# Methotrexate: A Patient Case Related to Risk of Concurrent Methotrexate and Trimethoprim Use on Dihydrofolate Reductase Binding

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## Abstract

Methotrexate is often used for the treatment of cancer, but it is also being used at low doses to treat rheumatoid arthritis. It has been thought that by preventing synthesis of purines and pyrimidines needed for DNA and RNA synthesis through the inhibition of dihydrofolate reductase, proliferation of quickly dividing lymphocytes and other cells that cause synovial inflammation can be prevented. Thus, patients could receive relief from symptoms of rheumatoid arthritis.[1] However, when using methotrexate in rheumatoid arthritis patients, it is important to be aware that methotrexate interacts with many other medications.

## Introduction

A patient came into the pharmacy to pick up a prescription for sulfamethoxazole/trimethoprim (Bactrim) for the treatment of a urinary tract infection. The pharmacist noted that this patient was also taking methotrexate for the treatment of rheumatoid arthritis. Both methotrexate and trimethoprim inhibit the enzyme dihydrofolate reductase at the same site, so problems with increased toxicity levels can occur if these two drugs are administered together.

This project investigates the molecular binding of methotrexate and trimethoprim to dihydrofolate reductase and discusses the mechanism of the toxicity associated with coadministration of the two drugs.

Dihydrofolate reductase, pictured in Figure 1, reduces dihydrofolic acid to tetrahydrofolic acid. Tetrahydrofolic acid is a coenzyme that is important for many reactions, especially in the metabolism of amino acids and nucleic acids.[2] Methotrexate and trimethoprim are both folate analogues that bind to and inhibit dihydrofolate reductase resulting in a decreased concentration of reduced folate cofactors. Figure 2 shows the region of methotrexate that binds to dihydrofolate reductase. These reduced folate cofactors are essential for the synthesis of nucleic acids..Therefore, coadministering methotrexate and trimethoprim results in an additive effect of inhibiting dihydrofolate reductase that can present serious adverse effects including bone marrow suppression and pancytopenia which is a deficiency of all blood cells.[3]

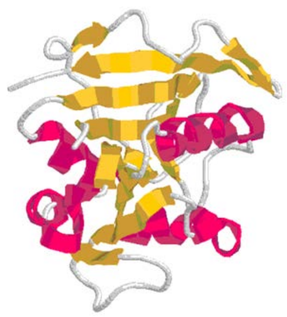

*Figure 1.* Dihydrofolate reductase enzyme structure.[4]

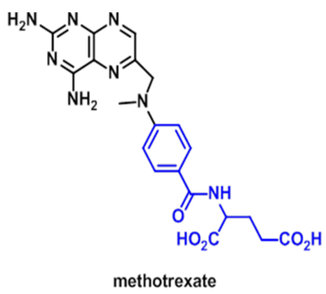


*Figure 2.* Structure of methotrexate; region in blue binds to dihydrofolate reductase.[5]

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## Molecular Story

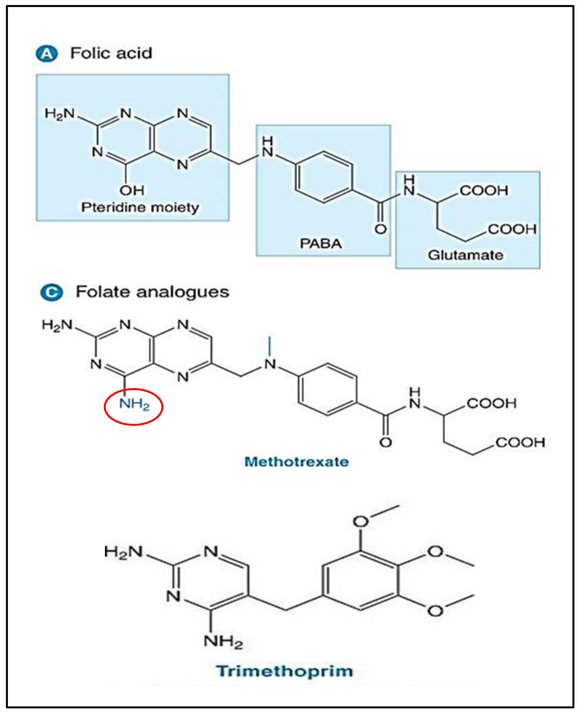
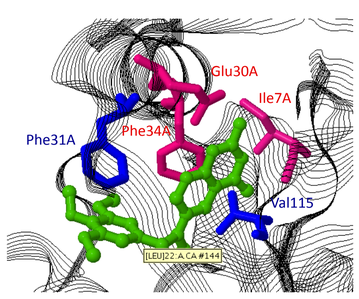



