Analyzing selected FDA approved drugs for effects on template switch mutagenesis in *E. coli*

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Abstract

Quasipalindromes (QPs) are DNA sequences that are imperfectly mirrored, known to form secondary structures like hairpins and cruciforms. These sites have been linked with a specific type of mutation called template-switch mutation (TSM). Certain drugs like 5-Azacytidine, Azidothymidine (AZT), and Ciprofloxacin are known to induce TSM. This study aims to assess the impact of five FDA-approved drugs—three antitumor drugs (CPT-11, Doxorubicin hydrochloride, and Gemcitabine hydrochloride) and two anti-inflammatories (Ibuprofen and Dexamethasone)—on template-switch mutagenesis. Studying FDA-approved drugs for their impact on TSM is important, as they are associated with diseases like hereditary angioneurotic edema, osteogenesis imperfecta, and biotinidase deficiency. The findings reveal no statistically significant effects on frequency of mutations after treatment with Gemcitabine hydrochloride, Ibuprofen, Dexamethasone, or Doxorubicin hydrochloride. However, CPT-11 treatment showed a notable decrease in TSM, suggesting a potential role in disrupting the template-switching process.

Keywords: quasi-palindrome; mutagenesis; DNA replication inhibitors; DNA repair

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Introduction

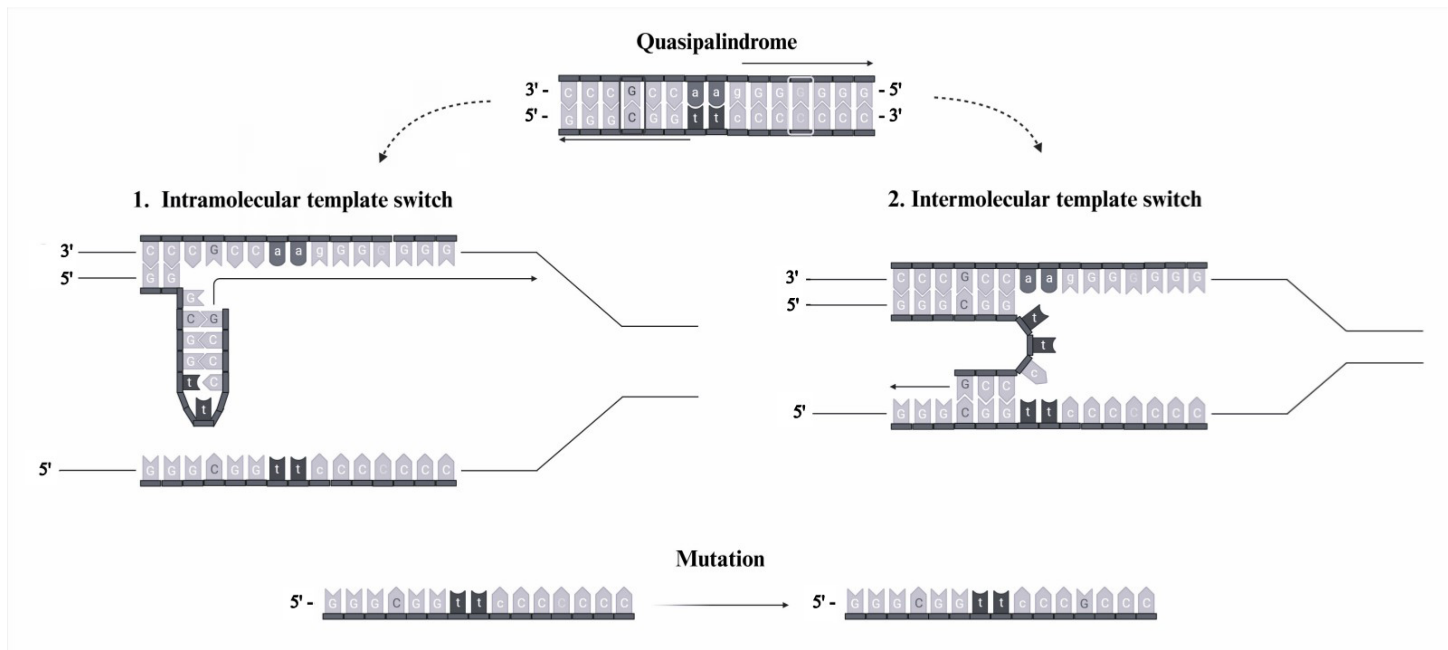
DNA mutations, changes that occur during DNA replication, can induce genetic instability. Among various mutation classes, this study investigates mutations caused by DNA secondary structures able to form quasi palindromes (QPs). QPs are imperfect inverted repeats of DNA that can give rise to hairpins and cruciform structures. DNA replication and gene expression are fundamental processes shared by all living organisms, including human cells. *E. coli* have simpler systems than human cells, making them ideal for preliminary studies. Their ease of genetic manipulation and rapid growth in the lab further enhance their utility.

