Saúde
(SANTA MARIA)

Case Report: Cross-reaction of phencyclidine in immunochromatographic tests for rapid drug detection using in silico analysis for investigation

Relato de Caso: Reação cruzada de fenciclidina em testes imunocromatográficos para detecção rápida de drogas utilizando análise in silico para investigação

Marcelo Henrique Nascimento Santana, Leonardo Corrêa Cardoso, Rachel Santos, Miguel Roehrs, Sarah Carobini Werner de Souza Eller Franco de Oliveira, Tiago Franco de Oliveira, André Valle de Bairros

Abstract:

Phencyclidine (PCP) is a dissociative drug previously used as an anesthetic agent but caused hallucinations due to its neurotoxic effects. A 49-year-old male farmer attempted suicide at home due to a massive intake of prescription drugs. Toxicological analysis was performed with an immunochromatography test and detected PCP (an uncommon drug in Brazil). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) and in silico models were employed to elucidate this case. Despite the presence of midazolam, fentanyl and lidocaine in urine and serum samples by LC-MS/MS, these drugs and respective metabolites were not able to promote a cross-reaction with PCP as demonstrated by in silico models as well as our practical experience. In fact, doxepin, trimipramine and promethazine and their respective metabolites showed a high correlation interaction in the binding site of 2PCP, especially promethazine. Predicted results obtained on docking show a high correlation interaction between PCP and promethazine, indicating a possible cross-reaction in the immunochromatography test.

Keywords: Immunochromatography, Case report, Phencyclidine, In silico analysis, Cross-reaction.

Resumo:

A fenciclidina (PCP) é uma droga dissociativa usada anteriormente como agente anestésico, mas causava alucinações devido aos seus efeitos neurotóxicos. Um agricultor de 49 anos tentou suicídio em casa devido a uma ingestão maciça de medicamentos prescritos. A análise toxicológica foi realizada com um teste de imunocromatografia e detectou PCP (uma droga incomum no Brasil). Cromatografia líquida-espectrometria de massa tandem (LC-MS/MS) e modelos in silico foram empregados para elucidar este caso. Apesar da presença de midazolam, fentanil e lidocaína em amostras de urina e soro por LC-MS/MS, essas drogas e seus respectivos metabólitos não foram capazes de promover reação cruzada com o PCP, conforme demonstrado por modelos in silico e também por nossa experiência prática. De fato, doxepina, trimipramina e prometazina e seus respectivos metabólitos apresentaram interação de alta correlação no sítio de ligação do 2PCP, especialmente prometazina. Os resultados previstos obtidos no docking mostram uma interação de alta correlação entre PCP e prometazina, indicando uma possível reação cruzada no teste de imunocromatografia.

Palavras-chave: Imunocromatografia, Relato de caso, Fenciclidina, Análise in silico, Reação cruzada

Como citar este artigo: SANTANA, M. H. N.; CARDOSO, L. C.; SANTOS, R.; ROEHRS, M; OLIVEIRA, S. C. W. S. E. F.; OLIVEIRA, T. F.; BAIRROS, A. V. Case Report: cross-reaction of phencyclidine in immunochromatographic tests for rapid drug detection using in silico analysis for investigation. Revista Saúde (Sta. Maria). 2023; 49.

Autor correspondente:
Nome: Leonardo Corrêa Cardoso
E-mail: leonardo.cardoso@acad.ufsm.br
Formação: Farmacêutico e Mestre em Ciências Farmacêuticas
Filiação: Universidade Federal de Santa Maria (UFSM)

Endereço: Rua Silva Jardim, 332 - 97010-491 - Santa Maria, Rio Grande do Sul.

Data de Submissão: 20/04/2023
Data de aceite: 31/08/2023

Conflito de Interesse: Não há conflito de interesse

DOI: 10.5902/2236583475385

Universidade Federal de Santa Maria 1960
CCS CENTRO DE CIÊNCIAS DA SAÚDE UFSM

INTRODUCTION

Phencyclidine (PCP) is a dissociative drug formerly used as an anesthetic agent. Its use causes hallucinations, and its medical use was suspended in the 1960s[1]. We reported a cross-reaction of PCP in the immunochromatographic test for screening drug detection during an investigation of a patient's suicide attempt. In recent decades, some cases of false-positive results for phencyclidine and other drugs in immunochromatographic assays have been reported[2,3]. However, none of the reports of these cross-reactions were used *in silico* analysis for their investigations.

