

Chapter 3

Trifluorothymidine induces cell death independently of p53

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ABSTRACT

Trifluorothymidine (TFT), a potent anticancer agent, inhibits thymidylate synthase (TS) and is incorporated into the DNA, both events resulting in cell death. Cell death induction related to DNA damage is often involves activation of p53. We determined the role of p53 in TFT cytotoxicity and cell death induction, using, respectively, the sulforhodamine B-assay and FACS analysis, in a panel of cell lines with either wild type, inactive, or mutated p53. Neither TFT cytotoxicity nor cell death induction changed with TFT exposure in cell lines with wt, inactive or mutated p53. Conclusion: sensitivity to TFT is not dependent on the expression of wt p53.

INTRODUCTION

Trifluorothymidine (TFT) is a thymidine analog which has been shown to bypass resistance pathways for 5-FU derivatives in model systems¹. TFT is given in combination with TPI, a specific inhibitor of thymidine phosphorylase (TP) to increase TFT bioavailability (Figure 1). This combination, TAS-102, is currently tested as an oral chemotherapeutic agent in phase II studies². TFT has been shown to inhibit TS in its monophosphorylated form³ (Figure 1). When further activated to its tri-phosphorylated form, TFT can be incorporated into the DNA^{4,5}, which will subsequently result in DNA damage⁶. Subsequently, cell death induction will follow.

Cell death induced by DNA damaging agents is often dependent on p53, which detects DNA damage⁷. Upon activation of p53, cell death will be induced by activation of the main executors of apoptosis, the caspases. In this study we describe the role of p53 in TFT cytotoxicity.

MATERIALS AND METHODS

The cell lines and stable transfected derivatives used in this study were described before⁸⁻¹¹. These are: WiDR (parental, mutant p53), WiDR B (transfected with wt p53), Lovo92 (parental, wt p53), Lovo li (functional inactive p53), Lovo 175X2 (transfected with mutant p53). To evaluate TFT cytotoxicity, the sulforhodamine B-assay was used¹². Cells were exposed to TFT for 72 h after which TCA (final concentration 10 %) was added. Cells were stained with SRB (0.4 %) and dye was dissolved in Tris (10 mM). Cell death induced by TFT

was determined by staining cells with propidium iodide (PI) stain and analyzed by FACS⁹. The sub-G₁ peak was used as indication for cell death induction.

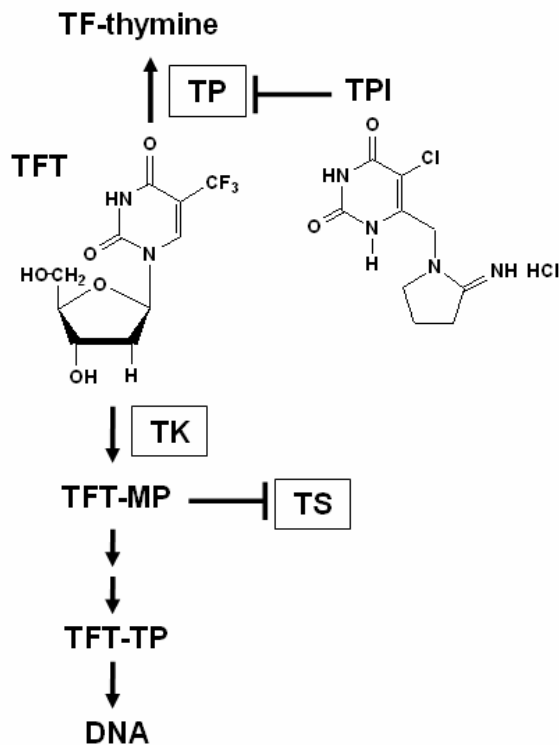


Figure 1 - Molecular structures of TFT and TPI. TFT can be degraded by thymidine phosphorylase (TP) to TF-thymine. TPI inhibits this degradation. Thymidine kinase (TK) activates TFT to its mono-phosphorylated form (TF-TMP). TFT-MP can inhibit thymidylate synthase (TS). When TFT is further activated it can be incorporated into the DNA.

RESULTS

TFT was active in both parental cell lines WiDR and Lovo92 (Figure 2). Cytotoxicity induced in the parental cell lines was compared to that of the derivatives. TFT cytotoxicity did not increase when wt p53 was transfected to WiDR (WiDR B). Also, cytotoxicity did not decrease when p53 was mutated or functionally inactive (Lovo 175 X2 and Lovo li).

Since p53 plays an important role in the induction of cell death after DNA damage induction by cytotoxic agents, the sub-G₁ peak as a measure of apoptotic cells was analyzed. In both the parental cell lines WiDR and Lovo92, cell death was induced (Figure 3). This induction of cell death was not increased when cells were transfected with wt p53 in WiDR cells. In Lovo92 cells, overexpression of a mutated and functionally inactive p53 did not result in a decrease in TFT induced cell death and rather a slight, non significant increase was observed in this cell line.

