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Modulation of 5-fluorouracil cytotoxicity through thymidylate synthase and NF-κB down-regulation and its application on the radiolabelled iododeoxyuridine therapy on human hepatoma cell

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Abstract

The inhibition of thymidylate synthase (TS) by 5-fluorouracil (5-FU) was known to increase the incorporation of radiolabelled iododeoxyuridine (IdUrd) into DNA. The relatively non-toxic compounds such as thiol-containing antioxidant pyrrolidinodithiocarbamte (PDTC) or aromatic fatty acid phenylbutyrate (PB) had been reported to enhance the cytotoxic efficacy of 5-FU. We designed a novel strategy through triplet combination of PB, PDTC and 5-FU to increase the radiolabelled IdUrd uptake and investigated the underlying mechanisms. The growth inhibition and [^{125}I]IdUrd-DNA incorporation by PB, PDTC, 5-FU in different combinations were tested on parent or p21^{Waf1} transfected Hep3B cells. The combination of PB and PDTC was more effective in enhancing 5-FU cytotoxicity than either drug alone. The combination of PB/PDTC and 5-FU blocked cells in S-phase and resulted in 8.5-fold increase of radiolabelled IdUrd-DNA incorporation. The transfection of p21^{Waf1} did not change the general pattern of enhancement. Intriguingly, the combination of PB and PDTC effectively down-regulated NF-κB and TS and prevented their up-regulation from 5-FU treatment than either drug alone through a p21^{Waf1}-independent mechanism. Based on this strategy, the 3-drug combination offered potential for improved radiolabelled IdUrd molecular radiotherapy for hepatoma treatment.

Keywords: Iododeoxyuridine; Thymidylate synthase; 5-FU; PDTC; PB; NF-κB

1. Introduction

IdUrd is not targeting on TS, clinically ineffective, and only used for radiosensitizer [1]. We and many others have demonstrated that radiolabelled IdUrd can produce much higher killing efficacy than cold IdUrd [2,3]. IdUrd is a carrier for targeting high-killing power of radioisotope to DNA. It was estimated that Auger electrons from single molecule ^{125}I may cause un-repairable double strand breaks. ^{131}I produce β-ray although less efficient which has wider range of cell killing. The combination of [^{125}I/^{131}I] IdUrd has proved to be most effective in targeting tumor in vivo [3–5]. Increasing radiolabelled IdUrd incorporation into DNA is the key of success.

Specific TS inhibitor like fluorodeoxyuridine, thymitaque or non-specific TS inhibitor like 5-FU can increase radiolabelled IdUrd uptake through inhibition of de novo thymidine synthesis [6,7]. The higher the TS inhibition, the higher the IdUrd-DNA incorporation can be seen [3]. 5-FU has been widely used in cancer therapy for many decades. Radiolabelled IdUrd is a representative drug taking the advantage of inhibition of TS from 5-FU which results in high cell kill through Auger electrons from ^{125}I labeled

Abbreviations: IdUrd, iododeoxyuridine; TS, thymidylate synthase; 5-FU, 5-fluorouracil; PDTC, pyrrolidinodithiocarbamte; PB, phenylbutyrate; PBS, phosphate buffered saline; DMEM, Dulbecco's modified Eagle's medium; dUMP, deoxyuridine monophosphate; dTMP, thymidine monophosphate

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IdUrd and the cross-fire effect from ^{131}I-IdUrd. The combination of relatively non-toxic cytostatic drugs to enhance the cytotoxicity of 5-FU has long been a promising clinical research area like leucovorin as a good example. We are particular interested in aromatic fatty acid, and antioxidant on the modulation of 5-FU cytotoxicity.

PB, an aromatic fatty acid, induced cytostasis and apoptosis on a variety of cancer types. PB has been reported to enhance the cytotoxic effect of 5-FU through an increased and sustained expression of p21^{Waf1} [8,9]. PDTC, a thiol-containing anti-oxidant decreased the tumorigenic properties of colon cells administered alone or together with 5-FU through a p53-independent p21^{Waf1} induction [10,11]. Although p21^{Waf1} induction was associated with their chemo-sensitization effect on 5-FU, there was no direct evidence that overexpression of p21^{Waf1} increased the 5-FU sensitivity. But there had direct evidences that overexpression of TS and NF-κB decreased the 5-FU sensitivity [12,13]. We are interested in how the TS and NF-κB activity changed under the concomitant use of PB, PDTC during 5-FU treatment.

The purpose of this study is to propose a novel strategy through triplet-combination of PB, PDTC with 5-FU to increase radiolabelled IdUrd uptake. The strategy may be useful in hepatoma treatment due to the relatively quiescent surrounding normal tissues as well as both 5-FU and radiolabelled IdUrd are suitable for intra-arterial infusion. We also found for the first time that PB and PDTC inhibit the expression of TS and NF-κB during 5-FU treatment through a p21^{Waf1} independent mechanism.