*Figure 3.* Comparison of structures of folic acid, methotrexate, and trimethoprim.[3]

As shown in Figure 3, both methotrexate and folic acid consist of a pteridine moiety, PABA, and glutamic acid. The main difference is that methotrexate has a 2,4-diaminopyrimidine unit, resulting from replacing the OH group with an amino group at the C-4 site.[3]



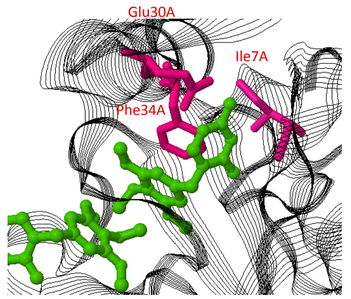




*Figure 4.* Active site of dihydrofolate reductase with bound drug (green). Left: methotrexate (1MVT.pdb); Right: trimethoprim (3S3v.pdb).

Both methotrexate and trimethoprim interact with the same amino acids (Figure 4; shown in pink).

- Glu30A via
  - -Hydrogen bond interactions
- Ile 7A as a
  - -Backbone contact
- Phe 34A via
  - -Pi-Pi interactions

Methotrexate also interacts with two additional amino acids (shown in blue).

- Phe 31A via
  - -Pi-Pi interactions
- Val 115 as a
  - -Backbone contact

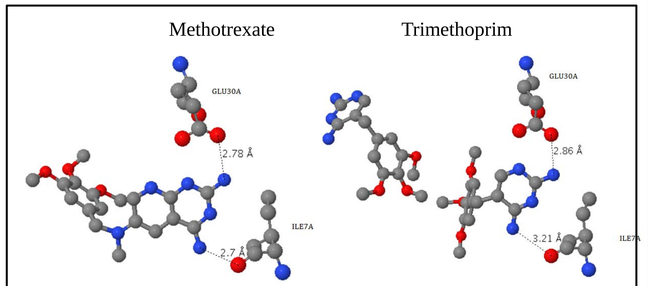



*Figure 5.* Methotrexate on the left and trimethoprim on the right. Blue are nitrogen, red are oxygen, and grey are carbons. Bond lengths between the drugs and dihydrofolate reductase show that methotrexate binds tighter to the receptor. (pdb files 1MVT and 3S3V, respectively)

## Future Work

Coadministration of methotrexate and trimethoprim results in an additive effect of inhibiting dihydrofolate reductase that can lead to serious adverse effects. Therefore, clinically, it is recommended to avoid administering these two medications together.

Research has been done to increase trimethoprim's affinity for bacterial dihydrofolate reductase to avoid drug-drug interactions at the human dihydrofolate reductase and reduce side effects.Trimethoprim's affinity for bacterial dihydrofolate reductase is currently several thousand times greater than its affinity for human dihydrofolate reductase.[6]

Therefore, there is not much that can be done structurally to improve trimethoprim without reducing its therapeutic effect. However, it might be beneficial to research the possibility of redesigning the structure of methotrexate to allow for tighter binding to human dihydrofolate reductase.

## Summary

Methotrexate inhibits dihydrofolate reductase which results in decreased concentrations of reduced folate cofactors. This slows growth of rapidly dividing cells in malignant tissues and reduced proliferation of other cells that cause synovial inflammation. Although methotrexate works well in treating cancer and rheumatoid arthritis, toxicity remains a concern due to drug-drug interactions and additive binding effects with other concomitant therapies.

## References

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