Roscovitine inhibits ongoing DNA synthesis in human cervical cancer

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Abstract

The effect of roscovitine, a purine analogue and cyclin dependent kinase inhibitor, on DNA synthesis rate in tissue mini-units obtained from human cervical cancers was investigated. Roscovitine (100 μM) gave a DNA synthesis rate inhibition by 61% (P < 0.0001; range 23–93%) within 30 min of incubation. This inhibitory effect was concentration-dependent. The results suggest that the inhibition of tumor DNA synthesis rate is due to a direct effect on the DNA synthesis machinery via presently unknown mechanisms. In addition, the potential application of CDKs inhibitors as preventive agents is discussed. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Cervical cancer is the second most frequent cancer among women worldwide and the most frequent cancer in Africa, Asia and South America [1]. Of the estimated 13 700 women in the United States in whom invasive cervical cancer was diagnosed in 1998, nearly 5000 will ultimately die due of the inadequacies of current treatment [2].

Early cervical cancers can be successfully treated with radical surgery but more advanced cancers (e.g. bulky stage IB) have poorer prognosis [3]. The ability of radiotherapy to cure locally advanced cervical cancer is limited by the size of the tumor, because the doses required to treat large tumors exceed the limit of toxicity in normal tissue [4]. To overcome this problem, concurrent radiotherapy and chemotherapy has been used. Radiotherapy combined with hydroxyurea compared with radiotherapy alone significantly increased the rate of complete response, progression-free survival, and overall survival [5].
Recently, regimens of radiotherapy and chemotherapy that contains cisplatin have been shown to improve the rates of survival and progression-free survival among women with locally advanced cervical cancer [3,6]. However, a significant percentage (31%) of the patients died during the 35 months follow-up [6]. In addition, most women who have been treated for cervical cancer have persistent vaginal changes that compromise sexual activity and result in considerable distress [7]. These data indicate that new therapeutical approaches are required to improve the survival rate and quality of life of patients carrying cervical cancer.

Roscovitine is a potent and specific inhibitor of the CDKs [8] that inhibits the growth of several human cancer cell lines [8,9] as well as the mesangial cell proliferation in experimental glomerulonephritis [10]. Previous data have shown that roscovitine inhibits the growth of several human cancer cell lines [8,9] and studies using roscovitine in rats [10] and flavopiridol in clinical trials [11] showed that CDK inhibitors have low in vivo systemic toxicities. Moreover, roscovitine blocks – with relatively high selectivity – DNA synthesis connected with replicative processes [12]. Abnormal regulation of cyclin dependent kinase inhibitors have been reported in cervical cancers [13,14]. In addition, increased CDK activity was found in E7-expressing oncoprotein [a protein that interacts with the cyclin dependent kinase inhibitor (CDKI) p21] [15], in differentiating keratinocytes [16]. These data suggest that CDK modulators could be of interest for the treatment of cervical tumors.

In this work we have used a proliferation assay based on the use of tissue mini-units [17] to study the effect of roscovitine on ongoing DNA synthesis in specimens obtained from human cervical cancers. Our result indicate that roscovitine inhibits ongoing DNA synthesis of human cervical tumor in a concentration-dependent manner.

2. Materials and methods

Residual cervical tissue specimens (totally 18 specimens; two of these specimens were technically impossible to process) were obtained from 15 patients at routine tumor biopsy. Clinical data for the 15 patients are summarised in Table 1.

Generation of tissue mini-units, determination of DNA synthesis rate and sensitivity to roscovitine were done as previously described [12]. The DNA synthesis rate of human cervical tumors and the effect of 100 μM roscovitine was studied as follow: mini-units of tumor tissue were incubated with DMEM containing 4 μCi/ml [methyl-3H]-thymidine plus DMSO alone (control) or 100 μM roscovitine for 90 min. This concentration of roscovitine was chosen since it has been shown to inhibit cell proliferation in different tissues [8,9,12,18]. A total of 90 min were chosen as standard incubation time since this time gave a suitable difference in radioactive precursor incorporation between control and experimental samples [12,18]. Furthermore, the 90 min incubation time minimizes metabolic alterations inherent to the tumor incubation system.

The effect of roscovitine was determined as the percentage of inhibition of DNA synthesis rate (Table 1) or the percentage of DNA synthesis (Fig. 1).

3. Results

As shown in Table 1, the 15 patients included in this study had a median age of 57 years (ranging from 30 to 72 years). The tumors were of Federation of Gynecology and Obstetrics (FIGO) stages IB1–IIIB.

A high (around 30-fold) intertumoral variation of the spontaneous DNA synthesis rate was observed (Table 1). Roscovitine at 100 μM decreased the DNA synthesis rate of all cervical tumors (Table 1). The percentage of inhibition was ≥50% in 10/16 (62%) cases (range 23–93%). The average percentage of inhibition was 61 ± 21% (P < 0.0001).