2. Materials and methods

2.1. Chemicals

PB, PDTC, and 5-FU were purchased from Sigma and freshly prepared 10 × working solution in PBS before experiments. IdUrd were purchased from Sigma and [^{125}I] IdUrd were synthesized from our laboratory with methods as previously published with some modifications [14]. Briefly, 100 μL of oxidizing agent (H_2O_2:1N HCl:H_2O = 4:1:95) was added to a 300 μL v-vial coated with 50 μg (0.1 μM) of 5-tributylstannyl-2′-deoxyuridine and containing 20 μL ethanol and 3.7–37 MBq (0.1–1 mCi) sodium [$^{125/131}$I]iodide. The reaction mixture was set aside and vortexed intermittently. After 8 min, the mixture was frozen in liquid nitrogen, lyophilized under vacuum for about 1 h to give the final product as a ''hot kit''. Unreacted [$^{125/131}$I] iodide (in form of I_2 in the presence of oxidizing agent), HCl, solvents (ethanol and H_2O) and oxidizing agent (H_2O_2) were removed during lyophilization. The lyophilized [$^{125/131}$I]IdUrd hot kit was redissolved in ethanol and the radiochemical purity was determined using TLC and HPLC. The retention time of [$^{125/131}$I]IdUrd was 7.2–7.4 min. The labeling yield was >95% and the radiochemical purity was >98%. The lyophilized [^{125}I]IdUrd product was stable up to 3 weeks. The lyophilized [$^{125/131}$I]IdUrd hot kit, if dissolved in physiological saline and eluted through a 0.22 μm apyogenic disk, was ready for biological or clinical application.

2.2. Growth inhibition assay comparing PB, PDTC and 5-FU against hepatoma cell line

Hep 3B hepatoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1 × non-essential amino acids, 2 mM sodium pyruvate and 10% (v/v) heat-inactivated fetal bovine serum (Life Technologies). To evaluate the combination effect of PB and/or PDTC on the cytotoxicity of 5-FU, we determined the growth inhibition effect of each drugs in the presence of PDTC 20 μM or PB 2 mM or both with or without 5-FU 20 μM for 72 h on Hep3B cells. Hep3B cells were seeded in 100 mm tissue culture dishes at a cell density of 1×10^6 cells per dishes, and cultured at 37 °C in a 5% CO_2 incubator. Indicated drugs were added 4 h after seeding. Viable cells were washed three times with phosphate buffered saline (PBS), trypsinized and counted by the trypan blue dye exclusion method. Each condition was expressed as an average of three determinations for the concentration of drugs. In order to understand whether p21^{Waf1} expression is responsible for the 5-FU-enhancing effect from PB and PDTC treatment, we use transient p21^{Waf1}-overexpressed Hep3B cell for experiments. The transfection was performed by liposome with cytomegalovirus-derived expression vector pCR3.1/p21^{Waf1}, containing the human p21^{Waf1} cDNA.

2.3. Growth inhibition of [^{125}I]IdUrd in combination with PB, PDTC and 5-FU

To evaluate the combination effect of [^{125}I]IdUrd on the 3-drug combination, Hep3B cells were pre-treated PB 2 mM, PDTC 20 μM and 5-FU 20 μM for 45 h before adding [^{125}I]IdUrd 0.37 MBq/mL (10 nmole/L), and cold IdUrd 10 μM for another 3 h incubation, then washed the cells with PBS twice and changed with fresh DMEM medium for another 24 h. Cells were washed three times with PBS, trypsinized and counted by the trypan blue dye exclusion method.

2.4. Cell cycle analysis

Hep3B cells treated with or without PB/PDTC/5-FU in different combinations for 48 h were trypsinised, washed twice with PBS. Cell pellets were suspended in 1 mL 70% ethanol for 30 min at −20 °C. 1×10^6 cells were centrifuged and resuspended in 1 mL of propidium iodide staining solution (0.04 mg/mL propidium iodide, 100 μg/mL DNase-free RNase A) and incubated at 37 °C for 20 min.

Flow cytometric analysis was performed using a FACScan cytometer (Becton Dickinson).

2.5. Western blot analysis

For protein analysis, cells were lysed in a buffer composed of 150 mmol/L NaCl, 50 mmol/L Tris (pH 8.0), 5 mmol/L EDTA, 1% (v/v) Nonidet p-40, 1 mmol/L phenylmethylsulfonyl fluoride, 20 μg/mL aprotinin, and 25 μg/mL leupeptin. Total protein concentration of lysates was measured using the Bio-Rad protein assay reagent. Cell lysate (100 μg) was electrophoresed on a 12% polyacrylamide gel, transferred onto Immobilon-P PVDF membrane (Millipore), blocking in the PBS-Tween 20 and 10% non-fat milk for 2 h at room temperature, then filter was incubated with specific antibodies to $p21^{Waf1}$ (Ab-3; Oncogene Science Inc.), TS (Ab-3; MDBio Inc.) and α-tubulin (A-2; MDBio Inc.) for 2 h at room temperature, then with second antibody labeled horseradish peroxidase for 1 h at room temperature. Blots were developed using a chemiluminescent detection system (ECL; Amersham Life Science). The prints were scanned by a VISTA8S scanner (U_{max}), and the optical density of the bands was computer analyzed by NIH 1.6 Image Software (NIH). The relative intensity of bands for the relevant protein was correlated by the relative intensity of the internal control α-tubulin.

