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Research article

Evaluation of roscovitine anticancer agent as a reference compound for cancer and apoptosis studies

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Abstract

Apoptosis induction is a well-known strategy to mitigate the growth of cancerous cells. The aim of this study is to determine the possibility of using roscovitine as a reference agent for evaluating novel pro-apoptotic agents. This was conducted through evaluating its cytotoxic activity of roscovitine using MTT assay on HCT-116, MCF-7 and HepG2 cancer cell lines, as well as WI-38 normal cell line, and its ability to induce apoptosis by means of measuring the concentration of p53 and caspase 3 using ELISA technique. Roscovitine was found to exhibit a potent cytotoxic activity on all the cancerous cells, while normal cells was less affected after roscovitine treatment. In addition, the levels of p53 and caspase 3 in HCT-116 cell line were raised by 18.25 and 10.77 folds, respectively, following treatment with roscovitine. Thus, it was assured that roscovitine is a potent apoptosis inducing agent, and so it could be used as a reference compound in studying novel pro-apoptotic compounds.

Introduction

Apoptosis is a type of programmed cell death, which is perceived as an essential mechanism for numerous vital processes such as normal turnover of cells and eradication of cells with DNA mutations [1]. Apoptosis suppression is the main hallmark of cancerous cells, thus its reactivation is considered a promising strategy in treating cancer [2]. It can be induced through an internal or an external stimuli that initiate a sequence of a precisely controlled reactions leading to cell death [3]. P53 is a tumor suppressor protein that is involved in controlling numerous vital cellular functions including cellular proliferation, differentiation, cell cycle, angiogenesis, DNA repair and apoptosis [4]. It can induce apoptosis as a result of different stress signals, such as DNA damage or mutation, which stabilize and activate p53 protein [5]. Mutation of p53 or loss of its function is associated with cancer growth and resistance, while its upregulation results in cell cycle arrest and induction of apoptosis [6]. The execution of p53-dependent apoptosis proceeds through the activation of caspases, which are a family of cysteine-dependent endoproteases. Two main pathways are involved in p53-dependent activation of caspases; the intrinsic and the extrinsic apoptotic pathways [7]. The intrinsic pathway is activated through stress stimuli such as lack of growth factors or DNA damage. Where, p53 upregulates the pro-apoptotic (BAX) and downregulates the anti-apoptotic (Bcl-2) members of Bcl-2 proteins family. These expression alterations allow the release of mitochondrial apoptogenic factor cytochrome c to the cytosol leading to apoptosome formation. Which is formed from both caspase 9 and the adapter molecule Apaf-1, in order to activated caspase 9 which then cleaves and activates the effector caspases (caspase 3, 6 and 7) [8,9]. Whereas, the extrinsic pathway is triggered by the ligation of members of death receptors family, such as CD95/Fas/APO-l and tumour necrosis factor receptors, with their ligands. However, p53 can regulate the expression of these receptors either in transcriptional or non-transcriptional manner. This interaction triggers the formation of the death inducing signalling complex (DISC) from caspase 8 and the adapter molecule FADD. Caspase 8 is activated upon DISC formation, then it activates the effector caspases [10].

Seliciclib (R-Roscovitine, (2R)-2- {[6-(Benzylamino)-9-isopropyl-9H-purin-2-yl] amino}- 1-butanol) is a substituted purine derivative that currently undergoes phase II clinical trials as an anticancer agent. It is a potent inhibitor of cyclin-dependent kinases (CDKs) as it competes with ATP for its binding site [11, 12]. It has been reported that roscovitine is suggested to cause cell cycle arrest in the G1 and G2/M phases and apoptosis in p53-dependent manner [13, 14]. The aim of this study is to evaluate the effect of a roscovitine on different cancer

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and normal cell lines, in order to use it as a reference compound in anticancer studies.

Materials and methods

Cell culture

Human breast adenocarcinoma MCF-7, human hepatocellular carcinoma HepG-2, human colorectal cancer HCT-116 and human diploid lung fibroblasts WI-38 cell lines were purchased from VACSERA Tissue Culture Unit (Giza, Egypt). HCT-116 and MCF-7 cells were maintained in RPMI 1640 medium (Lonza Verviers SPRL., Verviers, Belgium) at 5% CO_2 and 37°C, while HepG-2 and WI-38 cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM; Lonza Verviers SPRL., Verviers, Belgium) at 5% CO_2 and 37°C. Both media were supplemented with 10% fetal bovine serum (FBS; Life Science Group L, UK) and 1% penicillin/streptomycin (Lonza Verviers SPRL., Verviers, Belgium).

