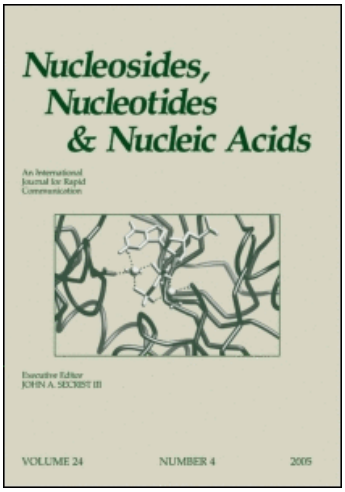

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Role of Platelet Derived Endothelial Cell Growth Factor/Thymidine Phosphorylase in Fluoropyrimidine Sensitivity and Potential Role of Deoxyribose-1-Phosphate

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ABSTRACT

Thymidine phosphorylase (TP) catalyzes the phosphorolytic cleavage of thymidine (TdR) to thymine and deoxyribose-1-phosphate (dR-1-P). TP, which is overexpressed in a wide variety of solid tumors, is involved in the activation and inactivation of fluoropyrimidines. We investigated the role of TP in 5′-deoxy-5-fluorouridine (5′DFUR), 5-fluorouracil (5FU) and trifluorothymidine (TFT) sensitivity. TP had no effect on TFT while it activated 5′DFUR and to a lesser extent 5FU. In order to provide an explanation for this difference in activation of 5′DFUR and 5FU, we studied the role of the 5FU co-substrate, dR-1-P, needed for its activation.

Key Words: Platelet derived endothelial cell growth factor; Thymidine phosphorylase; Deoxyribose-1-phosphate; Fluoropyrimidines.

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INTRODUCTION

TP is assumed to be identical to the angiogenic factor platelet derived endothelial cell growth factor (PD-ECGF), and its expression has been studied extensively in numerous immunohistochemical studies. It has been shown that TP is overexpressed in a wide variety of solid tumors and has been described as proangiogenic.[1] TP catalyzes the phosphorolytic cleavage of thymidine to thymine and dR-1-P. Besides this natural substrate it can use other substrates among which are several fluoropyrimidines. It can activate 5′DFUR to its active form 5FU; 5′DFUR is the last intermediate in the conversion of the orally administered fluoropyrimidine capecitabine (Xeloda). With this strategy, the overexpression of TP is used to obtain specific activation of capecitabine in the tumor.[2] TFT is a fluoropyrimidine which potentially can bypass 5FU resistance.[3] TFT can be inactivated by TP converting it to trifluorothymine. This conversion can be inhibited by a specific thymidine phosphorylase inhibitor (TPI)[4] thereby increasing the bioavailability of TFT. The combination of TFT and TPI (TAS-102) is currently tested in phase 1 trials.[5]

We investigated the role of TP in the activation of 5′DFUR and 5FU. In order to explain the observed differences in activation we also studied the co-substrate of 5FU activation, dR-1-P.

MATERIALS AND METHODS

In order to study the role of TP in the sensitivity of three different fluoropyrimidines, IC_{50}s (concentration which gives 50% growth inhibition) were determined with or without TPI in a panel of cell lines using the sulforhodamine assay.[6] Dose modifying factors (DMF) were calculated as: $(IC_{50} + TPI)/IC_{50}$. Furthermore two cell lines were transfected with human TP (Colo320 and H460 giving rise to transfected variants, Colo320TP1 and H460TP2).[6] TP activity was assessed in all the cell lines used.

The investigation of dR-1-P accumulation and metabolism was carried out in the Colo320 and Colo320TP1. Cells were incubated for 4 hr with 200 μM TdR, samples of the medium were analyzed on thymine content in order to obtain an estimate of the TdR conversion and consequently dR-1-P production.

