucerin or hascobase. These pH values secure the good stability of Co^{2+} and Ni^{2+} complexes with Na_2H_2EDTA (pβ1 Ni(EDTA) = 18.62, pβ1 Co(EDTA) = 16.31). In addition, Na_2H_2EDTA should form complexes with Co^{2+} and Ni^{2+} which are more stable than the metal protein conjugate antigen formed in epidermis and have more rapid complexation reaction kinetics with these metal ions, compared to the skin protein–metal ion complexation reaction that leads to the formation of metal-protein conjugate antigen (3). The eucerin-based ointments with the buffer of pH = 7.4 or pH = 7.8 had the greatest capacity for Co^{2+} binding. Moreover, the topical preparations of protective ointments with a higher pH value may run a risk of allergy reaction. The chelating agents should be dispersed in a vehicle that is suitable for cutaneous application and has a pH that favors ion metals complexation. The pH ranges of the epidermis and dermis are 4.2-6.5 and 7.2-7.3, respectively, so the conditions of the examined ointments were favorable for complexation reaction (9).

The utilization of barrier creams with Na_2H_2EDTA is an appropriate solution for the patients sensitized to metals who cannot thoroughly avoid allergen contact or who cannot wear protective gloves. Because the significant amounts of some metal ions (especially Ni^{2+}) remain bound to the epidermis after their contact with the skin, it is recommended to wash hands immediately after this contact (3). The effect can be improved by the use of cleaning solutions containing the chelating agent such as Na_2H_2EDTA or histidine. However, the protective creams with Na_2H_2EDTA can only give protection to Ni^{2+} or Co^{2+} sensitized individuals but they remain not useful among chromium sensitized individuals. Chromium is the only trivalent ion among metal allergens which forms the stable complex with Na_2H_2EDTA at very low pH (1-2) (10). The application of protective creams with Na_2H_2EDTA can, however, be limited as indicated by the latest reports (11) about Na_2H_2EDTA sensitized patients. The general rule is that water in oil (w/o) emulsions are effective against aqueous solution of irritant agents and oil in water (o/w) emulsions are effective against lipophilic materials. In this study no statistical differences between the protective ability of ointments containing hascobase or eucerin as a vehicle have been established. It should be noticed that the vehicle alone of barrier creams cannot have a positive effect on the skin status and the reduction of allergy reaction (12). Based on the presented results it can be stated that Na_2H_2EDTA was an active component of barrier creams prepared in the experiment. In conclusion, it is clear that Na_2H_2EDTA – hascobase formula should be choosen as more optimal due to its higher galenic stability in comparison to Na_2H_2EDTA – eucerin formula.

REFERENCES

1. Gawkrodger D., Healy J., Howe M.: Contact Dermatitis 32, 257 (1995).
2. Cohen D., Heidary N.: Dermatol. Ther. 17, 334 (2004).
3. Wohrl S., Kriechbaumer N., Hemmer W., Focke M., Brannath W., Gotz M., Jarisch R.: Contact Dermatitis 44, 224 (2001).
4. Zhai H., Maibach H.: J. Cosmet. Dermatol. 1, 20 (2002).
5. Basketter D., Dooms-Goossens A., Karlberg A., Leppoittevin J.: Contact Dermatitis 32, 65 (1995).
6. Kutting B., Drexler H.: Int. Arch. Occup. Environ. Health 76, 253 (2003).
7. Kresken J., Klotz A.: Int. Arch. Occup. Environ. Health 76, 355 (2003).
8. Drexler H.: Int. Arch. Occup. Environ. Health 76, 359 (2003).
9. Cavani A.: Toxicology 209, 119 (2005).
10. Saary J., Qureshi R., Palda V., DeKoven J., Pratt M., Skotnicki-Grant S., Holness L.: J. Am. Acad. Dermatol. 53, 845 (2005).
11. Bernedo N., Audicana M., Uriel O., Velasco M., Gastaminza G., Fernández E., Munoz.: D. Contact Dermatitis 50, 252 (2004).
12. Berndt U., Wigger-Alberti W., Gabard B., Elsner P.: Int. Arch. Occup. Environ. Health 76, 359 (2003).