The *thyA* hotspot in *E. coli* was the first natural QP hotspot studied for mutagenesis (1). These structures have been correlated with a specific type of mutation referred to as a template-switch mutation (TSM). TSMs are associated with several human diseases as reviewed (2), including hereditary angioneurotic edema (3), osteogenesis imperfecta (4), and biotinidase deficiency (5). Lynn Ripley was first to propose the two mechanisms for QP-induced TSM, intramolecular and intermolecular (Figure 1), both of which can involve a modification of the quasi-palindromic region into a perfected palindrome proposed to be after a momentary fork stalling event. (6)

While TSM occurs on both leading and lagging strands during DNA replication, the leading strand exhibits a bias towards TSM in both reversion (7) and TSM-specific assays (8). This bias is nullified in the absence of exonucleases (ExoI and ExoVII) (8), which are known to prevent TSM (1, 9) with ExoI playing the larger role (1). This suggests that both strands can undergo mutations,

Figure 1.

Quasi-palindrome Template-Switch Mutation Mechanisms



Two template-switch mutation models involving a temporary displacement of the nascent strand and relocation of the polymerase onto another strand different from the original template. In the intramolecular model, the nascent strand becomes the template, in the intermolecular model, the homologous strand becomes the template. Both mechanisms created a situation that can perfect an imperfect palindrome. (Created in https://BioRender.com)

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but the lagging strand is more adept at averting and correcting them (8). Additionally, research has demonstrated that exposure of QP sites to chemicals can influence the rate of TSM (10, 11). Azidothymidine (AZT), a thymidine analogue used as an antiretroviral medication to prevent and treat HIV/AIDS, has been seen to increase TSM rates (11). Other drugs found to increase rates of TSM include 5-Azacytidine (5-azaC), the antibiotic ciprofloxacin, and formaldehyde (10). 5-azaC interacts with cytosine methyltransferase (12) to cause DNA/protein crosslinks that stall replication (13), the antibiotic ciprofloxacin is a topoisomerase type II inhibitor, like DNA gyrase (14) and the chemical formaldehyde acts as a general mutagen, including creating DNA/Protein crosslinks in human cells (15). This study focuses on testing additional FDA-approved drugs, described below, for their impact on TSM frequency in *E. coli*.

A mutational reporter in the *lacZ* gene of *E. coli* has been used to identify the rate of quasi-palindrome mutations after exposure to various FDA-approved drugs. These mutations become apparent when *lacZ* alleles revert to Lac+, a phenomenon observed only if a template-switch event occurs. Additionally, since most studies on the influence of drug exposure on TSM have also used this mutational reporter in *E. coli*, continuing to use this system allows for consistent and fair comparisons across research.

All the FDA-approved therapeutics selected have at least one mechanism of action capable of causing replication fork stalling, a necessary condition for both intra- and intermolecular TSM. It is expected that these drugs are either inhibiting a process during DNA replication or disrupting DNA through intercalation and will lead to an increase in TSM frequency.

Gemcitabine Hydrochloride (dFdC)

Gemcitabine hydrochloride (dFdC) is an FDA-approved drug used in cancer treatment. Once inside cells, deoxycytidine kinase (dCK) phosphorylates dFdC, and subsequent phosphorylation steps lead to its active forms, dFdCDP and dFdTP. dFdCDP inhibits ribonucleotide reductase, reducing the pool of deoxynucleotides, mostly dCTPs, and enhancing the concentration of dFdCDP. This self-potentiation mechanism promotes masked chain termination that inhibits DNA synthesis and causes a fork stalling event (16). Although there are no previous studies focusing on gemcitabine's chain termination effects on TSM, studies conducted with the AZT chain terminator drug led to the hypothesis that gemcitabine might similarly be able to enhance TSM frequency (11).

Ibuprofen

Ibuprofen is an FDA-approved drug that treats inflammation and is used mostly to treat mild forms of pain and reduce fevers. A previous study found that the reactive oxygen species (ROS) generated by ibuprofen caused DNA strand scission and could cause DNA degradation (17). Furthermore, ibuprofen was identified to have intercalative binding with the DNA helix. This is where structures resembling base pairs will be stationed in the double helix rather than the appropriate base pairs and thereby distort the structure. In this sense, DNA replication is prevented from operating regularly. We have chosen to test ibuprofen due to the potential role it has in genetic instability.