2.6. [^{125}I]IdUrd-DNA incorporation

The cells treated with PB or/and PDTC or/and 5-FU in different combinations for 45 h were then exposed to [^{125}I]IdUrd 0.37 MBq/mL and 10 μM IdUrd for 3 h. The cells were harvested and the remaining isotopes were washed three times with PBS and pelleted. DNA was extracted twice with 100 μL of 0.2 N perchloric acid. The insoluble material was incubated at 37 °C for 20 min with 100 μL RNase solution. The reaction was terminated by the addition of 200 μL of 0.2 N perchloric acid and centrifuged at 14,000 rpm for 10 min. The pellet was then transferred for radioactivity counting with an IS-330 Beckman scintillation counter (Fullerton).

2.7. Thymidylate synthase catalytic activity assay

The assay determines the catalytic activity of TS by means of tritiated water released during the TS catalysed conversion of [5-^{3}H] dUMP to dTMP [15]. Cells were treated with indicated drugs for 48 h before harvested. Cell suspension (4×10^6 cells/mL) in 50 mM Tris–HCl buffer, pH 7.4, containing 2 mM dithiothreitol was sonicated on ice (15 s, 23 kHz). Cell extracts were immediately centrifuged at 100,000 × *g* for 30 min (4 °C). The protein concentration of supernatants was measured using the Bio-Rad protein assay reagent. Cytosols were incubated with [^{3}H] dUMP (100 nM final concentration) and 5,10-methylene-5.6.7.8-tetrahydrofolate (0.63 mM final concentration) in a total volume of 55 μL in Tris–HCl buffer. After 25 min of incubation at 37 °C, the reaction was stopped on ice. Excess of [^{3}H] dUMP was removed by adding 300 μL of activated charcoal (15%) containing 4% trichloroacetic acid. After centrifugation at 5000 × *g* for 10 min the radioactivity presented in 150 μL of the supernatants was determined by liquid scintillation counting.

2.8. Transfections and luciferase assay

2×10^5 Hep 3B cells in six well tissue culture plate were transfected with 1 μg of the reporter plasmid pNFκB-Luc (Clontech Laboratories) as previously described [16]. To assure identical transfection efficiency in control and treated cell, cells were replated 24 h after tranfection into 6-well plates, and after attachment they were treated with PDTC 20 μM or PB 2 mM or both for 12 h with or without 5-FU 20 μM added at the last hour as indicated. After cells were harvested, luciferase activity was determined according to the manufacturer's instructions using Dual-Luciferase Reporter Assay System (Promega). Total amounts of plasmids transfected were adjusted to be constant in each experiment by adding an empty vector plasmid. The luciferase activity was normalized by the Renilla luciferase activity and expressed in arbitrary units. Results were performed in duplicate and repeated at least three times with reproducible results.

2.9. Statistical analysis

Results are expressed as mean ± S.D. Statistical difference was assessed by the paired two-tailed Student *t*-test $p < 0.05$ was considered significant.

3. Result

3.1. Effect of PB, PDTC on 5-FU cytotoxicity

Fig. 1 illustrated that either PB or PDTC enhanced the growth inhibition effect of 5-FU. The combination of PB and PDTC with 5-FU had resulted in greater growth inhibition (26.2% of control) than either drug alone. There was a statistically significant sensitization effect on 5-FU by the combination of PB and PDTC when individual growth inhibition effects from PB (86%) or PDTC (66.3%) were normalized back (89% on 5-FU alone group versus 51.6% on PB/PDTC/5-FU combination group, $p < 0.01$). Flow cytometric analysis of cell cycle distribution showed substantial accumulations of cells in S-phase from 5-FU treatment. As shown in Fig. 2, the combination of PB/5-FU, or PDTC/5-FU showed higher percentage of S-phase blockages than 5-FU alone. The 3-drug combination showed the most significant accumulation of S-phase blockage than 5-FU alone (62.4% versus 39.0%, $p < 0.05$).