In order to establish if the effect of roscovitine was concentration-dependent, the tissue mini-units prepared from cases numbers 11 and 15 were incubated during 90 min in DMEM containing 4 μCi/ml [methyl-3H]-thymidine and 0, 10, 50 and 100 μM roscovitine. Roscovitine treated mini-units showed a concentration-dependent inhibition of the DNA synthesis (Fig. 1) that grossly correlates with the sample sensitivity.

In addition, the temporal effect of roscovitine on incorporation of the radioactive precursor was studied in tissue mini-units prepared from two different patients (cases numbers 1 and 12). The tissue mini-units were incubated with DMEM containing 4 μCi/
ml [methyl-3H]-thymidine plus DMSO alone (control) or 100 μM roscovitine for 30, 60, and 90 min. The result showed that roscovitine decreased DNA synthesis within 30 min (Fig. 2).

4. Discussion

In this paper, we show that roscovitine inhibits the DNA synthesis rate in tissue mini-units prepared from human cervical cancer (Table 1) in a concentration-dependent manner (Fig. 1) and with a short-time onset (Fig. 2). The concentration needed to inhibit the spontaneous DNA synthesis rate by 50% (IC50) was ≤10 μM for case no. 11 and ~100 μM for case number 15. The reduced inhibition of DNA synthesis in case number 15 could be due to an insufficient roscovitine concentration rather than to a roscovitine-insensitive DNA synthesis. A concentration of 100 μM roscovitine inhibited the DNA synthesis rate in all specimens and gave an average inhibition of ~61%.
The inhibitory effect of roscovitine reported here is in agreement with those previously reported for normal developing rat cerebral cortex [18] as well as human gliomas [12].

Roscovitine could decrease the DNA synthesis rate of human cervical cancer cells by at least two mechanisms: (1) a short-time onset mechanism as reported here as well as for human gliomas [12] and probably mediated by a direct effect on the DNA replication machinery; and (2) a long-time onset mechanism of action probably due to inhibition of cell cycle transitions by inhibition of CDK activities [8,9] or other possible targets of roscovitine such as Erk 1, Erk2, S6 kinase and CaM kinase II [19]. Finally, we hypothesised that roscovitine (or other/s CDKs inhibitor/s) might also be useful as a preventive drug by inhibiting the replication of HPV and thereby inhibiting malignant progression of lesions. Our hypothesis is based on the following data: Cervical cancer has been shown to have human papillomavirus (HPV) infection as a central causal agent with more of 95% of all cervical carcinomas harboring high risk HPVs [20]. Low risk HPVs are associated with lesions with a very low frequency of malignant progression, whereas the lesions caused by the high risk HPVs can undergo carcinogenic progression [21]. The highest risk group HPVs encode two viral oncoproteins, E6 and E7, which are expressed consistently in cervical cancers [16]. The HPV E7 oncoprotein: (1) interacts with the cyclin dependent kinase inhibitor (CDKI) p21 and blocks the inhibition of CDK activity and PCNA-dependent DNA replication [15]; and (2) uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21cip1-mediated inhibition of CDK2 [16]. There is evidence that malignant progression of cervical cancers could be due to elevated CDK activities by the interaction of the HPV E7 oncoprotein with P21 [16]. It has recently been reported that HPV E7 oncoprotein rapidly convert telomerase negative and TGFβ sensitive conditionally immortal cells to a fully immortal phenotype [22]. The role of HPV E7 in malignant transformation is still unclear since CKI neutralization by HPV E7 oncoprotein seems to be required for viral DNA replication rather than for malignant transformation of the host cells [23]. However, the available data suggest that the interactions between the HPV E7 oncoprotein and cell cycle proteins are important for the aetiology of cervical cancers. It is known that the HPV E7 oncoprotein can overcome several forms of G1 arrest that are induced by various antiproliferative signals [23]. In addition, Duensing et al. reported that treatment of E7 expressing U2OS cells (a human osteosarcoma cell line) with a low roscovitine concentration as 5 μM decreased the proportion of cells with abnormal centrosome number within 24 h. This effect was probably mediated through the inhibition of cdk2 activity [24]. Abnormal centrosome synthesis induced by HPV type 16 E7 oncoprotein was shown to be an early event during neoplastic progression leading genetic instability [25]. The genetic instability probably plays some role in the viral life cycle by contri-
buting to the integration of the HPV DNA into the host genome [24]. Although the authors did not measure the effect of roscovitine on HPV replication it is tempting to speculate that the reduced proportion of cells with abnormal centrosome number will result in a reduced number of viral particles that in turn, might further reduce malignant progression. Such an effect is possible since CDK activity is required for the efficient replication of other viruses such as herpes simplex [26,27], adenovirus [28] and HIV-1 [29] and might represent a potential application of CDKs inhibitors.

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References


