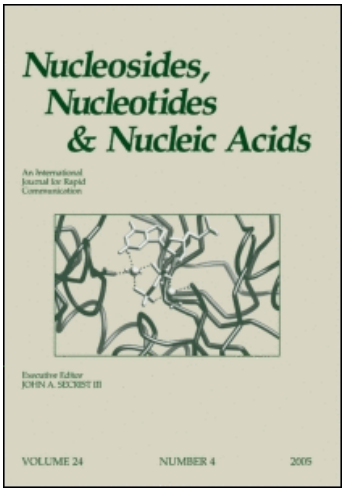

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Intracellular Thymidylate Synthase Inhibition by Trifluorothymidine in FM3A Cells

O. H. Temmink[a]; E. M. Comijn[a]; M. Fukushima[b]; G. J. Peters[a]
[a] Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands [b] Taiho Pharmaceuticals, Hanno, Japan

Online publication date: 27 October 2004

To cite this Article Temmink, O. H. , Comijn, E. M. , Fukushima, M. and Peters, G. J.(2004) 'Intracellular Thymidylate Synthase Inhibition by Trifluorothymidine in FM3A Cells', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1491 — 1494
To link to this Article: DOI: 10.1081/NCN-200027707
URL: http://dx.doi.org/10.1081/NCN-200027707

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1491–1494, 2004

Intracellular Thymidylate Synthase Inhibition by Trifluorothymidine in FM3A Cells

O. H. Temmink,[1,*] E. M. Comijn,[1] M. Fukushima,[2] and G. J. Peters[1]

[1]Department of Medical Oncology, VU University Medical Center
Amsterdam, The Netherlands
[2]Taiho Pharmaceuticals, Hanno, Japan

ABSTRACT

Trifluorothymidine (TFT) can be phosphorylated by thymidine kinase (TK) to TFTMP which can inhibit thymidylate synthase (TS), resulting in depletion of thymidine nucleotides. TFT can be degraded by thymidine phosphorylase (TP) which can be inhibited by thymidine phosphorylase inhibitor (TPI). Using the TS in situ Inhibition Assay (TSIA) FM3A breast cancer cells were exposed 4 h or 24 h to TFT and 5-Fluorouracil (5FU). TS activity reduced to 9% (0.1 μM TFT) and 58% (1 μM 5FU) after 4 h exposure and to 6% (TFT) and 21% (5FU) after 24 h exposure. TPI did not affect TS inhibition by TFT. FM3A cells lacking TK or TS activity (FM3A/TK$^-$) were far less sensitive to TFT compared to FM3A cells. Conclusion: TFT can be taken up and activated very rapidly by FM3A cancer cells, probably due to favourable TK enzyme properties, and TPI did not influence this.

Key Words: Trifluorothymidine; Thymidylate synthase; Thymidine phosphorylase inhibitor.

*Correspondence: O. H. Temmink, Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands.

DOI: 10.1081/NCN-200027707
1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

The very potent anticancer agent trifluorothymidine (TFT) can inhibit TS via the metabolite TFTMP. In contrast to 5FU, TFT only needs one conversion step to become a potent TS inhibitor.[1] The degradation of TFT to trifluorothymine (TF-Thy) by TP can be prevented by TPI. TPI was originally synthesized to increase TFT cytotoxicity in cancer cells.[2] Therefore we questioned whether TPI could increase TS inhibition by TFTMP in intact cancer cells. TS inhibition by TFTMP was compared with that of FdUMP, the metabolite derived from 5FU.