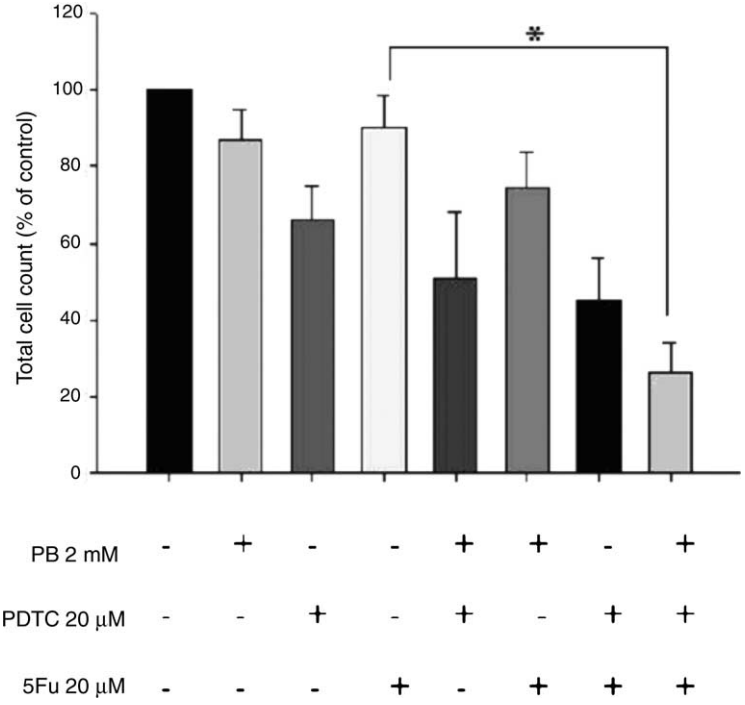

Fig. 1. Effect of PB, PDTC on 5-FU induced growth inhibition. Hep3B cells were treated with single agent PB 2 mM, PDTC 20 μM, 5-FU 20 μM or in different combinations for 72 h before counting for total cell number. Each percentage of growth relative to untreated control represents mean data of three experiments. All data shown are mean ± 1 S.D. (*) Indicates $p < 0.05$.

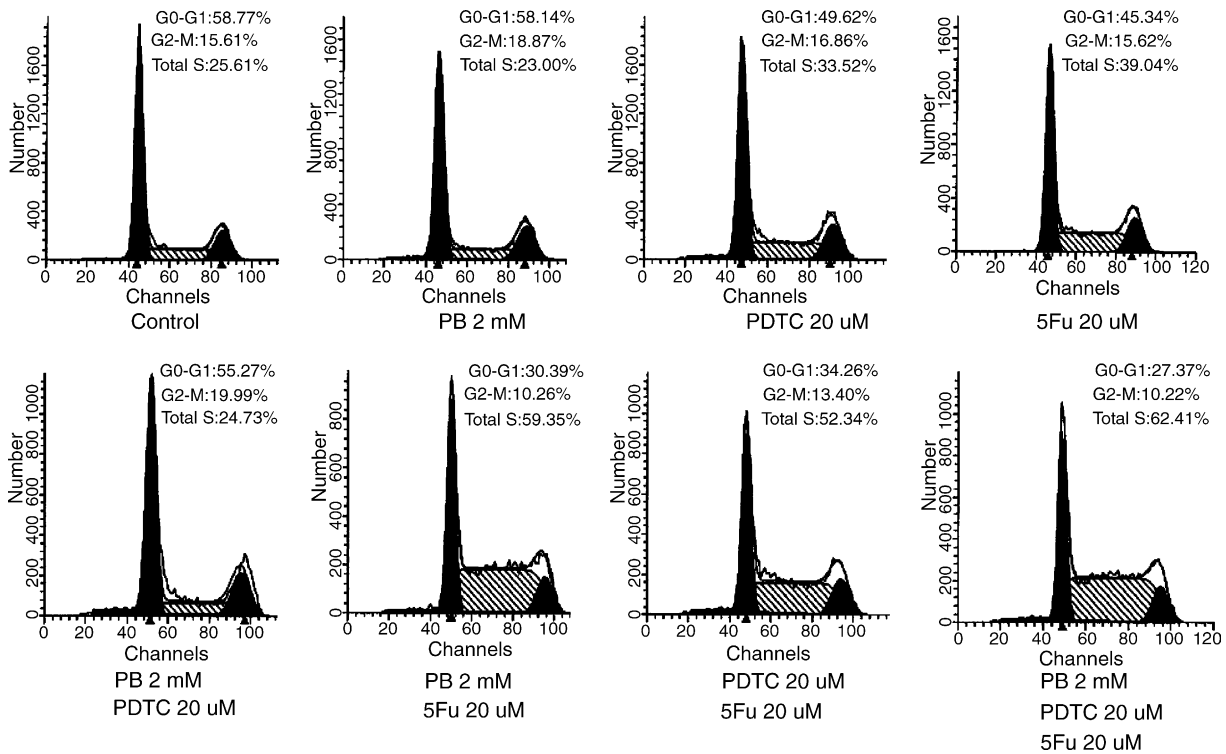

Fig. 2. Representative flow cytometry graphs showed increased S-phase blockage of Hep3B cells from 5-FU treatment. Hep3B cells treated with PB 2 mM, PDTC 20 μM or 5-FU 20 μM in different combinations for 48 h before analyzed for cell cycle distribution. Numbers represent the percentage of cells in each phase of the cell cycle.

3.2. Effect of [^{125}I]IdUrd on the cytotoxicity of PB, PDTC and 5-FU combination

It has been suggested that cold IdUrd increase the incorporation of [^{125}I]IdUrd [17]. We add 0.37 MBq/mL [^{125}I]IdUrd (10 nmole/L) in a concentration of 1/1000 of its surrounding milieu (10 μM/L cold IdUrd) to PB/PDTC/5-FU combination had resulted in a 8.3% growth inhibition of control. As shown in Fig. 3, a statistically significant growth inhibition effect of [^{125}I]IdUrd on the combination of PB, PDTC and 5-FU on Hep3B cells can be observed when growth inhibition effect from [^{125}I]IdUrd (83.3%) was normalized back (28.1% on PB/PDTC/5-FU combination group versus 9.97% on PB/PDTC/5-FU/[^{125}I]IdUrd group, $p < 0.01$).