Dexamethasone

Dexamethasone is an FDA-approved hormonal corticosteroid commonly prescribed to treat inflammation and swelling. Dexamethasone is comprised of multiple aromatics and hydroxy, methyl, and oxo groups, making it similar in structure to other corticosteroids such as hydrocortisone and prednisolone. Further studies examining the interaction between the dexamethasone-glucocorticoid receptor complex, and DNA have suggested the potential for dexamethasone to behave as an intercalating agent (18), giving it the potential to cause template switch mutations.

Doxorubicin Hydrochloride

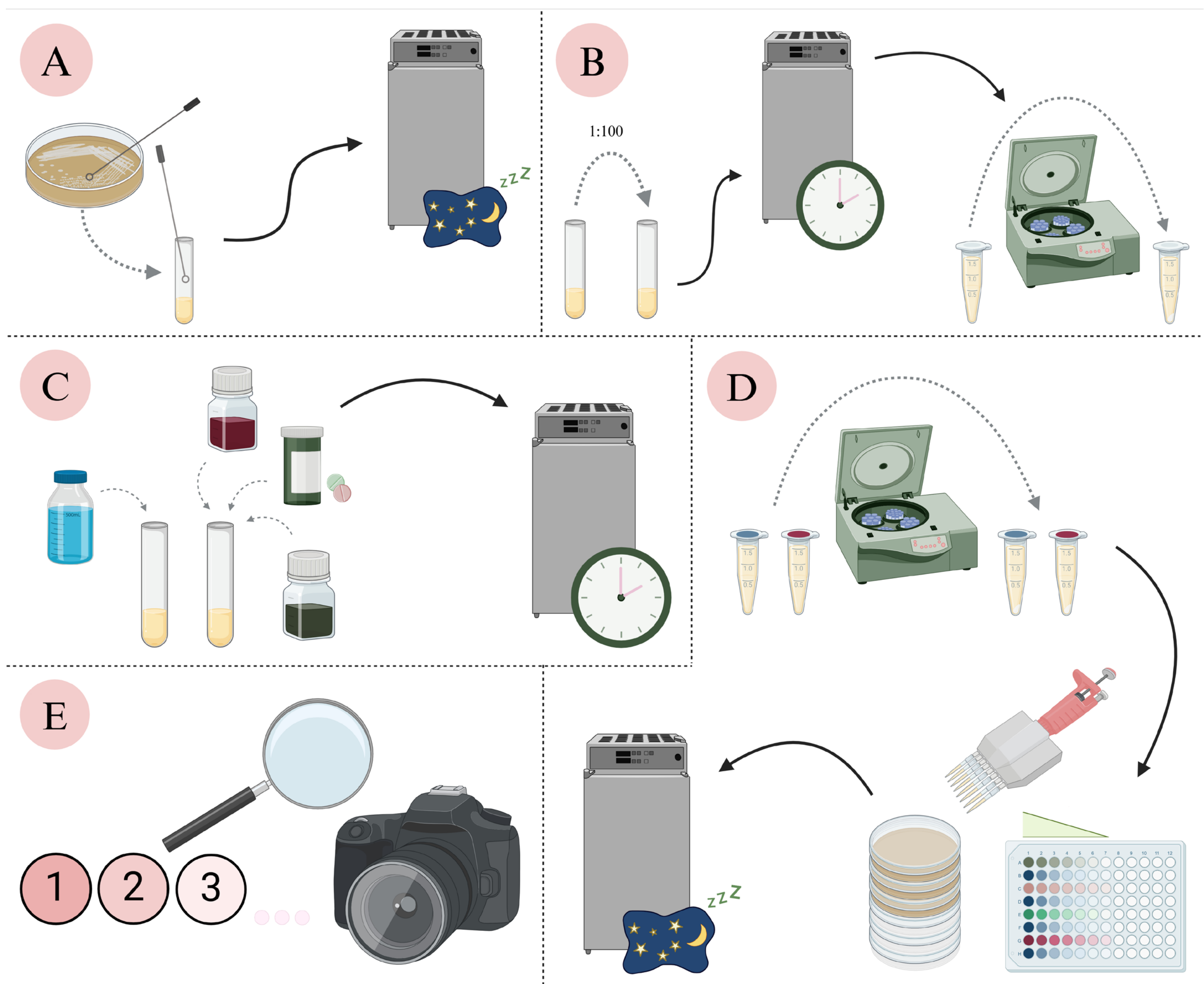
Doxorubicin hydrochloride (Doxo) is a chemical capable of inhibiting topoisomerase II (19), generating reactive oxygen species (20), and intercalating DNA (21). Here we evaluate the potential role of Doxo in TSM due to inhibiting topoisomerases and Doxo mediated DNA-protein crosslinks, similarly to how 5-azaC and ciprofloxacin have been shown to promote TSM by stalling replication (10) implicating the Dcm-DNA covalent complex trapped by 5-azaC as the initiator for mutagenesis. The leading strand of replication is more mutable than the lagging strand, which can be explained by blocks to the replicative helicase and/or fork regression. We find that template-switch mutagenesis induced by 5-azaC does not require double strand break repair via RecABCD; the ability to induce the SOS response is anti-mutagenic. Mutants in recB, but not recA, exhibit high constitutive rates of template-switching, and we suggest that RecBCD-mediated DNA degradation prevents template-switching associated with fork regression. A mutation in the DnaB fork helicase also promotes high levels of template-switching. We also find that other DPC-inducers, formaldehyde (a non-specific crosslinker.

