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New antimicrobial properties of "old" 5-fluorouracil

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Aim. The objective of this study was to determine the activity of 5-fluorouracil (5-FU) against five pathogenic bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 433 and two fungi: *Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 208821. **Methods.** 5-FU was tested by the broth microdilution against selected microorganisms. **Results.** It was found that 5-FU significantly inhibits the growth of *S. aureus* ATCC 43300 and *C. neoformans* ATCC 208821 (MIC ≤ 0.25 mg/L). *P. aeruginosa* ATCC27853, *K. pneumonia* ATCC 700603 and *C. albicans* ATCC90028 were considered to be resistant to 5-FU (MIC > 32mg/L), whereas *A. baumanii* ATCC 19606 and *E. coli* ATCC 25922 were considered to be intermediate (MIC = 16mg/L). **Conclusion.** These findings suggest that 5-FU could be a promising drug candidate for the treatment of infection diseases caused by methicillin-resistant *S. aureus* (MRSA) ATCC 43300 and *C. neoformans* ATCC 208821.

Keywords: 5-fluorouracil, antibacterial activity, antifungal activity.

Introduction

5-Fluorouracil (5-FU) is the third most commonly used chemotherapeutic drug throughout the world for the treatment of cancer [1–4], which has been shown to be effective against several bacterial and fungal species. According to ChEMBL database, the antibacterial activity of this compound was reported toward *Staphylococcus aureus* strains ATCC 4163, ATCC 25923, ATCC 6538, ATCC 29213; *S. epidermidis* strains ATCC 12228, ATCC 35984 as well as against several hospital isolates of these pathogens obtained in the Warsaw Medical University Hospital [5]. Steinfeld *et al.* studied the anti-yeast activity of 5-FU toward *Saccharomyces cerevisiae* 9763, *Candida albicans* 1-V, *C. albicans*

WD 18-4 and *C. krusei* 1-T [6]. Other authors investigated the 5-FU activity toward three yeast cultures *C. albicans*: BC759, B311 and 3153A [7]. Schwarz *et al.* reported the anti-yeast activity of 5-FU toward several isolates of *Cryptococcus neoformans*: 98.0176, 18476, 285, 550, 98.0673, 99.1026, 98.1121, 276, 280 and 533 [8]. Yasumoto *et. al.* tested 5-FU toward *S. aureus* strains ATCC 6538P, *Sarcina lutea* ATCC 9341, *Micrococcus flavus* ATCC 10240, *Bacillus subtilis* ATCC 6633, *Escherichia coli* NIHJ, *Klebsiella pneumonia* ATCC 10031, *Proteus vulgaris* IFO 3045 and *Pseudomonas diminute* IAM 1513 [9]. Several studies report the antibacterial activity of 5-FU toward *Pseudomonas aeruginosa* cystic fibrosis isolates [10, 11].

The objective of this study was investigation of the antimicrobial activity of 5-FU against five bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 433 and two fungi: *Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 208821.

Materials and Methods

5-FU synthesis

5-FU was synthesized as described earlier [12, 13].

Antibacterial assay

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (CAMHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1.5–3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD_{600}), then added to each well of the plate containing compound dissolved in DMSO, giving a cell density of 5×10^5 CFU/mL and a total volume of 50 µL. All the plates were covered and incubated at 37 °C for 18 h without shaking.

Growth inhibition of all bacteria was determined by measuring absorbance at 600 nm (OD_{600}), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and the positive control (bacteria without inhibitors) on the same plate as references.

Antifungal assay

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C. A yeast suspension of 1×10^6 to 5×10^6 CFU/mL (as determined by OD_{530}) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5×10^3 CFU/mL and a total volume of 50 µL. All plates were covered and incubated at 35 °C for 36 h without shaking.

Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD_{530}), whereas the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm ($OD_{600\text{-}570}$), after the addition of resazurin (0.001 % final concentration) and incubation at 35 °C for additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive

control (fungi without inhibitors) on the same plate.

The minimum inhibitory concentration (MIC) was determined following the CLSI guidelines, identifying the lowest concentration, at which full inhibition of the bacteria or fungi has been detected.

The growth rate for all bacteria and fungi has a variation of ± 10 %, which is within the reported normal distribution of bacterial/fungal growth. Any significant variation (or outliers/hits) is identified by the modified Z-Score, and the actives are selected by a combination of inhibition value and Z-Score.