3.3. The effect of PB, PDTC, 5-FU on [^{125}I]IdUrd-DNA incorporation

As shown from Fig. 4, the radioactivity from [^{125}I]IdUrd incorporated in the DNA was increased by 5-FU treatment. PB and PDTC alone have little effect on the [^{125}I]IdUrd-DNA incorporation. The combination of PB and 5-FU produces higher [^{125}I]IdUrd-DNA incorporation than 5-FU alone, so as the PDTC/5-FU combination. We noticed a mean of 8.5-fold increase in [^{125}I]IdUrd radioactivity after PB/PDTC/5-FU 3-drug combination treatment, which indicated the 3-drug modulation strategy was very effective in increasing IdUrd-DNA incorporation. The p21^{Waf1}-overexpressed cells has similar [^{125}I]IdUrd-DNA incorporation as control Hep3B cells with or without 5-FU (data not shown).

3.4. Effect of PB, PDTC and 5-FU on p21^{Waf1}, TS protein levels and TS enzyme catalytic activity

To ascertain whether PB, PDTC and 5-FU influenced the protein expression of p21^{Waf1} or TS, Western blot was performed. As shown in Fig. 5A, a non-significant change of p21^{Waf1} or TS expressions was induced by PB or PDTC treatment alone. Intriguingly, the combination of PB and PDTC showed a profound down-regulation of TS protein while prevented the TS protein up-regulation from 5-FU treatment. The p21^{Waf1}-overexpressed Hep3B cells did not influence TS protein expression nor did it prevent of TS induction after 5-FU treatment (data not shown). As shown in Fig. 5B, TS catalytic activity assessed by measuring of tritiated water released during the TS catalysed conversion of [5-^{3}H] dUMP to dTMP from free-form TS in cell extract disclosed an increased TS activity from 5-FU treatment. The combination of PB and PDTC decreased the TS catalytic activity and prevented the TS activation from 5-FU. Over expression of p21^{Waf1} had no effect on the prevention of TS activation from 5-FU treatment.

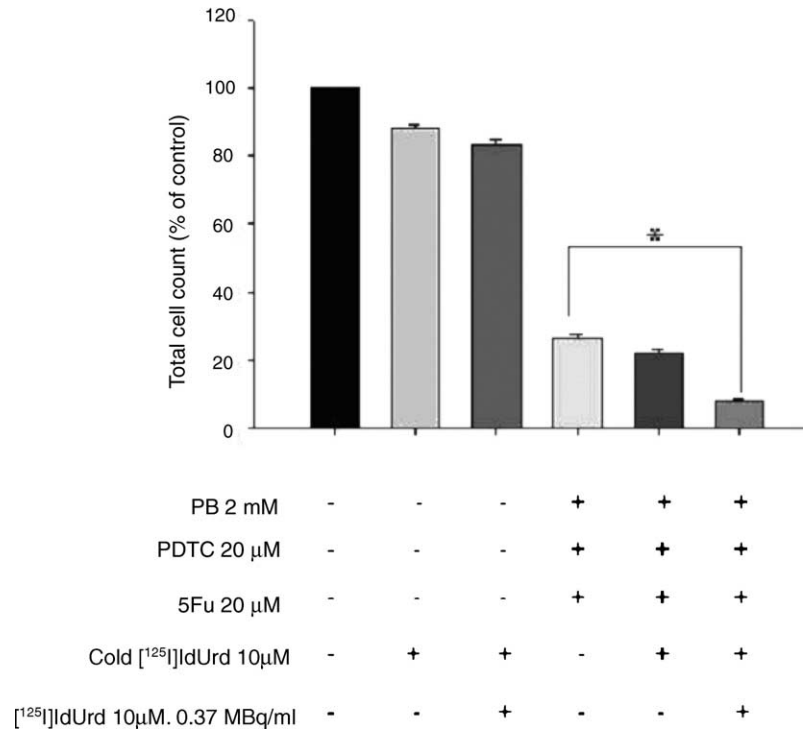

Fig. 3. In vitro growth inhibition of [^{125}I]IdUrd with PB/PDTC/5-FU 3-drug combination. Hep3B cells were treated with drug free medium, PB 2 mM, PDTC 20 μM and 5-FU 20 μM for 45 h before adding cold IdUrd 10 μM with or without [^{125}I]IdUrd 0.37 MBq/mL for another 3 h. Then changed to fresh DMEM medium for 24 h incubation and counting for total cell numbers. Each percentage of growth relative to untreated control represents mean data of three experiments. All data shown are mean ± 1 S.D. (*) Indicates $p < 0.05$.