MATERIALS AND METHODS

To evaluate intracellular TS inhibition we used the TS in situ Inhibition Assay (TSIA).[3] For the TSIA assay tritiated deoxycytidine was used, which after cellular uptake can be converted to dCMP and subsequently to tritiated dUMP, the substrate for TS. TS converts tritiated dUMP to dTMP thereby releasing tritiated water, which can be measured. We measured TS activity in intact FM3A mouse mammary cancer cells and its TK deficient variant FM3A/TK$^-$.[4] These cells were cultured in RPMI medium supplemented with 10% fetal calf serum. TSIA was evaluated after exposure of the cells to 1 μM 5FU or 0.1 μM TFT (±10 μM TPI, 5-chloro-6-(2-iminopyrrolidin-1-yl)methyl-2,4(1H,3H)-pyrimidine dio ne hydrochloride) for either 4 h, 24 h or 4 h followed by a 20 h incubation in drug-free medium (DFM). The concentrations of 5FU and TFT used were chosen because these concentrations caused cell growth inhibition of about 50%. This growth inhibition was determined using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay.[5] Growth inhibition was also determined in FM3A/TS$^-$ cells, a TS deficient variant requiring 20 μM thymidine to survive.

RESULTS

Cytotoxicity of 5FU and TFT in the FM3A variants varied considerably (Table 1). The FM3A/TK$^-$ and FM3A/TS$^-$ variants were far less sensitive to TFT than the parental

Table 1. Effect of 5FU and TFT (±TPI) on growth inhibition in FM3A, FM3A/TK$^-$ and FM3A/TS$^-$ cells (IC_{50} values, μM).

	Cell line		
Drug	FM3A	FM3A/TK$^-$	FM3A/TS$^-$
5FU	0.1 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
TFT	0.2 ± 0.1	79 ± 20*	246 ± 75*
TFT + 10 μM TPI	0.2 ± 0.04	79 ± 16*	245 ± 75*

Values are expressed as means of 3–4 separate experiments ± SEM. Significance when compared to parentals: *$p < 0.01$. TPI was added during whole 72 h incubation period.

Table 2. Effect of 5FU and TFT (±TPI) on the inhibition of TS in FM3A and FM3A/TK$^-$ cells using the TSIA.

Drug	Cell line	Drug exposure period		
		4 h	4 h + 20 h DFM	24 h
Controls		100	100	100
1 μM 5FU	FM3A	58 ± 8	48 ± 13	21 ± 5
1 μM 5FU	FM3A/TK$^-$	80 ± 6	83 ± 3	67 ± 6
0.1 μM TFT	FM3A	9 ± 2	74 ± 4	6 ± 5
0.1 μM TFT	FM3A/TK$^-$	85 ± 3	99 ± 5	95 ± 7
0.1 μM TFT + 10 μM TPI	FM3A	6 ± 3	75 ± 4	19 ± 7
0.1 μM TFT + 10 μM TPI	FM3A/TK$^-$	84 ± 5	91 ± 2	92 ± 8

Values are % of untreated control cells and are expressed as means of at least 3 separate experiments ± SEM. For more details: see Materials and Methods.

cells, in contrast to 5FU, where no significant differences were found between the cell lines. Addition of 10 μM TPI did not change cytotoxicity to TFT in these cell lines.

The effects of 5FU and TFT (with or without TPI) on intracellular TS inhibition was compared in FM3A cancer cells with intact or deficient TK when exposed for 4 h or 24 h (see Table 2). TS was strongly inhibited in FM3A cells after 4 h when compared to controls. TS activity was reduced to 9% and 58% after exposure to TFT or 5FU, respectively. After 24 h exposure TS activity was almost completely inhibited by TFT (6%), in contrast to 5FU (21%). In FM3A/TK$^-$ cells 5FU could inhibit TS, although less compared to FM3A cells. TFT hardly affected TS activity in FM3A/TK$^-$ cells. TS activity was not affected when 10 μM TPI was added to the medium. Table 2 also shows that TS activity recovers rapidly in FM3A cells after 4 h exposure to TFT (back to 74%), but not when exposed to 5FU. Naturally, TSIA could not be evaluated for FM3A/TS$^-$.

