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The influence of p53 status on the cytotoxicity of fluorinated pyrimidine L-nucleosides

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ABSTRACT

Fluorinated nucleoside analogues are a major class of cancer chemotherapy agents, and include the drugs 5-fluorouracil (5FU) and 5-fluoro-2′-deoxyuridine (FdUrd). The aim of this study was to examine the cellular toxicity of two novel fluorinated pyrimidine L-nucleosides that are enantiomers of D-nucleosides and may be able to increase selectivity for cancer cells as a result of their unnatural L-configuration. Two fluorinated pyrimidine L-nucleosides were examined in this study, L110 ([β-L, β-D]-5-fluoro-2′-deoxyuridine) and L117 (β-L-deoxyuridine:β-D-5′-fluoro-2′-deoxyuridine). The cytotoxicity of these L-nucleoside was determined in primary mouse fibroblasts and was compared with 5FU and FdUrd. In addition, the influence of p53 status on cytotoxicity was investigated. These cytotoxicity assays were performed on a matched set of primary mouse fibroblasts that were either wild type or null for the p53 tumour suppressor gene. It was found that cells lacking functional p53 were over 7500 times more sensitive to the drugs L110, L117 and FdUrd than cells containing wild type p53.

1. Introduction

Nucleoside analogues are utilised as cancer chemotherapeutic agents and include the drugs 5-fluorouracil (5FU) and 5-fluoro-2′-deoxyuridine (FdUrd) (Fig. 1) [1,2]. The drug 5FU is currently used in the treatment of gastrointestinal tract, pancreatic, head and neck, skin and prostate cancers [3,4]; and has been particularly successful in the treatment of colon and breast cancers [3,5,6]. The nucleoside of 5FU, FdUrd, is used to treat hepatic malignancies in addition to colorectal, pancreatic, breast and head and neck cancers [3,5,6].

As tumour cells actively divide, they are heavily reliant on *de novo* synthesis for their source of nucleotides [4]. This characteristic of tumour cells is the basis of the selective toxicity achieved by fluorinated pyrimidines. However, this selectivity is incomplete, as the drugs also target normal dividing cells. As such, toxic side effects such as myelosuppression, stomatitis, nausea, vomiting, diarrhoea, and angor pextoris occur as a result of chemotherapy using 5FU and FdUrd [4]. An additional problem with fluorinated pyrimidines is the development of drug resistance in response to p53 mutation [7]. Consequently, a chemotherapeutic agent with increased selectivity for cancer cells and p53-independent toxicity is required. L-nucleoside analogues could meet these requirements and were investigated in this study.

1.1. Mechanism of action of 5FU and FdUrd

The mechanisms of 5FU and FdUrd cytotoxicity are related, since FdUrd is the nucleoside of 5FU. Once inside the cell, 5FU can be converted into one of four metabolites: fluorouridine triphosphate (FUTP); fluorodeoxyuridine triphosphate (FdUTP); fluorodeoxyuridine monophosphate (FdUMP); or dihydrofluorouracil (DHFU) [1].

5FU exerts its cytotoxic affects in three ways, by inhibition of thymidylate synthase (TS), and by incorporation into DNA, and/or RNA [1,2,8]. All three mechanisms can result in the induction of apoptosis. The most characterised effect of 5FU is the direct inhibition of the enzyme TS by FdUMP. Thymidylate synthase is responsible for the *de novo* synthesis of thymidylate (dTMP) from deoxyuridine monophosphate (dUMP) [3]. This disruption of the nucleotide pools severely disrupts DNA synthesis [3] and leads to apoptosis.

Abbreviations: 5FU, 5-fluorouracil; ANOVA, analysis of variance; DHFU, dihydrofluorouracil; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; FdUMP, fluorodeoxyuridine monophosphate; FdUrd, 5-fluoro-2′-deoxyuridine; FdUTP, fluorodeoxyuridine triphosphate; FUTP, fluorouridine triphosphate; IC_{50}, half maximal inhibitory concentration; L110, [β-L, β-D]-5-fluoro-2′-deoxyuridine; L117, β-L-deoxyuridine: β-D-5′-fluoro-2′-deoxyuridine; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; TS, thymidylate synthase.

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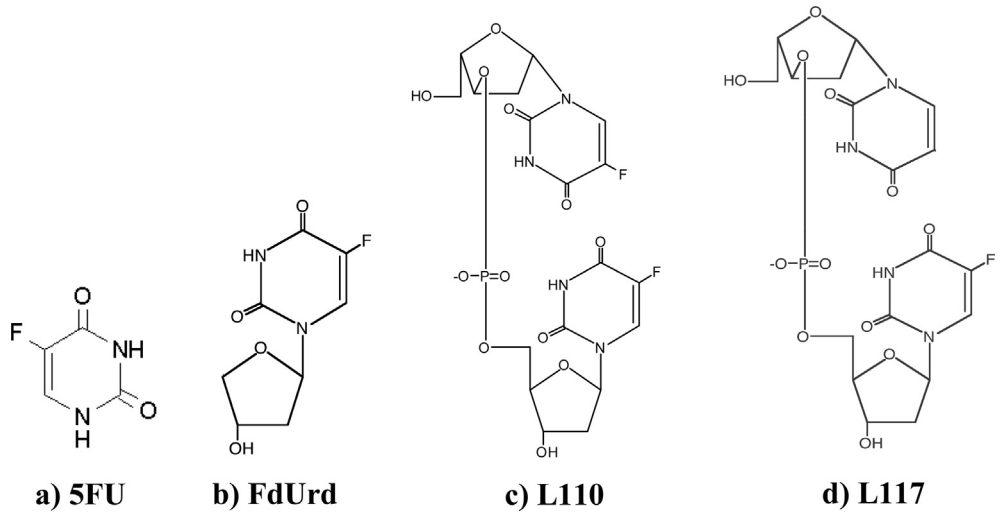