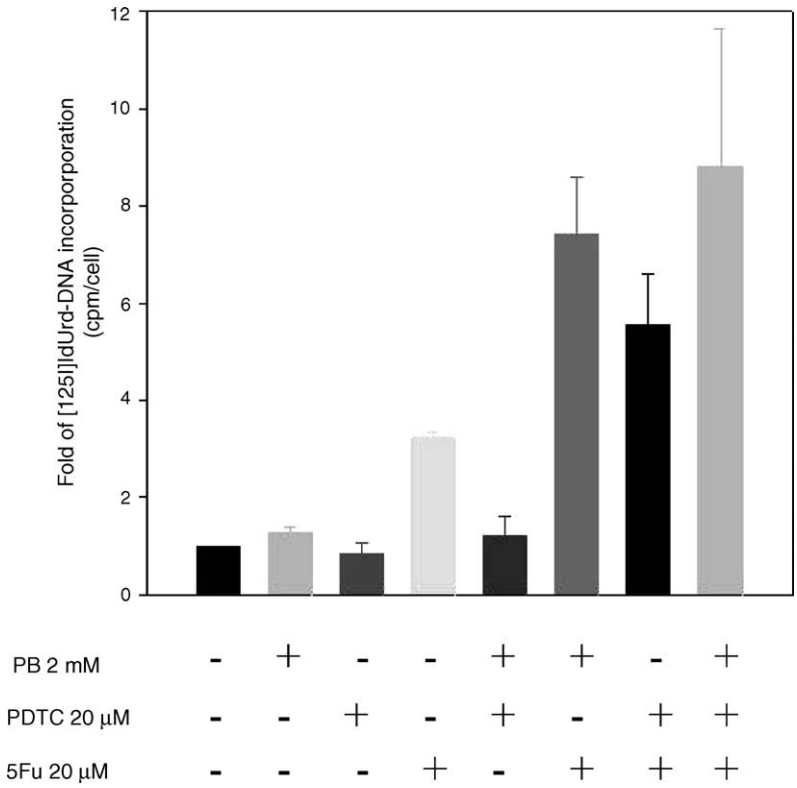

Fig. 4. Effect of PB, PDTC, 5-FU or different combinations on the incorporation of [^{125}I]IdUrd-DNA in Hep3B cells. Hep3B cells were treated with PB 2 mM, PDTC 20 μM and 5-FU 20 μM in different combinations for 45 h before adding cold IdUrd 10 μM with [^{125}I]IdUrd 0.37 MBq/mL for another 3 h. DNA was extracted by PCA precipitation after counting to equal cell number. The pellets were sent for radioactivity counting. Bars represent mean ± 1 S.D. of three independent experiments in triplicate. Results have been represented as fold of control.

3.5. Effect of PB, PDTC and 5-FU on nuclear NF-κB transcriptional activity

NF-κB is an important regulator of programmed cell death and drug resistance. The effect of PB and PDTC on the transcriptional activity of NF-κB was assessed by luciferase reporter assay system. As shown in Fig. 6, the luciferase activity was about 1.25 fold higher, relative to untreated control after 5-FU treatment. The luciferase activity from PB treatment was decreased to 95% ($p = 0.4$) and to 65% by PDTC ($p < 0.05$). However, the combination of PB and PDTC has resulted in a relative luciferase activity of 20% ($p < 0.01$) and 22% without or with concomitant 5-FU treatment, respectively. The data demonstrated that the combination of PB and PDTC inhibited the NF-κB activity and prevented its activation from 5-FU treatment.

4. Discussion

Radiolabelled IdUrd therapy has potential in overcoming chemo-resistant cells through ''reversed-role chemotherapy'' and through its high tumor cytocidal activity by only a few molecules incorporated into tumor DNA [18]. Based on the strategy of ''the higher the TS are inhibited, the higher the IdUrd be incorporated'', we demonstrated here a non-toxic modulation strategy for enhancing of 5-FU cytotoxicity with PB and PDTC for radiolabelled IdUrd therapy. We also found for the first time that the combination of PB and PDTC effectively down-regulated TS and NF-κB through a $p21^{Waf1}$-independent mechanism to enhance 5-FU cytotoxicity.

FU treatment increases S-phase accumulation. The S-phase arrest induced by 5-FU treatment resulted from DNA replication blockage caused by thymidineless environment [19]. The degree of S-phase blockage from different combinations of PB/PDTC and 5-FU were correlated with the degree of cytotoxicities. We did not disclose an induced $p21^{Waf1}$ expression from PB or PDTC treatment alone, which may explain why there was no G0/G1 blockage in Hep3B cells by PB/PDTC treatment.

Antioxidants can not only reduce free radical-dependent DNA damages but also have direct cancer-killing effects. PDTC showed pro-oxidant activity depending on the specific micro-ambient [20,21]. PDTC induced apoptosis in a variety of human cancer cell lines [10,22]. PDTC is a potent inhibitor to prevent NF-κB activation [23]. PB had been reported to induce $p21^{Waf1}$ and led to the appearance of hypophosphorylated Rb and G1 phase arrest, suggesting a role for $p21^{Waf1}$ in PB-induced cytostasis [24]. Both PDTC and PB were known to enhance 5-FU cytotoxicity through $p21^{Waf1}$ induction [8–11]. The $p21^{Waf1}$ was