CASE REPORT

A 49-year-old farmer was admitted to the University Hospital of Santa Maria (HUSM) with a sharp force laceration in the cervical region and mental confusion, for which the medical team requested a toxicological analysis. The patient's urine and serum samples were tested by immunochromatography screening for 12 drugs (Abon Biopharm™) which serum sample demonstrated reagents for tricyclic antidepressants (TCAs) while benzodiazepines, TCAs and PCP were reagents in the urine sample. The presence of PCP, an unusual illicit drug in Brazil, required specific analyses employing liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and two distinct methodologies[4,5] were used to confirm PCP in these samples. The methods did not detect PCP in the patient's samples. However, it was detected the presence of midazolam, fentanyl, and lidocaine in both samples by LC-MS/MS. So, *in silico* studies were performed to analyze a possible cross-reaction in the immunochromatographic test. The iGemDOCK™ software was used for the docking analysis to predict the main pharmacological interactions such as hydrogen bonds (H-bonds), Van Der Waals and electrostatics binding energies between active sites and the respective molecules. For the docking analysis study, it was employed binding site 1PC of the antibody FAB model complexed with PCP (PDB ID: **2PCP**) according to the structure code from the software. Docking accuracy settings and genetic algorithm parameters (GA Parameter) were set at 200, 70, and 10 for population size, generations, and the number of solutions, respectively. In addition, it was calculated hydrophobic and electrostatic set score functions at 1:1[6]. Considering the immunochroma-

tographic screening, LC-MS/MS results and probable drugs ingested by the patient and respective metabolites, *in silico* evaluation was applied for clomipramine, desmethylclomipramine, desmethylmaprotiline, desmethyltrimipramine, doxepin, fentanyl, lidocaine, maprotiline, monoethylglycinexylidide (MEGX), midazolam, nordoxepin, norfentanil, 4-hydroxy-midazolam (4-OH-midazolam), promethazine, promethazine sulfoxide Dextromethorphan, Diphenhydramine, Desvenlafaxine, Tramadol, Lamotrigine and trimipramine compared to PCP and 4-hydroxy-phencyclidine (4-OH-PCP). These molecules have the 3D structure geometries that were optimized in ORCA™ 5.0[7], using def2-SVP basis, DFT method and B3LYP data. BIOVIA Discovery Studio®[8] was used to visualize 3D interactions between molecules and binding sites (Figure 1-C).

DISCUSSION

The result of the statistical calculations found in the *in-silico* evaluation is converted by the software into a dendrogram format. Dendrogram (Figure 1-B) graphically shows the interactions similarity between our target molecules in groups based on his correlations and amino acid residues of the binding site 1PC from **2PCP** structure by consensus (term used where the data from the samples are statistically chosen). So, this approach allows the visualization of the clusters and thus informs which molecules are closest to the PCP. Our study is based on residues that showed some binding and that are common to all molecules where all of them showed some binding, and which are common to all suspect molecules. Using a color scale from green to red for negative and positive energy values respectively, light colors represent the high values and black for zero. After docking analysis, it was observed a high correlation between PCP, promethazine, doxepin and nordoxepin (Cluster 1) interactions with the most important amino acid residues of the **2PCP** bidding site[9]. In cluster 2, the Tramadol and its metabolite showed a correlation with the 4-OH-PCP, considering this correlation with Phencyclidine metabolite, the Tramadol was tested in the immunoassay. Midazolam has a considerable correlation and is closer to Cluster 1. However, midazolam as well as fentanyl does not cross-react with said immunoassay according to the immunochromatographic test data sheet (Abon Biopharm™). Also, midazolam and fentanyl are common drugs utilized in various medical protocols[10]

and it was applied to the patient as soon as he was admitted to the HUSM. In addition, we observed that these drugs do not promote the aforementioned cross-reaction with PCP based on our laboratory tests and practical experience in toxicological analysis for hospital purposes. In 3D molecule design, it is possible to visualize the pharmacophore groups of ligands interacting with amino acid residues in the binding site.

