BIOCHEMICAL PHARMACOLOGY 73 (2007) 1548–1557

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journal homepage: www.elsevier.com/locate/biochempharm

The synergistic interaction of gemcitabine and cytosine arabinoside with the ribonucleotide reductase inhibitor triapine is schedule dependent

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ARTICLE INFO

Article history:
Received 31 October 2006
Accepted 18 January 2007

Keywords:
Gemcitabine
Cytosine arabinoside
Triapine
Synergistic interaction
Ribonucleotide reductase
Deoxycytidine kinase

ABSTRACT

Gemcitabine and ara-C have multiple mechanisms of action: DNA incorporation and for gemcitabine also ribonucleotide reductase (RNR) inhibition. Since dCTP competes with their incorporation into DNA, dCTP depletion can potentiate their cytotoxicity. We investigated whether additional RNR inhibition by Triapine (3-AP), a new potent RNR inhibitor, enhanced cytotoxicity of gemcitabine and ara-C in four non-small-cell-lung-cancer (NSCLC) cell lines, using the multiple-drug-effect analysis.

Simultaneous and sequential exposure (preexposure to 3-AP for 24 h) in a constant molar ratio of 3-AP and gemcitabine was antagonistic/additive in all cell lines. Preexposure to 3-AP at an IC_{25} concentration for 24 h before variable concentrations of gemcitabine was synergistic. RNR inhibition by 3-AP resulted in a more synergistic interaction in combination with ara-C, which does not inhibit RNR.

Two cell lines with pronounced synergism (SW1573) or antagonism (H460) for gemcitabine/3-AP, were evaluated for accumulation of the active metabolites, dFdCTP and ara-CTP. Simultaneous exposure induced no or a small increase, but ara-CTP increased after pretreatment with 3-AP, 4-fold in SW1573 cells, but not in H460 (<1.5 fold). Ara-C and gemcitabine incorporation into DNA were more pronounced (about 2-fold increased) for sequential treatment in SW1573 compared to H460 cells (<1.5 fold). This was not related to the activity and expression of deoxycytidine kinase and the M2 subunit of RNR.

In conclusion, RNR inhibition by 3-AP prior to gemcitabine or ara-C exposure stimulates accumulation of the active metabolites and incorporation into DNA. The combination 3-AP/Ara-C is more synergistic than 3-AP/gemcitabine possibly because gemcitabine already inhibits RNR, but ara-C does not.

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Abbreviations: dFdC, gemcitabine (2′,2′-difluorodeoxycytidine; Gemzar); Ara-C, cytosine arabinoside; dCK, deoxycytidine kinase; dFdCDP, gemcitabine diphosphate; dFdCTP, gemcitabine triphosphate; dCTP, deoxycytidine triphosphate; RNR, ribonucleotide reductase; dNTP, deoxynucleoside triphosphate; dCDA, deoxycytidine deaminase; dCMPD, deoxycytidylate deaminase; HU, hydroxyurea; HCT, a-(N)-heterocyclic carboxaldehyde thiosemicarbazone; Fe, iron; 5-HP, 5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone; 3-AP; Triapine, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid; FCS, fetal calf serum; HBSS, Hank's balanced salt solution; TCA, trichloroacetic acid; SRB, sulforhodamine-B; CI, Combination Index; dATP, deoxyadenosine triphosphate; dGTP, deoxyguanosine triphosphate; dTTP, deoxythymidine triphosphate

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.01.025

1. Introduction

Gemcitabine (2′,2′-difluorodeoxycytidine, dFdC, Gemzar) is a deoxynucleoside analogue used in the treatment of non-small-cell-lung-cancer (NSCLC) [1]. It is frequently combined with other anti-tumor agents such as taxanes and platinum based analogues [1]. Gemcitabine is transported into the cell by nucleoside transporters where it is phosphorylated to its monophosphate by deoxycytidine kinase (dCK) and subsequently to its active metabolites gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP) [2] (Fig. 1). The main mechanism of action of gemcitabine is incorporation into DNA, for which it competes with deoxycytidine triphosphate (dCTP) [3]. dFdCDP inhibits ribonucleotide reductase (RNR) [4], which is increased in several in vitro [2] and in vivo [5] models of acquired resistance to gemcitabine (Fig. 1). RNR is responsible for catalyzing the reduction of ribonucleotides to their corresponding deoxyribonucleotides essential for DNA synthesis and repair of DNA damage. Inhibition of RNR leads to decreased cellular deoxynucleoside triphosphate (dNTP) pools, including dCTP [6]. The latter effect leads to self-potentiation because dCTP competes with dFdCTP for incorporation into DNA [7]. In addition, since dCTP is a feedback inhibitor of dCK, a decrease in dCTP pools will stimulate gemcitabine phosphorylation. Gemcitabine can be inactivated by deoxycytidine deaminase (dCDA) and deoxycytidylate deaminase (dCMPD) [7].