DISCUSSION

In this paper we demonstrate that TFT can inhibit TS rapidly and effectively and that its activation is dependent on TK. Presumably TFTMP is the active metabolite of TFT, which inhibits TS directly.[6] In addition, TFTMP can be further phosphorylated to its triphosphate form to be incorporated into DNA. TS inhibition by TFTMP was shown to be dependent on TK activity, and in contrast to 5FU, it only needs one conversion step to become active.

5FU is also active in FM3A/TK$^-$ cells indicating that its activation is not dependent on the TP/TK pathway. Its activity against the FM3A/TS$^-$ cells indicates that in this cell line 5FU acts via incorporation into RNA, also because the FM3A/TS$^-$ cells are grown in medium containing thymidine.

The results show that already within 4 h TFT reduced TS activity to below 10% and that TP inhibition by TPI did not affect this. Probably a low concentration of TFT is required to induce dTTP depletion and is not influenced by degradation by TP. Possibly

TFT is a preferential substrate for TK rather than TP, because at low concentration (0.1 μM) the Km acts in favour of TK.

Also after 24 h exposure TS was still inhibited to below 10% indicating that the constant influx of TFT was not decreased. It is possible that TS inhibition by TFTMP is tumor-specific, because tumor cells resistant to nucleoside analogs may have elevated TS levels.[1] The retention of TS inhibition in cells exposed 4 h to 5FU followed by 20 h DFM demonstrates that the complex between FdUMP-TS-folate is very stable in these cells, in contrast to the complex TFTMP-TS since culturing in DFM restores TS activity. TS inhibition by TFT via TFTMP seems very rapid but requires a continuous exposure. Future studies should attempt to characterize the contribution of all TFT-metabolizing enzymes involved and to investigate this in tumor cells of different tissue origin.

ACKNOWLEDGMENT

This study was supported by Taiho Pharmaceuticals, Hanno, Japan.

REFERENCES

1. Murakami, Y.; Kazuno, H.; Emura, T.; Tsujimoto, H.; Suzuki, N.; Fukushima, M. Different mechanisms of acquired resistance to fluorinated pyrimidines in human colorectal cancer cells. Int. J. Oncol. **2000**, *17* (2), 277–283.
2. Fukushima, M.; Suzuki, N.; Emura, T.; Yano, S.; Kazuno, H.; Tada, Y.; Yamada, Y.; Asao, T. Structure and activity of specific inhibitors of thymidine phosphorylase to potentiate the function of antitumor 2′-deoxyribonucleosides. Biochem. Pharmacol. **2000**, *59* (10), 1227–1236.
3. Rots, M.G.; Pieters, R.; Kaspers, G.J.; van Zantwijk, C.H.; Noordhuis, P.; Mauritz, R.; Veerman, A.J.; Jansen, G.; Peters, G.J. Differential methotrexate resistance in childhood T-versus common/preB-acute lymphoblastic leukemia can be measured by an in situ thymidylate synthase inhibition assay, but not by the MTT assay. Blood **1999**, *93* (3), 1067–1074.
4. Ayusawa, D.; Koyama, H.; Seno, T. Resistance to methotrexate in thymidylate synthetase-deficient mutants of cultured mouse mammary tumor FM3A cells. Cancer Res. **1981**, *41* (4), 1497–1501.
5. Keepers, Y.P.; Pizao, P.E.; Peters, G.J.; van Ark-Otte, J.; Winograd, B.; Pinedo, H.M. Comparison of the sulforhodamine B protein and tetrazolium (MTT) assays for in vitro chemosensitivity testing. Eur. J. Cancer **1991**, *27* (7), 897–900.
6. Eckstein, J.W.; Foster, P.G.; Finer-Moore, J.; Wataya, Y.; Santi, D.V. Mechanism-based inhibition of thymidylate synthase by 5-(trifluoromethyl)-2′-deoxyuridine 5′-monophosphate. Biochemistry **1994**, *33* (50), 15086–15094.