Z-Score analysis

The Z-Score is calculated based on the sample population using a modified Z-Score method, which accounts for possible skewed sample population. The modified method uses median and MAD (median average deviation) instead of average and sd, and a scaling factor: M(i) = 0.6745*(x(i) – median(x))/MAD) [14]. The compounds with the inhibition values equal to or about 80 % and abs(Z-Score) about 2.5 for either replicate (n = 2 on different plates) were considered active. The compounds with inhibition values between 50.9–79.9 % or abs(Z-Score) below 2.5 were classified as partially active. The compounds with inhibition values below 50 % and abs(Z-Score) below 2.5 were considered as inactive.

Results and Discussion

Drug repositioning is a promising strategy for the development of novel antibacterial drugs, since pharmacokinetics, pharmacodynamics and toxicity have been already established. In this study we investigated the antimicrobial activity of widely used anticancer drug 5-FU. It inhibits thymidylate synthase and thus prevents the synthesis of thymidine monophosphate (dTMP), which is an important nucleotide for DNA replication. Therefore, 5-FU induces apoptosis of rapidly dividing cancer cells by dTMP starvation [15]. It has also been reported that 5-FU mainly causes the thymineless death of bacteria by inhibiting thymidine synthesis, which, in turn, blocks DNA and RNA synthesis. However, it is still too early to say about the same molecular mechanisms for inhibiting the tumor cells and bacteria [16]. Earlier, the authors Ahmad *et al.* [17] reported the direct and indirect effects caused by the thymine starvation in bacteria. The direct effects include single- and double-strand DNA breaks leading to the bacterial death. The indirect effects involve plasmids elimination, lack of transforming ability, bacterial cell filamentation, *etc.* Yeast cells exhibit the effects similar to those of bacteria during thymine starvation [17]. Recently, it was shown that 5-FU inhibits the synthesis of common qourum-sensing communication signal autoinducer-2 (AI-2), which is responsible for the biofilm formation in methicillin-resistant *Staphylococcus aureus* (MRSA) [16]. Therefore, 5-FU can possess various mechanisms of action to reveal the antimicrobial effects.

The antimicrobial activities of 5-FU toward five bacteria — *S. aureus* ATCC 433, *A. baumannii* ATCC 19606, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, and two fungi — *C. albicans* ATCC 90028, *C. neoformans* ATCC 208821, disclosed by us are presented in Table 1.

As it can be seen from the Table 1, 5-FU significantly inhibits growth of methicillin-

*Table 1.***Antimicrobial activity of 5-FU**

Organism	Growth inhibition, %	Z-Score	MIC, mg/L	MIC, μM
Staphylococcus aureus ATCC 43300	91.6; 92.6	–6.9, –8.0	≤ 0.25	≤ 1.9
Acinetobacter baumannii ATCC 19606	84.3; 91.0	–10.7; –15.7	16	122.9
Escherichia coli ATCC 25922	87.3; 92.7	–25.7; –27.7	16	122.9
Pseudomonas aeruginosa ATCC 27853	48.8; 57.2	–10.3; –8.9	> 32	> 245.8
Klebsiella pneumoniae ATCC 700603	65.6; 72.6	–7.6; –9.4	> 32	> 245.8
Cryptococcus neoformans ATCC 208821	111.4; 113.3	–10.8; –15.3	≤ 0.25	≤ 1.9
*Candida albicans*ATCC 90028	14.8; 6.5	–1.1; –3.0	> 32	> 245.8

resistant *S. aureus* ATCC 43300 and *C. neoformans* ATCC 208821 with MIC value of ≤0.25 mg/L (1.9 μM). Earlier, the same activity of 5-FU was demonstrated toward *S. aureus* strains ATCC 4163 and *S. aureus* ATCC 25923, two times lower activity of 5-FU was found toward *S. aureus* ATCC 6538, *S. aureus* ATCC 29213, hospital isolates *S. aureus* 452/11, *S. aureus* 456/11, *S. aureus* 514/11, *S. aureus* 572/12, *S. aureus* 573/12, *S. aureus* 585/12, *S. aureus* 586/12 and four times lower activity was shown toward *S. aureus* 462/11, *S. aureus* 522/12 and *S. aureus* 537/12 [5]. Other authors found that 5-FU inhibits *S. aureus* ATCC 6538P with MIC value of 9.4 mg/L [9]. Previous studies demonstrated that 5-FU possesses high inhibitory activity toward *C. neoformans* 98.0176 (MIC = 0.008 mg/L), *C. neoformans* 18476 (MIC = 0.25 mg/L), *C. neoformans* 550 (MIC = 0.5 mg/L), moderate inhibitory activity toward *C. neoformans* 285 (MIC = 4 mg/L), *C. neoformans* 99.1026 (MIC = 8 mg/L), *C. neoformans* 98.0673 (MIC = 16 mg/L) and low inhibitory activity toward *C. neoformans* 98.1121 (MIC = 256 mg/L), *C. neoformans* 276 (MIC = 256 mg/L), *C. neoformans* 280 (MIC = 256 mg/L) and *C. neoformans* 533 (MIC = 256 mg/L) [8].