Irinotecan Hydrochloride (CPT-11)

Irinotecan hydrochloride (CPT-11), derived from camptothecin, is a chemotherapeutic drug that inhibits topoisomerase I (22, 23). The inhibition of topoisomerase I by CPT-11 results in the formation of highly cytotoxic topoisomerase I-DNA cleavage complexes, importantly triggering various cellular responses, including protein-linked DNA breaks [23] and replication fork arrest[24]. Due to the similarities of properties with other TSM causing drugs, we have included the evaluation of CPT-11.

Figure 2.

Mutational Assay Methods



A. Single colonies were selected, inoculated, and cultured overnight. B. Cultures were diluted and incubated for 2 hours before pelleting *via* centrifugation. C. Cultures were divided into treated and control tubes before adding drug or control at the provided concentrations. Cultures were then incubated for 2 hours. D. Cultures were pelleted *via* centrifugation and the cell pellets were washed before resuspending the cells and performing serial dilutions. Dilutions were plated on LB and minimum lactose plates and incubated overnight. E. Total colony forming units (CFUs)/mL were determined and percentage of reverting CFUs were calculated. (Created in https://BioRender.com)

Methods

Bacterial Strains, Growth Conditions, and Media

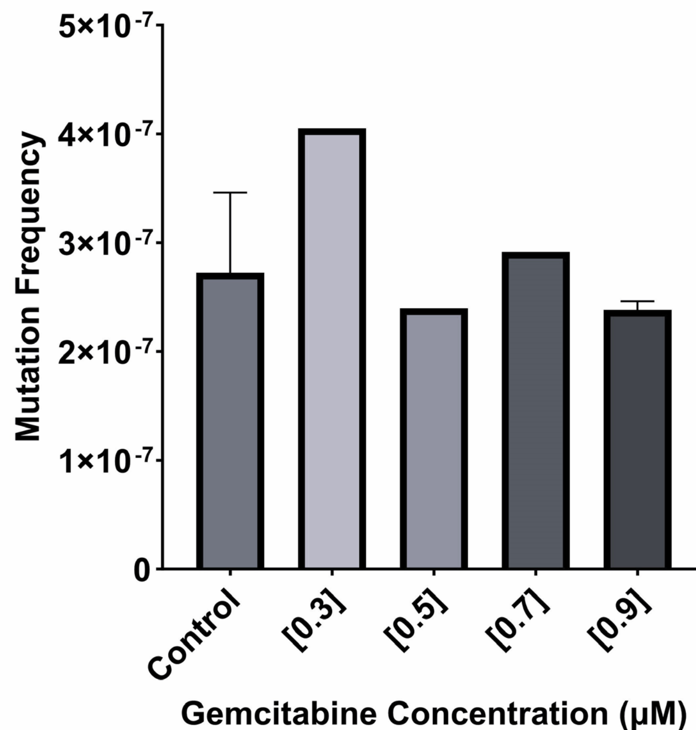
A *lacZ* mutational reporter strain of *Escherichia coli,* QP5, that reverts to Lac+ when a 4 base pair deletion occurs due to a template switch mutation was acquired from Dr. Susan T. Lovett (used in Laranjo et al. 2017) [25]. Standard growth medium was Luria Broth (LB) medium, comprised of 1% Bacto-tryptone, 0.5% yeast extract, and 0.5% sodium chloride (Fisher Scientific, Waltham, MA, USA) and minimum lactose solid medium, comprised of 1x M9 salts, 0.2% lactose, 1mM Magnesium Sulfate, and 0.1mM Calcium Chloride (Alpha Teknova, Hollister, CA, USA). Plates were also comprised of 1.5% Bacto-agar.