To investigate the fate of dR-1-P Colo32OTP1 cell lysates were spiked with 500 pmol dR-1-P and incubated for 0, 5 and 10 minutes, in the presence of TPI preventing dR-1-P conversion to TdR by TP. After the incubation periods the lysates were analyzed for dR-1-P.[7] This experiment was repeated in the presence of TPI and dR-5-P or TPI and deoxyribose (dR), to see if the flux of dR-1-P could be influenced by either of the two sugar moieties and to obtain a clue where the dR-1-P is disappearing to.

RESULTS

In Fig. 1 the TP activity in the cell lines is shown. Colo320 had the lowest TP activity and its transfected variant Colo320TP1 had the highest activity, H460TP2 and H460 were the second and third most active cell lines, respectively. The results of the growth inhibition experiments expressed as DMFs are shown in Fig. 2. For 5′DFUR

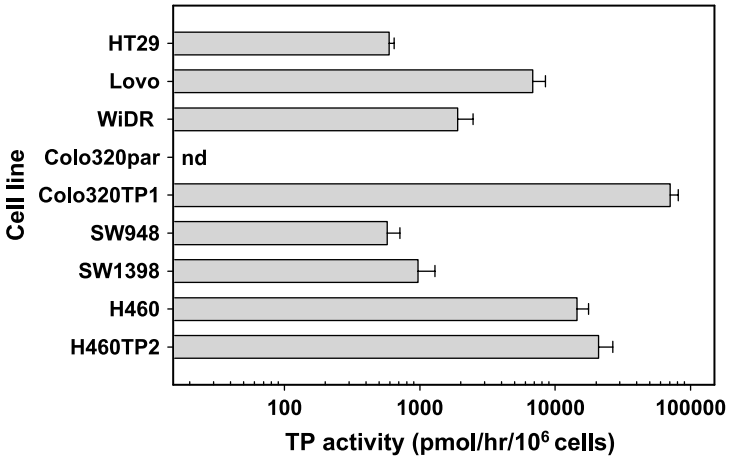

Figure 1. TP activity in the cell lines used. All cell lines are derived from colon cancer, except for H460 and H460TP2, which are non small cell lung cancer. Values are means ± SEM. (From Ref. [6].)

there is a good correlation ($r = 0.9$ $p < 0.01$) between the extent of the DMF and the TP activity in the cells, that is high activity corresponds with a high DMF.

Surprisingly there was no effect of either TPI or transfection on the IC_{50}s and DMFs of TFT in the cell panel although lower IC_{50}s were expected since TPI was added to prevent TFT breakdown in the cells.

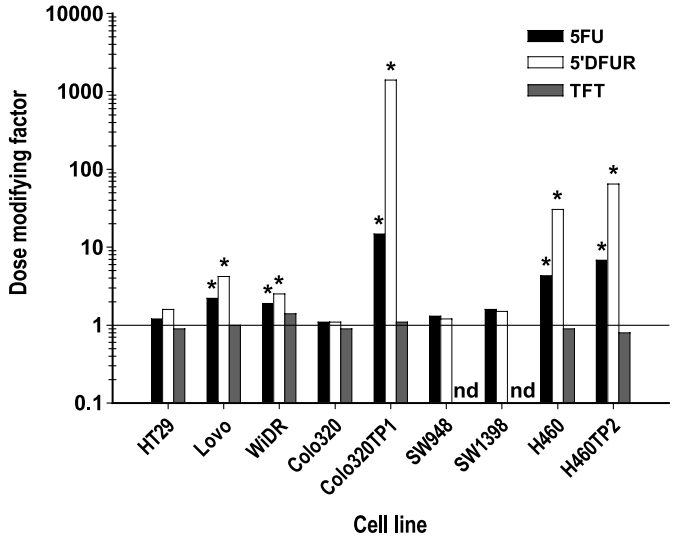

Figure 2. The DMF calculated as: (IC_{50} + TPI)/IC_{50} for the fluoropyrimidines: 5FU, 5′DFUR and TFT in the cell panel. Nd: not done.