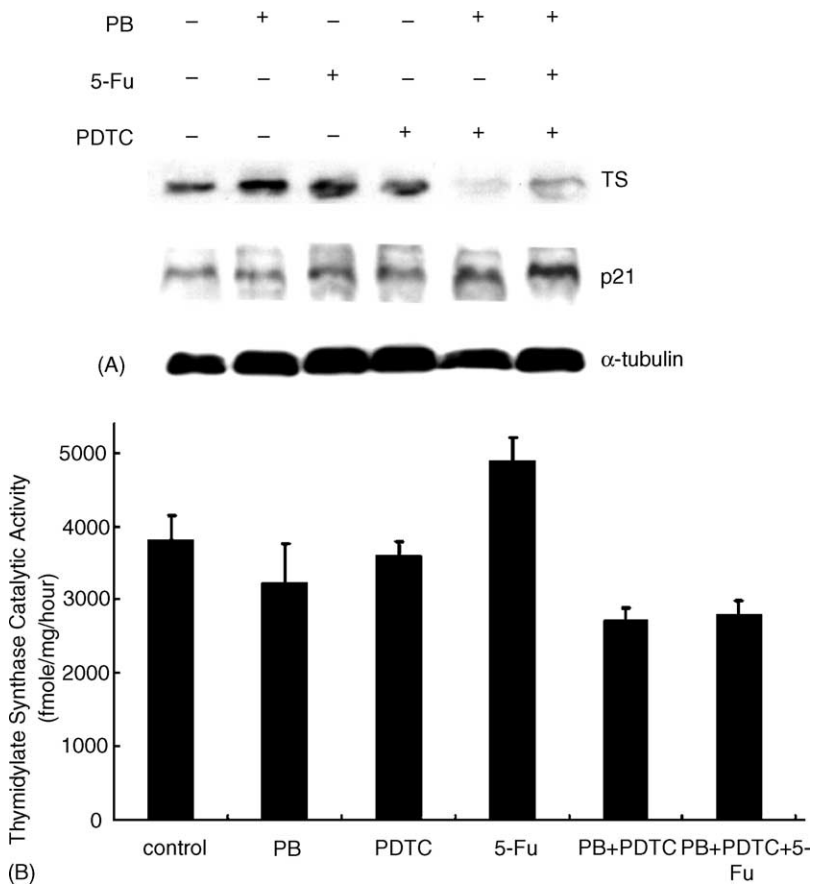

Fig. 5. Effect of PB, PDTC and 5-FU on $p21^{Waf1}$, TS protein expression and TS enzyme catalytic activity. Protein were prepared from Hep 3B cells treated with indicated drugs for 48 h: (A) Western blot analysis was performed using TS, $p21^{Waf1}$ antibody as described in Section 2. α-tubulin used as an internal control, (B) TS catalytic activity was determined as described under Section 2 and was measured in pmol/mg/min.

slightly induced by PB/PDTC combination and to a higher extent by 5-FU/PB/PDTC 3-drug combination, but $p21^{Waf1}$ was not induced under low dose PB or PDTC treatment alone. We have investigated whether $p21^{Waf1}$-overexpressed cell line increased the sensitivity to 5-FU through establishing a $p21^{Waf1}$-overexpressed Hep3B cell line by $p21^{Waf1}$ gene transfer since there was no direct evidence that overexpression of $p21^{Waf1}$ increased the 5-FU sensitivity in the literatures. We could not detect any extra-sensitivity to 5-FU or extra IdUrd-DNA incorporation from 5-FU treatment compared between parent and $p21^{Waf1}$-transfected Hep3B cells. We could not detect overexpressed $p21^{Waf1}$ cells be able to down regulate TS or NF-κB by $p21^{Waf1}$ transfection itself, nor can we detect extra TS and NF-κB down-regulation by PB/PDTC treatment (data not shown). Clearly, there would be other mechanisms involved besides $p21^{Waf1}$ induction.

The literatures regarding TS and its potential role as prognostic and predictive marker in different cancers type remains confusing. Many investigators have demonstrated that a low tumoral TS expression in colorectal cancer patients receiving 5-FU based chemotherapy was related to clinical responsiveness as well as to longer survival [25–27], while many other studies failed to confirm the association between TS intensity and colon cancer outcomes [28,29]. But most in vitro and in vivo study have shown an association between increase TS expression and the development of 5-FU resistance [30,31]. Generally speaking, the major mechanism of acquired-resistance to 5-FU is thought to be overexpression of TS [32]. Besides intrinsic high TS-expression, acute increase in TS expression during 5-FU treatment is associated with decrease sensitivity to 5-FU [33]. The elevation of TS protein levels after 5-FU treatment was found to be the inhibition of a TS autoregulatory translational feedback loop [34]. Down-regulate TS during 5-FU treatment is associated with 5-FU sensitization. Bras-Goncalves et al. had first demonstrated that butyrate combined with 5-FU was able to down-regulate TS expression and sensitized 5-FU [35]. We did not observe TS down-regulation by low dose PB or PDTC treatment alone, but significant down-regulation by the combination of PB and PDTC was observed. The TS induction after 5-FU treatment was also inhibited by the combination treatment.