Mutational Assay

Single colonies were inoculated and cultured in 1.5 mL of Luria Broth (LB) medium and grown overnight at 37°C with aeration (Figure 2A). The culture underwent a 1:100 dilution in fresh 1.5 mL LB medium, followed by a 2-hour incubation period at 37°C with aeration. Following incubation, the entire culture was pelleted by centrifugation at approximately 7000 rcf for two minutes so that the latent medium could be removed, and the pellet was resuspended in 150 µL of fresh LB (Figure 2B). The resuspended culture was divided evenly (75 µL in each) among two tubes followed

Figure 3.

Mutation frequency of Gemcitabine at varying concentrations



Gemcitabine was tested at 0.3 (n=1), 0.5 (n=1), 0.7 (n=1), and 0.9 µM (n=2) and mutation frequency were calculated. The fold increase of the samples was 0.67, 1.14, 0.93, and 1.14 respectively compared to the control showing no statistical difference. Error bars represent 95% CI based on the average mutation frequency.

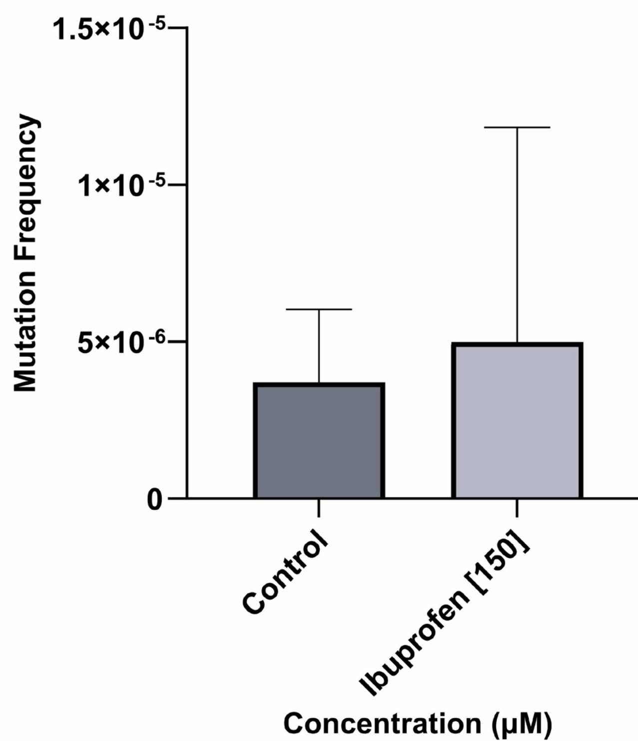
by an addition of 1.5 mL of fresh LB to each tube (Figure 2C). The specified concentration of the selected drug – CPT-11 (0.5 µM, 1 µM), Dexamethasone (60 µM), Ibuprofen (150 µM), Doxorubicin (0.025 µM, 0.4 µM, 0.5 µM) – was added to one tube while an equal amount of water was added to the other tube. Both tubes were placed back in the incubator for an additional 2 hours to grow at 37°C with aeration. Subsequently, both cultures were pelleted by centrifugation once more and the cell pellet was washed twice with 1 mL LB, followed by resuspension in 200, 400, or 600 µL LB. Cultures were then subject to serial dilutions in a 96-well plate to obtain 10-fold dilutions up to a 1:100,000 dilution, spotted onto LB and minimum lactose plates and grown overnight at 37°C to determine the CFU (colony forming units)/mL (Figure 2D).

Data Collection and Analysis

The percentage of reverting CFUs of the total was calculated for each drug by dividing the number of total CFU/mL on the LAC plates by the number of total CFU/mL on the LB plates (Figure 2E). All corresponding data points were averaged to obtain the average mutation frequency of each drug. Statistical analysis was done using an unpaired t-test that does not assume equal standard deviation among populations (Welch's t-test) using GraphPad Prism version 10.2.0 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com.

Figure 4.

Mutation frequency of Ibuprofen at 150 µM



Ibuprofen demonstrated a fold increase of 0.74 compared to the control (n=3). This was not statistically different than the control group. Error bars represent 95% CI based on the average mutation frequency.