Earlier, Ueda *et al.* demonstrated that 5-FU at 10 μM concentration inhibits the biofilm formation of *P. aeruginosa* PA14 and at higher concentration (25 μM) also inhibits bacterial growth in planktonic cultures [18]. In our experiments, *P. aeruginosa* ATCC 27853 is considered to be resistant to 5-FU (MIC > 32 mg/L).

There are several literature data concerning the activity of 5-FU toward different strains of *C. albicans*. Steinfeld *et al.* demonstrated that 5-FU inhibits *C. albicans* WD 18-4 with MIC value of 2.5 mg/L, while *C. albicans* 1-V is resistant to 5-FU (MIC > 20 mg/L) [6]. Other authors have shown that 5-FU efficiently inhibits the growth of *C. albicans* BC759 (MIC = = 1.0 mg/L), *C. albicans* B311 (MIC = = 4.0 mg/L) and *C. albicans* 3153A (MIC = = 1.0 mg/L) [7] while in our experiments *C. albicans* ATCC 90028 is completely resistant to 5-FU.

Conclusion

Therefore, the tests performed have clearly shown that 5-FU significantly inhibits the growth of methicillin-resistant *S. aureus*

ATCC 43300 and *C. neoformans* ATCC 208821. *P. aeruginosa* ATCC27853, *K. pneumonia* ATCC 700603 and *C. albicans* ATCC 90028 were shown to be resistant to 5-FU wheares *A. baumanii* ATCC 19606 and *E. coli* ATCC 25922 are intermediate. These results suggest that the anticancer drug 5-FU could be considered for anti-infective therapy of MRSA infections and cryptococcosis.