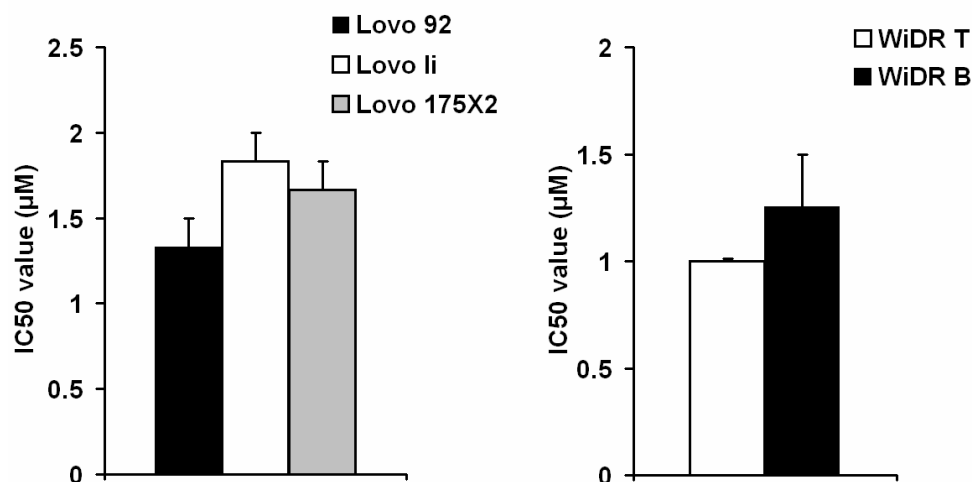


Figure 2 - TFT cytotoxicity using cells with various p53 status. IC50 values were determined from graphs and are expressed as means of 3 independent experiments \pm SEM.

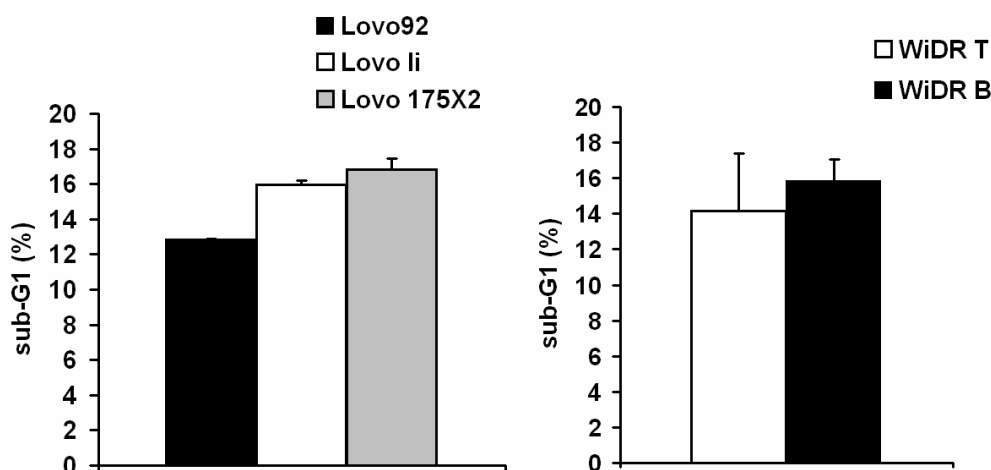


Figure 3 - Cell death induction (sub-G1) after TFT exposure using cells with various p53 status. Values are % and are expressed as means of 3 independent experiments \pm SEM.

DISCUSSION

In this report we show that the activity of TFT is not dependent on the presence of wild-type p53. TFT cytotoxicity and apoptosis induction (sub-G₁) did not differ between the cell lines with wild type, mutated, or non-functional p53.

The tumor suppressor gene p53 is absent or mutated in 85% of colorectal cancer¹³. Mutations in p53 have been related with resistance to chemotherapy¹⁴. In the whole NCI cell

line panel¹⁵ and more specifically colon cancer cells it was shown that when p53 was mutated, cells were less sensitive to 5FU and TS-directed antifolates^{15,16}. Since TFT cytotoxicity and cell death induction did not alter between cells with different p53 status, this can be a major advantage over 5-FU and antifolates.

Many DNA damage inducing agents in current use have been reported to induce apoptosis in a p53 dependent manner, including 5-FU¹⁷. Previously we demonstrated that in Lovo cells, cell death seemed to be apoptotic in contrast to WiDR which showed less apoptotic features after exposure to 5-FU and antifolates¹⁸. Since TFT sensitivity was not dependent on p53 expression, p53 related repair of DNA damage may not be the main mechanism of cell death induced in the panel of cell lines we have tested. Other reported mechanisms of TFT have been inhibition of TS. However, in the same panel of cell lines we observed a difference in sensitivity to antifolates and 5-FU^{10,11,18}. Maybe another mechanism than TS inhibition is also important in TFT activity.

Bcl-2 is an anti-apoptotic protein that can be inactivated by pro-apoptotic Bcl-2 family members that are induced by p53, thereby destabilizing mitochondria causing the release of apoptogenic factors. Many gastrointestinal tumors have been reported to have mutated or absent Bcl-2. Previously, WiDR cells were shown to lack Bcl-2 expression, while Lovo92 cells have functional Bcl-2¹⁸. Since TFT sensitivity and cell death induction between WiDR and Lovo92 cells did not vary much, we conclude that Bcl-2 itself is likely not a crucial regulator for TFT-induced apoptosis, although other Bcl-family members could be important in this process.

Several studies have suggested that death receptor-dependent apoptosis might also play a role in drug sensitivity¹⁹. Since TFT induced p53 independent cell death, the death receptor pathway may play a role, perhaps involving Fas or TNF-related apoptosis-inducing ligand (TRAIL). In conclusion, TFT induced p53 independent cell death, in contrast to 5-FU. These findings provide a basis for further investigation of TFT as therapy against colorectal cancer.

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