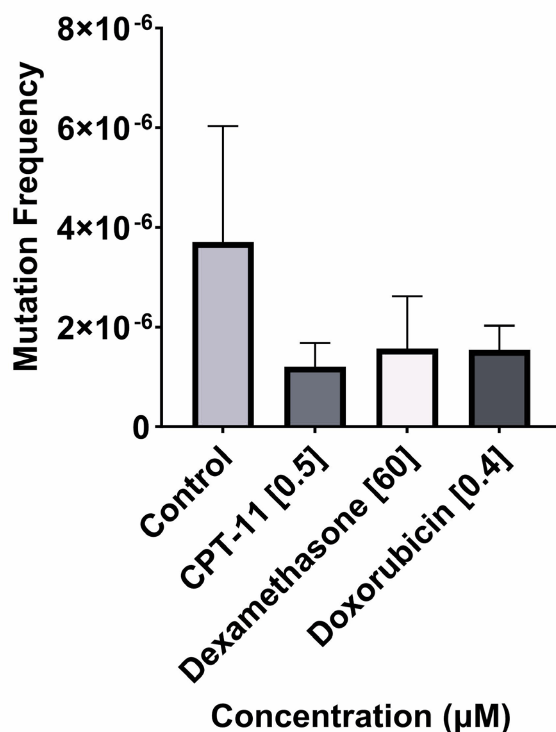
Results

Of the four concentrations of gemcitabine hydrochloride tested (0.3 µM, 0.5 µM, 0.7 µM, and 0.9 µM), the fold change was only between 0.67 and 1.14 (Figure 3) showing no statistical difference from the control group. Similarly, ibuprofen showed a fold change of 0.74 and no statistical difference from the control (Figure 4).

When compared to the control group, dexamethasone resulted in a lower average rate of template-switch mutations, though not statistically different from the control. Dexamethasone was tested at a concentration of 60 µM and resulted in a 2.37-fold change in mutation frequency, with the average rate of mutation 1.56E-06 compared to the control of 3.71E-06 (Figure 5). This was similar to the results of doxorubicin hydrochloride at a concentration of 0.4 µM which had a fold change of 2.41 and an average rate of mutation of 1.54E-06 (Figure 5). Testing done with CPT-11 showed a statistically significant (p=0.0383) change in reported template-switch mutation rate, producing a 3.08-fold decrease when tested at a concentration of 0.5 µM with an average rate of mutation of 1.20E-06 (Figure 5).

Figure 5.

Comparison of mutation frequency of CPT-11, Dexamethasone, and Doxorubicin



CPT-11 was tested at a concentration of 0.5 µM (n=17) and resulted in a statistically significant fold change of 3.08 compared to the control with a p-value of 0.0383. Dexamethasone, at a concentration of 60 µM (n=5), showed a statistically insignificant fold change of 2.37 compared to the control. Doxorubicin was also statistically insignificant, tested at a concentration of 0.4 µM (n=24) and displaying a fold change of 2.41 compared to the control. Error bars represent 95% CI based on the average mutation frequency.

Discussion

This study aims to expand our understanding of which FDA-approved drugs contribute to TSM. It is important to examine how these therapeutics, including some commonly used over-the-counter drugs, may affect mutation frequency, as TSM have been linked to the development of various diseases. There is still a significant gap in understanding TSM, their causes, and the mechanisms behind these mutations.

The expected outcomes for these experimental drugs were that they would either inhibit a process during DNA replication or act as intercalating agents to disrupt DNA, leading to an increase in TSM frequency. However, for most of the drugs tested (Ibuprofen, Dexamethasone, Doxorubicin hydrochloride, and Gemcitabine hydrochloride), no statistically significant change in mutation frequency was observed.

Our preliminary investigation into the effects of gemcitabine hydrochloride on TSM frequency suggests no significant impact on the leading strand. Contrary to the initial hypothesis, due to gemcitabine hydrochloride's chain termination effects during DNA replication, our results from limited trials do not support this notion. Instead, it appears that gemcitabine hydrochloride may have no effect on TSM frequency, aligning with its primary role in inhibiting DNA synthesis rather than causing replication fork stalling [26]. Similarly, although ibuprofen's potential to intercalate within DNA [17] could theoretically stall the replication machinery, our trials did not show a statistical increase in TSM frequency. Ibuprofen, hypothesized to act as an intercalating agent, likely failed to intercalate the DNA or caused excessive damage due to its relatively large size. This could explain the lack of change in mutation frequency, as the DNA either remained unchanged or was too damaged to synthesize properly.

Both dexamethasone and doxorubicin hydrochloride did not result in a statistically significant change in TSM rate when compared to the control group. Dexamethasone and doxorubicin hydrochloride, also predicted to intercalate DNA and stall the replication fork, may have caused enough damage to prevent successful replication, leading to no observed change in TSM frequency, though additional trials may provide further insights. In contrast, CPT-11 showed a statistically significant decrease in TSM frequency. This supports the hypothesis that CPT-11 inhibits topoisomerase I and induces DNA strand breaks. These breaks likely prevent the displacement of the nascent DNA strand and instead promote DNA damage repair through resection and homology search rather than Quasi-Palindrome hairpin formation, thereby reducing polymerase displacement onto the opposite strand. This highlights its potential role in mitigating TSM occurrence. We plan to further explore the potential impact of FDA-approved drugs on TSM using our extensive FDA-approved drug library. This ongoing investigation aims to investigate on the mechanisms underlying TSM, including both natural occurrences and drug-induced cases.