Cancer cells with activation of NF-κB nuclear activity have demonstrated chemo- and radio-resistance to apoptosis [36,37]. NF-κB inhibition restores sensitivity to Fas-mediated apoptosis by chemotherapy [38]. Wang et al. had

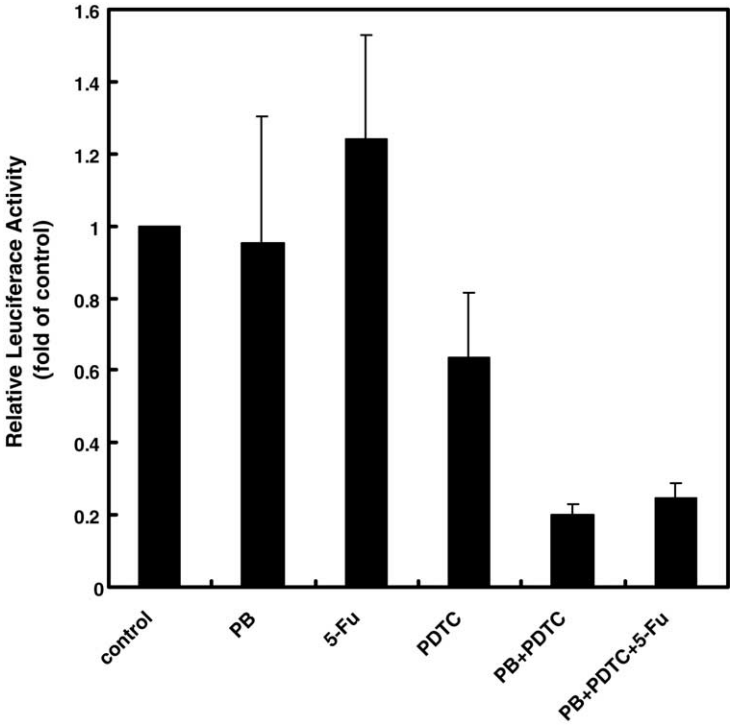

Fig. 6. Effect of PB, PDTC and 5-FU on NF-κB transcriptional activity. Hep 3B cells were treated with PDTC 20 μM or PB 2 mM or both for 12 h with or without 5-FU 20 μM added at the last hour. NF-κB transcriptional activity was assessed by luciferase activity as described in Section 2. Promoter activity is expressed as firefly luciferase activity relative to the internal control (pRL.SV40 *Renilla* luciferase activity). Results are expressed as the mean ± S.D. from three separate experiments.

demonstrated that high constitutive NF-κB activity caused by its gene over expression is an intrinsic character of TS inhibitor-resistant cells [39]. We found in this experiment that the combination of PB and PDTC effectively down-regulate NF-κB activity. The activation of NF-κB from 5-FU treatment was also inhibited by the concomitant use of PB and PDTC. The cytotoxicity of 5-FU is therefore enhanced when NF-κB is down-regulated. One potential advantage of combination PB and PDTC with 5-FU treatment is relatively non-toxic of PB and PDTC to normal tissue. The mechanism by which PDTC and PB increase apoptosis in transformed cells but not their normal component is not known. One possibility maybe due to constitutively active NF-κB transcription factors is required for the survival of many malignant cells [40]. The imbalance condition disturbed from PB/PDTC combination treatment make cancer cells more vulnerable to NF-κB inhibition agents than normal cells.

The combination of PB/PDTC, although is capable to enhance 5-FU cytotoxicity, it may not be able to eradicate hepatoma because hepatoma is resistant to most chemotherapy including 5-FU. The Auger electrons emitted by [^{125}I]IdUrd inside DNA molecules are very toxic to cells and had potential used as targeted radionucleotide therapy. Cancer cells in cycling can be killed by Auger electrons emitted from [^{125}I]IdUrd or β-emitter from [^{131}I]IdUrd, the non-cycling cells can be killed by β-emitter or by by-stander effects [3,41]. Despite promising results from many in vitro and in vivo experiments, the clinical benefits of radiolabelled IdUrd are not impressive. The limitations of radiolabelled IdUrd therapy are the dose-limiting toxicity from bone marrow uptake of IdUrd by systemic delivery and low proportions of S-phase tumor cells for targeting during injection. This cell cycle specific molecular radiotherapy strategy is especially attractive in hepatoma treatment due to the relatively quiescent surrounding normal tissues and the good feasibility of intra-arterial injection. Intermittent intra-arterial delivery of radiolabelled IdUrd through hepatic artery pump had been reported successful for metastatic liver diseases [42]. Buchegger et al. had recently reported close to 20% injected dose was incorporated in tumor DNA by intratumoral injection with fluorodeoxyuridine pre-treatment [43]. We had proved in mice hepatoma model that in situ electrogene transferring of antisense TS gene could down-regulate TS and increase the therapeutic effect of radiolabeled IdUrd therapy [44].

In conclusion, the study supports the use of PDTC and PB in combination with 5-FU through down-regulation of TS and NF-κB. Since both PB and PDTC have been used safely in clinic [45–49], the combination should encourage future clinical trials. Taking the advantage of stronger TS inhibition resulted in higher radiolabelled IdUrd-DNA incorporation, the combination of PB/PDTC/5-FU and [^{125}I/^{131}I]IdUrd might have therapeutic implications for the treatment of hepatoma.

Acknowledgement

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