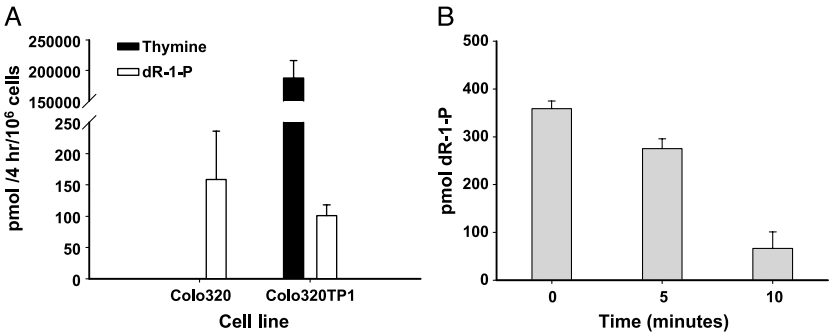

Figure 3. A) Intact cells were incubated with 200 μM thymidine, and subsequently assayed for dR-1-P. B) To lysates of Colo320TP1 500 pmol of dR-1-P was added and incubated for different time periods, to study disappearance of dR-1-P. TPI was also added to prevent (re)formation of TdR and loss of dR-1-P in this reaction. (From Ref. [7].)

In the case of 5FU similar results were found as for 5′DFUR , but the DMFs were lower although they still corresponded with the activity data.

The study of the co-substrate for 5FU activation in Colo320 and Colo320TP1 is shown in Fig. 3A, and although large amounts of dR-1-P were produced in the transfected cell line less than 99% was found in the dR-1-P assay. This indicates that there is a rapid disappearance of dR-1-P in the cells, which was confirmed in cell lysates of Colo320TP1 which were incubated with dR-1-P and TPI. In this incubation dR-1-P disappeared rapidly within minutes (Fig. 3B). In order to obtain insight in where the dR-1-P is disappearing to we tried to intervene in the disappearance by adding deoxyribose or dR-5-P influencing the dephosphorylation or conversion into

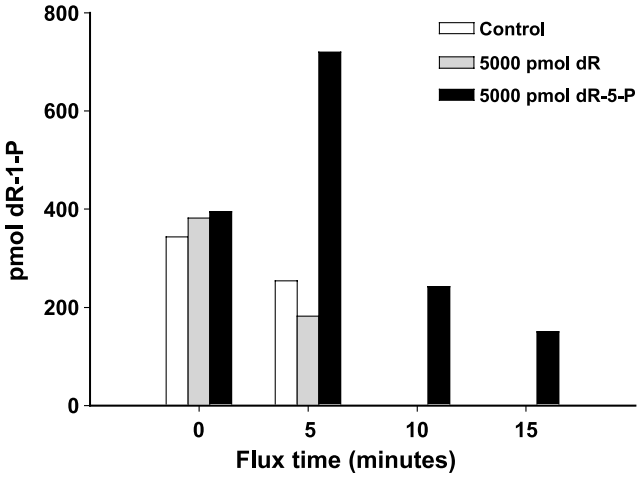

Figure 4. The effect of 5000 pmol dR or dR-5-p on the flux of 500 pmol dR-1-P in Colo320TP1 cell lysates. To all incubations 10 μM TPI was added to prevent formation of TdR.

dR-5-P, respectively. Deoxyribose did not influence the disappearance whereas dR-5-P resulted in an initial increase followed by an decrease (Fig. 4).