Additional research on the impact of drug exposure on TSM should be conducted in mammalian cells. While this experiment focused on *E. coli* and provided valuable insights, the effects on mammalian cells remain unknown. Testing these drugs in mammalian cells would allow for a more direct evaluation of the conclusions drawn from this study in a more complex system. This approach would also enable observations in cells that are susceptible to TSM-associated diseases. Such findings would be particularly compelling given that the drugs tested here are also used to treat other diseases not directly related to TSM. This could open discussions about the potential consequences of using these therapeutics, especially regarding their broader impact on mutation frequency.

References

1. Viswanathan, M., Lacirignola, J. J., Hurley, R. L., & Lovett, S. T. (2000). A novel mutational hotspot in a natural quasipalindrome in Escherichia coli. *Journal of Molecular Biology, 302*(3), 553–564. https://doi.org/10.1006/jmbi.2000.4088

2. Bissler, J. J. (1998). DNA inverted repeats and human disease. *Frontiers in Bioscience*, *3*, d408-18. https://doi.org/10.2741/a284

3. Bissler, J. J., Donaldson, V. H., & Davis, A. E. (1994). Contiguous deletion and duplication mutations resulting in type 1 hereditary angioneurotic edema. *Human Genetics*, *93*(3), 265–269. https://doi.org/10.1007/BF00212020

4. Bateman, J. F., Lamande, S. R., Dahl, H. H., Chan, D., Mascara, T., & Cole, W. G. (1989). A Frameshift Mutation Results in a Truncated Nonfunctional Carboxyl-terminal Proα1(I) Propeptide of Type I Collagen in Osteogenesis Imperfecta. *Journal of Biological Chemistry*, *264*(19), 10960–10964. https://doi.org/10.1016/S0021-9258(18)60412-0

5. Pomponio, R. J., Narasimhan, V., Reynolds, T. R., Buck, G. A., Povirk, L. F., & Wolf, B. (1996). Deletion/insertion mutation that causes biotinidase deficiency may result from the formation of a quasipalindromic structure. *Human Molecular Genetics*, *5*(10), 1657–1661. https://doi.org/10.1093/hmg/5.10.1657

6. Ripley, L. S. (1982). Model for the participation of quasi-palindromic DNA sequences in frame-shift mutation. *Proceedings of the National Academy of Sciences*, *79*(13), 4128–4132. https://doi.org/10.1073/pnas.79.13.4128

7. Rosche, W. A., Trinh, T. Q., & Sinden, R. R. (1997). Leading strand specific spontaneous mutation corrects a quasipalindrome by an intermolecular strand switch mechanism1. *Journal of Molecular Biology*, *269*(2), 176–187. https://doi.org/10.1006/jmbi.1997.1034

8. Seier, T., Padgett, D. R., Zilberberg, G., Sutera, V. A., Toha, N., & Lovett, S. T. (2011). Insights Into Mutagenesis Using *Escherichia coli* Chromosomal *lacZ* Strains That Enable Detection of a Wide Spectrum of Mutational Events. *Genetics*, *188*(2), 247–262. https://doi.org/10.1534/genetics.111.127746

9. Dutra, B. E., & Lovett, S. T. (2006). Cis and Trans-acting Effects on a Mutational Hotspot Involving a Replication Template Switch. *Journal of Molecular Biology*, *356*(2), 300–311. https://doi.org/10.1016/j.jmb.2005.11.071

10. Laranjo, L. T., Klaric, J. A., Pearlman, L. R., & Lovett, S. T. (2018). Stimulation of Replication Template-Switching by DNA-Protein Crosslinks. *Genes*, *10*(1), 14. https://doi.org/10.3390/genes10010014

11. Seier, T., Zilberberg, G., Zeiger, D. M., & Lovett, S. T. (2012). Azidothymidine and other chain terminators are mutagenic for template-switch-generated genetic mutations. *Proceedings of the National Academy of Sciences*, *109*(16), 6171–6174. https://doi.org/10.1073/pnas.1116160109

12. Santi, D. V., Norment, A., & Garrett, C. E. (1984). Covalent bond formation between a DNA-cytosine methyltransferase and DNA containing 5-azacytosine. *Proceedings of the National Academy of Sciences*, *81*(22), 6993–6997. https://doi.org/10.1073/pnas.81.22.6993

13. Kuo, H. K., Griffith, J. D., & Kreuzer, K. N. (2007). 5-Azacytidine–Induced Methyltransferase-DNA Adducts Block DNA Replication In vivo. *Cancer Research*, *67*(17), 8248–8254. https://doi.org/10.1158/0008-5472.CAN-07-1038