Cytosine arabinoside (Ara-C) is a deoxynucleoside analogue which has no activity against solid tumors, but is frequently used in the treatment of hematological malignancies [8]. The initial activation of Ara-C is also mediated by dCK, while its active metabolite ara-CTP is also incorporated into DNA [9], leading to inhibition of DNA polymerase (Fig. 1). Similar to gemcitabine, ara-C is also transported into the cell by nucleoside transporters and can also be inactivated by dCDA and dCMPD. In contrast to gemcitabine, ara-C is not able to inhibit RNR (Fig. 1).

RNR is a tetramer consisting of two non-identical homodimers. The two identical M2 subunits regulate the substrate specificity of the enzyme, while the other two identical M1 subunits are responsible for the activity by binding the ribonucleotides and allosteric effectors [10,11]. Two types of RNR exist with two different M2 homologues; the p53 independent form (hRRM2) that is linked with the cell cycle and growth control mechanisms and the recently identified p53 dependent RNR (p53R2) that forms a complex with p53 and is thought to be involved in DNA repair in both proliferating and resting cells [12–14]. P53 can interact with p53R2 and hRRM2 at the protein level to regulate RNR activity [15]. The classic RNR inhibitor hydroxyurea (HU) is a poor inhibitor of RNR and has moderate anticancer activity [16]. Other types of RNR inhibitors include deoxynucleoside analogues, polyhydroxy-substituted benzohydroxamic acid derivatives such as amidox, didox and trimidox [17] and iron chelators such as the thiocarbazones. These chelators target the iron in the M2 subunit of the RNR enzyme. 5-Hydroxypyridine-2-carboxaldehyde thiosemicarbazone (5-HP) is 1000-fold more potent than HU, but clinical results were disappointing because it was rapidly glucuronidated and excreted [18,19]. Its analogue 3-Aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine) is resistant to glucuronidation, while HU resistant cancer cells are still sensitive to 3-AP [20,21]. Initial in vitro and in vivo anti-tumor activity were promising [20,22] and the compound is currently being evaluated in several phase I and

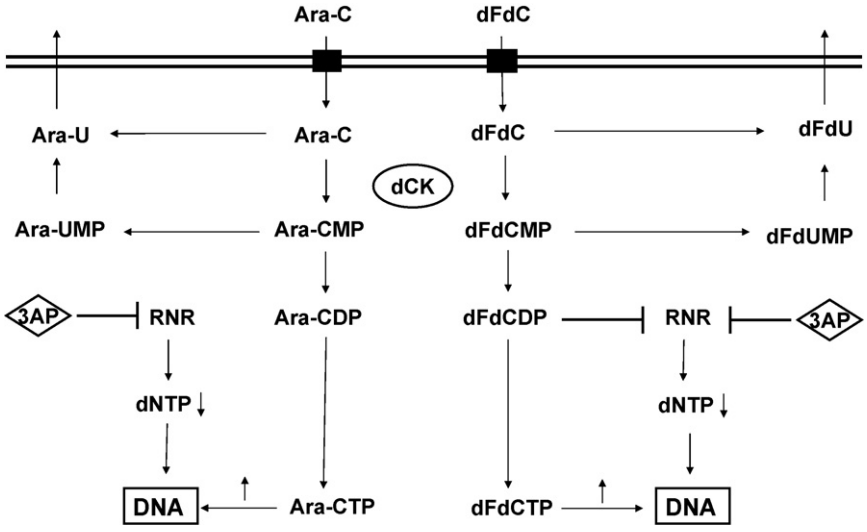

Fig. 1 – Metabolism and hypothetical interaction of deoxynucleoside analogues ara-C (left) or gemcitabine (right) with the RNR inhibitor 3-AP. Both ara-C and gemcitabine are transported into the cell by nucleoside analogue transporters and are phosphorylated by deoxycytidine kinase (dCK) to their monophosphates (ara-CMP, dFdCMP) and subsequently to its diphosphate (ara-CDP dFdCDP) and triphosphate (ara-CTP dFdCTP). Gemcitabine and ara-C triphosphates are incorporated into the DNA leading to DNA damage. Gemcitabine can reduce dNTP pools, but ara-C not. The ribonucleotide reductase (RNR) inhibitor 3-AP will deplete dNTP pools. Decrease of dCTP levels will lead to enhanced ara-C and gemcitabine incorporation into the DNA, since the feedback inhibition by dCTP is diminished. The gemcitabine metabolite dFdCDP already inhibits RNR and can cause some dCTP depletion, but less then for 3-AP. Ara-C does not affect RNR, therefore the interaction between 3-AP and ara-C might to be more synergistic than with gemcitabine.