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REFERENCES

1. *More LA, Lane S, Asnani A.* 5-FU cardiotoxicity: vasospasm, myocarditis, and sudden death. *Curr Cardiol Rep.* 2021; **23**(3):17.
2. *Sara JD, Kaur J, Khodadadi R, Rehman M, Lobo R, Chakrabarti S, Herrmann J, Lerman A, Grothey A.* 5-fluorouracil and cardiotoxicity: a review. *Ther Adv Med Oncol.* 2018; **10**:1758835918780140.
3. *Christensen S, Van der Roest B, Besselink N, Janssen R, Boymans S, Martens JWM, Yaspo ML, Priestley P, Kuijk E, Cuppen E, Van Hoeck A.* 5-Fluorouracil treatment induces characteristic T>G mutations in human cancer. *Nat Commun.* 2019; **10**(1):4571.
4. *Grem JL.* 5-Fluorouracil: forty-plus and still ticking. A review of its preclinical and clinical development. *Invest New Drugs.* 2000; **18**(4):299–313.
5. *Huczyński A, Antoszczak M, Kleczewska N, Lewandowska M, Maj E, Stefańska J, Wietrzyk J, Janczak J, Celewicz L.* Synthesis and biological activity of salinomycin conjugates with floxuridine. *Eur J Med Chem.* 2015; **93**:33–41.
6. *Steinfeld AS, Naider F, Becker JM.* Anticandidal activity of 5-fluorocytosine-peptide conjugates. *J Med Chem.* 1979; **22**(9):1104–9.
7. *Kingsbury WD, Boehm JC, Mehta RJ, Grappel SF, Gilvarg C.* A novel peptide delivery system involving peptidase activated prodrugs as antimicrobial agents. *Synthesis and biological activity of peptidyl derivatives of 5-fluorouracil. J Med Chem.* 1984; **27**(11):1447–51.
8. *Schwarz P, Janbon G, Dromer F, Lortholary O, Dannaoui E.* Combination of amphotericin B with flucytosine is active *in vitro* against flucytosine-resistant isolates of *Cryptococcus neoformans*. *Antimicrob Agents Chemother.* 2007; **51**(1):383–5.
9. *Yasumoto M, Moriyama A, Unemi N, Hashimoto S, Suzue T.* Studies of antitumor agents. *1.* Resolution of racemic 1-(tetrahydro-2-furanyl)-k-fluorouracil into the R and S isomers and examination of the biological activities of the isomers. *J Med Chem.* 1977; **20**(12):1592–4.
10. *Imperi F, Fiscarelli EV, Visaggio D, Leoni L, Visca P.* Activity and impact on resistance development of two antivirulence fluoropyrimidine drugs in *Pseudomonas aeruginosa*. *Front Cell Infect Microbiol.* 2019; **9**:49.
11. *Di Bonaventura G, Lupetti V, De Fabritiis S, Piccirilli A, Porreca A, Di Nicola M, Pompilio A.* Giving drugs a second chance: antibacterial and antibiofilm effects of ciclopirox and ribavirin against cystic fibrosis *Pseudomonas aeruginosa* strains. *Int J Mol Sci.* 2022; **23**(9):5029.
12. *Duschinsky R, Pleven E, Heidelberger C.* The synthesis of 5-fluoropyrimidines. *J. Am. Chem Soc.* 1957; **79**(16), 4559–60.
13. *Barton DH, Hesse RH, To HT, Pechet MM.* A convenient synthesis of 5-fluorouracil. *J Org Chem.* 1972; **37**(2):329–30.
14. *Iglewicz B, Hoaglin D.* (1993), Vol. 16: How to Detect and Handle Outliers", The ASQC Basic References in Quality Control: Statistical Techniques, Edward F. Mykytka, Ph.D., Editor.
15. *Wilson PM, Danenberg PV, Johnston PG, Lenz HJ, Ladner RD.* Standing the test of time: targeting thymidylate biosynthesis in cancer therapy. *Nat Rev Clin Oncol.* 2014; **11**(5):282–98.
16. *Oe C, Hayashi H, Hirata K, Kawaji K, Hashima F, Sasano M, Furuichi M, Usui E, Katsumi M, Suzuki Y, Nakajima C, Kaku M, Kodama EN.* Pyrimidine

analogues as a new class of gram-positive antibiotics, mainly targeting thymineless-death related proteins. *ACS Infect Dis.* 2020; **6**(6):1490–500.
17. *Ahmad SI, Kirk SH, Eisenstark A.* Thymine metabolism and thymineless death in prokaryotes and eukaryotes. *Annu Rev Microbiol.* 1998; **52**:591–625.
18. *Ueda A, Attila C, Whiteley M, Wood TK.* Uracil influences quorum sensing and biofilm formation in *Pseudomonas aeruginosa* and fluorouracil is an antagonist. *Microb Biotechnol.* 2009; **2**(1):62–74.
19. *Blaskovich MA, Zuegg J, Elliott AG, Cooper MA.* Helping chemists discover new antibiotics. *ACS Infect Dis.* 2015; **1**(7):285–7.

Нові антимікробні властивості 5-фторурацилу

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Мета. Дослідити протимікробну активність 5-фторурацилу щодо п'яти штамів патогенних бактерій: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 433 і двох штамів грибів: *Candida albicans* ATCC 90028 та *Cryptococcus neoformans* ATCC 208821. **Методи.** Протимікробну активність 5-фторурацилу досліджували методом серійних мікророзведень. **Результати.** Було встановлено, що 5-фторурацил значним чином інгібує ріст метицилін-резистентного штаму *S. aureus* ATCC 43300 та *C. neoformans* ATCC 208821 (MIC ≤ 0.25 мг/л), тоді як штами *A. baumanii* ATCC 19606 та *E. coli* ATCC 25922 пригнічує із середньою ефективністю (MIC = 16 мг/л). *P. aeruginosa* ATCC27853, *K. pneumonia* ATCC 700603 і *C. albicans* ATCC90028 виявилися резистентними до 5-фторурацилу (MIC > 32 мг/л). **Висновки.** Отримані результати свідчать про те, що 5-фторурацил може бути перспективним препаратом для лікування інфекційних захворювань, викликаних метицилін-резистентним штамом *S. aureus* ATCC 43300 та *C. neoformans* ATCC 208821.

Ключові слова: 5-фторурацил, антибактеріальна активність, протигрибкова активність.

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