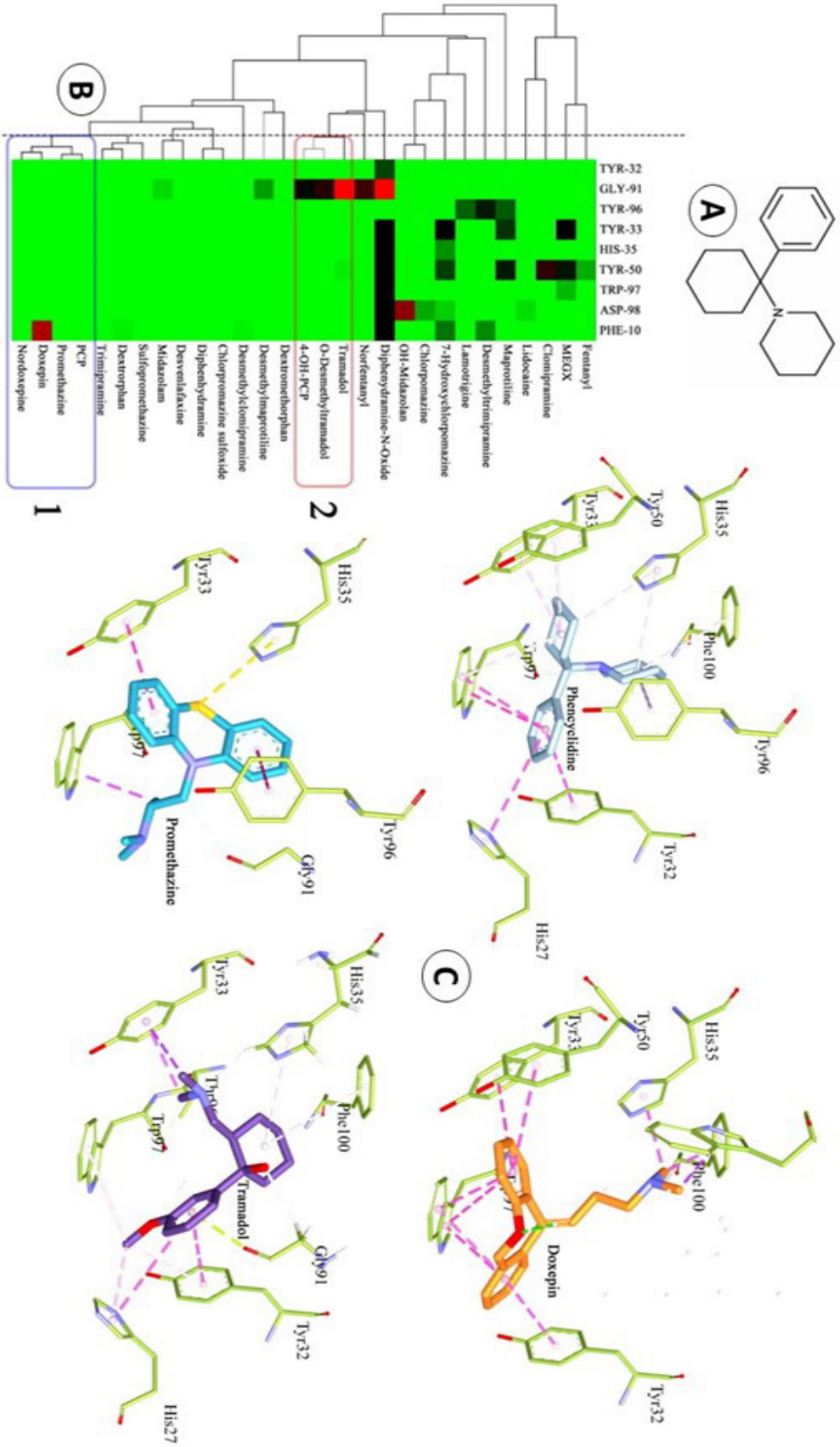

Figure 1. General scheme for *in silico* analysis. **A)** Chemical structure of PCP. **B)** Dendrogram. The dashed line defines the 2 main clusters closer to PCP and 4-OH-PCP according to amino acid residues 2PCP and 1PC binding site. Cluster 1, PCP, Promethazine, Doxepin and Nordoxepin. Cluster 2, Tramadol, O-Desmethyltramadol and 4-OH-PCP. **C)** 3D molecule design and respective interactions between the molecules and the active site (1PC). All images are projected at the same angle for visualization. Dashed lines indicate these interactions. Yellow, pink and red interaction indicates pi-sulfuric, pi-pi and carbon-hydrogen bonds, respectively.

Immunochromatographic screening from Abon Biopharm™ can detect doxepin, trimipramine and their metabolites as TCAs, however, LC-MS/MS methodologies did not detect these analytes as well as promethazine and its metabolites. Incidentally, these molecules showed a high binding capacity at the PCP site of the immunoassay available according to *in silico* evaluation.

Nevertheless, the limitation of the *in silico* model employed in the study is the inability to assess whether the molecule concentrations may have some influence on the cross-reaction to PCP as well as the possible interaction of these molecules together in the same active site. To verify the results obtained in the *in silico* studies, it is necessary to test the possible molecules that interact with the antibody in the immunoassay obtained in the docking process. It is worth mentioning that the docking was performed using a crystallographic model of FAB antibody in a single software, so future studies are needed using more *in silico* methodologies for a more accurate statistical evaluation of possible ligands. Furthermore, the *in silico* study was realized using the major suspected compounds and their metabolites. The closest compounds to PCP in the dendrogram were tested in immunoassay, and all of them are negative. It is possible that the molecule responsible for the positive result comes from an *in vivo* reaction in the patient and/or a molecule that has not been tested in the immunoassay.

CONCLUSIONS

The predicted results obtained on the docking analysis study show us high a correlation interaction of tramadol, o-desmethyltramadol, promethazine, doxepin and nordoxepin in the 1PC biding site. However, these molecules do not have a positive result in the immunoassay test. Considering these results, more studies are necessary for knowledge of the positive sample for PCP in the immunoassay.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of the Coordination for the Improvement of Higher Education Personnel (CAPES) and the Research Incentive Fund of the Health Sciences Center (FIPE/CCS).

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