14. Sreedharan, S., Oram, M., Jensen, B., Peterson, L. R., & Fisher, L. M. (1990). DNA gyrase gyrA mutations in ciprofloxacin-resistant strains of Staphylococcus aureus: Close similarity with quinolone resistance mutations in Escherichia coli. *Journal of Bacteriology*, *172*(12), 7260–7262. https://doi.org/10.1128/jb.172.12.7260-7262.1990

15. Craft, T. R., Bermudez, E., & Skopek, T. R. (1987). Formaldehyde mutagenesis and formation of DNA-protein crosslinks in human lymphoblasts in vitro. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *176*(1), 147–155. https://doi.org/10.1016/0027-5107(87)90262-4

16. Heinemann, V., Hertel, L. W., Grindey, G. B., & Plunkett, W. (1988). Comparison of the cellular pharmacokinetics and toxicity of 2',2'-difluorodeoxycytidine and 1-beta-D-arabinofuranosylcytosine. *Cancer Research*, *48*(14), 4024–4031.

17. Husain, M. A., Sarwar, T., Rehman, S. U., Ishqi, H. M., & Tabish, M. (2015). Ibuprofen causes photocleavage through ROS generation and intercalates with DNA: A combined biophysical and molecular docking approach. *Physical Chemistry Chemical Physics: PCCP*, *17*(21), 13837–13850. https://doi.org/10.1039/c5cp00272a

18. Soumya, S., & Joe, I. H. (2021). A combined experimental and quantum chemical study on molecular structure, spectroscopic properties and biological activity of anti-inflammatory Glucocorticosteroid drug, Dexamethasone. *Journal of Molecular Structure*, *1245*, 130999. https://doi.org/10.1016/j.molstruc.2021.130999

19. Tewey, K. M., Rowe, T. C., Yang, L., Halligan, B. D., & Liu, L. F. (1984). Adriamycin-Induced DNA Damage Mediated by Mammalian DNA Topoisomerase II. *Science*, *226*(4673), 466–468. https://doi.org/10.1126/science.6093249

20. Mimnaugh, E. G., Trush, M. A., Bhatnagar, M., & Gram, T. E. (1985). Enhancement of reactive oxygen-dependent mitochondrial membrane lipid peroxidation by the anticancer drug adriamycin. *Biochemical Pharmacology*, *34*(6), 847–856. https://doi.org/10.1016/0006-2952(85)90766-X

21. Zhang, H., Gao, Y. G., van der Marel, G. A., van Boom, J. H., & Wang, A. H. (1993). Simultaneous incorporations of two anticancer drugs into DNA. The structures of formaldehyde-cross-linked adducts of daunorubicin-d(CG(araC)GCG) and doxorubicin-d(CA(araC)GTG) complexes at high resolution. *Journal of Biological Chemistry*, *268*(14), 10095–10101. https://doi.org/10.1016/S0021-9258(18)82176-7

22. Du, H., Huang, Y., Hou, X., Quan, X., Jiang, J., Wei, X., Liu, Y., Li, H., Wang, P., Zhan, M., Ai, X., Lu, L., Yuan, S., & Sun, L. (2018). Two novel camptothecin derivatives inhibit colorectal cancer proliferation via induction of cell cycle arrest and apoptosis in vitro and in vivo. *European Journal of Pharmaceutical Sciences*, *123*, 546–559. https://doi.org/10.1016/j.ejps.2018.08.018

23. Hsiang, Y. H., Hertzberg, R., Hecht, S., & Liu, L. F. (1985). Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *Journal of Biological Chemistry*, *260*(27), 14873–14878. https://doi.org/10.1016/S0021-9258(17)38654-4

24. Hsiang, Y.-H., Lihou, M. G., & Liu, L. F. (1989). Arrest of Replication Forks by Drug-stabilized Topoisomerase I-DNA Cleavable Complexes as a Mechanism of Cell Killing by Camptothecin1. *Cancer Research*, *49*(18), 5077–5082.

25. Laranjo, L. T., Gross, S. J., Zeiger, D. M., & Lovett, S. T. (2017). SSB recruitment of Exonuclease I aborts template-switching in Escherichia coli. *DNA Repair*, *57*, 12–16. h ttps://doi.org/10.1016/j.dnarep.2017.05.007

26. de Sousa Cavalcante, L., & Monteiro, G. (2014). Gemcitabine: Metabolism and molecular mechanisms of action, sensitivity and chemoresistance in pancreatic cancer. *European Journal of Pharmacology*, *741*, 8–16. https://doi.org/10.1016/j.ejphar.2014.07.041