DISCUSSION

We observed a substantial effect of TP on the sensitivity to 5′DFUR, which appears to be its main route of activation. The DMF correspond with the TP activity of the cells. We did not find an effect of TP on TFT sensitivity, a surprising finding since Fukushima et al.[4] and we (data not shown) demonstrated that TFT is an excellent substrate for TP. Apparently TPI has no effect on the cellular cytotoxicity of TFT possibly due to rapid activation by thymidine kinase (TK) (Temmink et al., these proceedings) or that only certain threshold levels of TF-TMP, the active metabolite are needed to exert its effect. TPI was still functional, since TPI had effect in parrallel performed cytoxicity assays with 5′DFUR in the same cells excluding the possibility of transport/diffusion inhibition or interaction with the culture medium. 5FU was affected by TP in a similar way as 5′DFUR was, only to a much lesser extent, questioning the role of TP for 5FU activation under physiological conditions.

In order to explain this we studied dR-1-P the co-substrate in the activation reaction of 5FU. We found that despite a huge production of dR-1-P as measured by thymine production in the Colo320TP1 cells we detected less than 1% of the total amount produced. Furthermore dR-1-P disappeared very rapidly from Colo320TP1 cell lysates. This disappearance could be slowed by dR-5-P and not by deoxyribose indicating that dR-1-P is converted to dR-5-P and is then en route to be metabolised in the glycolysis or pentose phosphate pathway.

In conclusion, TP plays an important role in the activation of 5′DFUR and does not affect the cytoxicicity of TFT on a cellular level. 5FU can be activated by TP but its role appears to be limited due to the lack of the co-substrate; dR-1-P. Despite being formed in Colo320TP1 cells there is no net accumulation of dR-1-P, indicating a rapid disappearance. This was confirmed by flux assays in cell lysates in which at least part of the metabolic pathway of dR-1-P is active. Disappearance of dR-1-P in cell lysates was momentarily blocked by dR-5-P but not by deoxyribose indicating that a route in the direction of the glycolysis or pentose phosphate phathway might be favoured.

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REFERENCES

1. Ackland, S.P.; Peters, G.J. Thymidine phosphorylase: its role in sensitivity and resistance to anticancer drugs. Drug Resist. Updat. **1999**, *2*, 205–214.
2. Miwa, M.; Ura, M.; Nishida, M.; Sawada, N.; Ishikawa, T.; Mori, K.; Shimma, N.; Umeda, I.; Ishitsuka, H. Design of a novel oral fluoropyrimidine carbamate,

capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. Eur. J. Cancer **1998**, *34*, 1274–1281.
3. Satake, H.; Suzuki, H.; Emura, T.; Takeda, S.; Wataya, Y.; Yamada, Y.; Fukushima, M. A novel functional antitumor nucleoside, TAS-102,combined form of F_3Thd and its modulator (1): functional mechanims of the cytotoxicity of AS-102 effective against FUra-resistant tumors. Proc. Am. Assoc. Cancer Res. **1998**, *39*, (# 3187).
4. 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*, 1227–1236.
5. Thomas, M.B.; Hoff, P.M.; Carter, S.; Bland, G.; Lassere, Y.; Wolff, R.; Xiong, H.; Hayakawa, T.; Abbruzzese, J. A dose-finding, safety and pharmacokinetics study of TAS-102, an antitumor/antiangiogenic agent given orally on a once-daily schedule for five days every three weeks in patients with solid tumors. Proc. Am. Assoc. Cancer Res. **2002**, *43*, 554 (#2754).
6. De Bruin, M.; Van Capel, T.; Van Der Born, K.; Kruyt, F.A.; Fukushima, M.; Hoekman, K.; Pinedo, H.M.; Peters, G.J. Role of platelet-derived endothelial cell growth factor/thymidine phosphorylase in fluoropyrimidine sensitivity. Br. J. Cancer **2003**, *88*, 957–964.
7. De Bruin, M.; Smid, K.; Laan, A.C.; Noordhuis, P.; Fukushima, M.; Hoekman, K.; Pinedo, H.M.; Peters, G.J. Rapid disappearance of deoxyribose-1-phosphate in platelet derived endothelial cell growth factor/thymidine phosphorylase over-expressing cells. Biochem. Biophys. Res. Commun. **2003**, *301*, 675–679.