Fig. 1. Chemical structure of the nucleoside analogues used in this study. (a) 5FU: 5-fluorouracil; (b) FdUrd: 5-fluoro-2′-deoxyuridine; (c) L110: [β-L, β-D]-5-Fluoro-2′-deoxyuridine; (d) L117: β-L-deoxyuridine:β-D-5′-Fluoro-2′-deoxyuridine.

One consequence of the severe imbalance in the deoxynucleotide pool caused by TS inhibition is the conversion of FdUMP and dUMP to their respective triphosphate forms, FdUTP and dUTP, and incorporation into DNA [1]. After futile repair [8], single- and double-strand breaks form in the DNA [3]. Apoptosis is again induced as a result of this DNA damage.

The incorporation of 5FU into RNA occurs at a much higher level than its incorporation into DNA [1]. Extensive incorporation of FUTP into RNA disrupts normal RNA processing and function in a variety of ways, affecting both transcription and translation. This has profound effects on cellular metabolism and viability, and can result in the induction of apoptosis [3].

The toxicity of FdUrd is more restricted, with inhibition of TS the sole mechanism of toxicity. FdUrd is phosphorylated by thymidine kinase to FdUMP, which inhibits the enzyme TS. Consequently, FdUrd is only capable of DNA-directed cytotoxicity that results from TS inhibition.

1.2. L-nucleosides

Tumour cells have been found to alter the expression of nucleoside transporters [9,10], a characteristic that could be exploited by the use of nucleoside analogues to target these cells more specifically. The differential expression of nucleoside transporters in cancer cells has lead to the development of a novel strategy aimed at targeting an anti-cancer drug more effectively: the identification and use of a drug that enters cells by a facilitated transport mechanism unique to cancer cells.

L-nucleosides are the stereoisomers of D-nucleosides, which are the normal physiological nucleoside. All nucleoside analogues currently in use as anticancer agents are in the D configuration [11]. Considering the extensive use of D-nucleoside analogues in anticancer chemotherapy, the potential of L-nucleoside analogues as anticancer agents was investigated [12,13]. To date, only one L-nucleoside analogue with anticancer activity has been developed, troxacitabine, and it has been found to exhibit potent antitumour activity against a broad range of tumours [12–14].

L-nucleosides are promising anticancer drugs for a number of reasons. Firstly, they show toxicity against a number of solid tumours, such as hepatocellular and prostate tumours, which are unresponsive to D-nucleoside analogues [12]. Secondly, L-nucleosides exhibit greater metabolic stability than their D-nucleoside counterparts [15]. Thirdly, it is theorised that nucleoside transporters of normal cells will exhibit stereospecificity to permit entry of only the D-enantiomer. As changes in nucleoside transport and metabolism occur with neoplastic transformation, it is possible that only tumour cells that have developed altered transporters will be capable of taking up the L-nucleoside analogue. It is proposed that L-nucleoside analogues may be able to reduce the occurrence of toxic side effects by targeting cancer cells more selectively.

1.3. The p53 tumour suppressor gene

The p53 tumour suppressor gene encodes a 53 kDa phosphoprotein which accumulates in the nucleus in response to cellular stress [16–18]. Functional p53 acts as a transcription factor that regulates the expression of a large number of genes involved in cell cycle control, the induction of apoptosis, genetic stability, DNA repair, differentiation control, and angiogenesis. The p53 gene is mutated in over 50% of human tumours, making it the most frequently mutated gene in human cancer [19–21].

The induction of apoptosis in cancer cells is a common mechanism of action for a number of chemotherapy drugs, including the fluorinated pyrimidines [15]. Consequently, the loss of the p53 apoptotic pathway may influence the sensitivity of the cells to the drug. This appears to be the case for 5FU. *In vitro* studies have reported that loss of p53 function reduces cellular sensitivity to 5FU, if not abolishing it entirely [22–24]. For example, the loss of functional p53 in a colon cancer cell line made cells strikingly resistant to apoptosis induced by 5FU compared to the parental cell lines [22]. In addition, p53 null mice were found to be resistant to apoptosis triggered by 5FU [25]. The correlation between inactivation of p53 and resistance to 5FU has now been reasonably well established, both in patients and in experimental systems [9,10]. 5FU has been shown in human lymphoblastoid TK6 cells to induce genes associated with the p53 pathway [26].

Considering the fact that p53 is mutated in over 50% of human cancers, the resistance described above significantly inhibits the efficacy of the nucleoside analogue 5FU in chemotherapy. Although apoptosis of cells negative for p53 can still occur via other apoptotic pathways remaining in the cancer cell [15], an anticancer drug that