In vitro cytotoxicity assay

The cytotoxic effect of roscovitine was assessed on MCF-7, HepG-2, HCT-116 and WI-38 cell lines through MTT assay. MTT [3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide)] reagent was supplied by Tocris Bioscience (Bristol, UK). Cells were plated at a sub-confluent density in a 96-well microtiter plate, and incubated overnight. Then, 100 µL of different concentrations of roscovitine (LC Laboratories Massachusetts, USA) ranging from 100 to 0.1 µM were added to the test wells in triplicates, while 100 µL of the culture media was added to the untreated control wells. Later, the plates had been incubated for 24 hrs before MTT assay protocol was carried out adopting the MTT assay protocol previously described [15]. Optical density was measured at 590 nm by a Varioskan micro plate reader (Thermo Fisher Scientific, K.K., Kanagawa, Japan). Finally, the drug's dose-response curves were traced, in order to determine roscovitine 20, 50, and 80% inhibitory concentrations (IC_{20}, IC_{50}, and IC_{80}, respectively) and standard deviation (S.D) values, using Origin-Lab software (9.7.0.185 trial version, OriginLab Corporation, MA, USA).

In vitro detection of p53 using ELISA immunoassay

Effect of roscovitine on the expression level of P53 in HCT-116 cell line at the IC_{50} concentration was evaluated via Human p53 ELISA Kit (RAB0500 Sigma, Merck KGaA, Germany), against untreated cells (negative control), according to the manufacturer instructions. Optical densities were measured at 450 nm using Robonik P2000 ELISA reader [16, 17].

In vitro detection of caspase 3 using ELISA immunoassay

Effect of roscovitine on caspases-3 at HCT-116 cell line at its IC_{50} concentration was measured using Caspase-3 (active) human ELISA kit (Cat. No. KHO1091; Invitrogen Corporation, California, USA) against untreated control cells (negative control), according to the manufacturer instructions. Optical densities were measured at 450 nm using Robonik P2000 ELISA reader [18, 19].

Results and discussion

In vitro cytotoxicity assay

The cytotoxic effect of roscovitine was evaluated through calculating the average growth percentage of each concentration on HCT-116, MCF-7, Hepg-2 and WI-38 cell lines and tracing the dose response curves, figure 1, then calculating IC_{20}, IC_{50} and IC_{80} ± S.D values, figure 2. According to these results, the two most sensitive cell lines for roscovitine treatment were MCF-7 and HCT-116 with IC_{50} values = 9.32 ± 0.49 and 12.24 ± 1.17 µM, respectively. Whereas the least affected cell line was WI-38 cell line as roscovitine IC_{50} = 29.5 ± 3.84 µM. Moreover, the effect of roscovitine on all the cancerous cell lines, MCF-7, HCT-116 and HepG-2 was significantly higher than its effect on normal cell line, WI-38. This shows that roscovitine might have a selective cytotoxic activity towards cancer cells.

In vitro detection of p53 using ELISA immunoassay

Owing to the effect of p53 protein on both the intrinsic and the extrinsic apoptotic pathways, the effect of roscovitine on the level of p53 tumour suppressor protein in HCT-116 was assessed using ELISA technique to evaluate its pro-apoptotic activity. Results showed that the level of p53 was raised by 18.25 folds from 43.55 ± 4.98 pg/mL in untreated cells to 794.9 ± 23.71 in roscovitine treated cells, figure 3. These findings indicate that roscovitine could induce apoptosis in cancer cells in p53-dependent manner.

In vitro detection of caspase 3 using ELISA immunoassay

Since caspase 3 is considered the executioner caspase which activation is the optimum goal for any apoptotic pathway, the level of caspase 3 protease was estimated in HCT-116 roscovitine treated and untreated cells. Roscovitine was found to be able to increase caspase 3 level by 10.77 folds to 496.2 ± 8.63 in treated cells compared to 46.08 ± 0.76 in control cells, figure 3. These results shows that roscovitine has a significant pro-apoptotic effect on HCT-116 colon cancer cells.

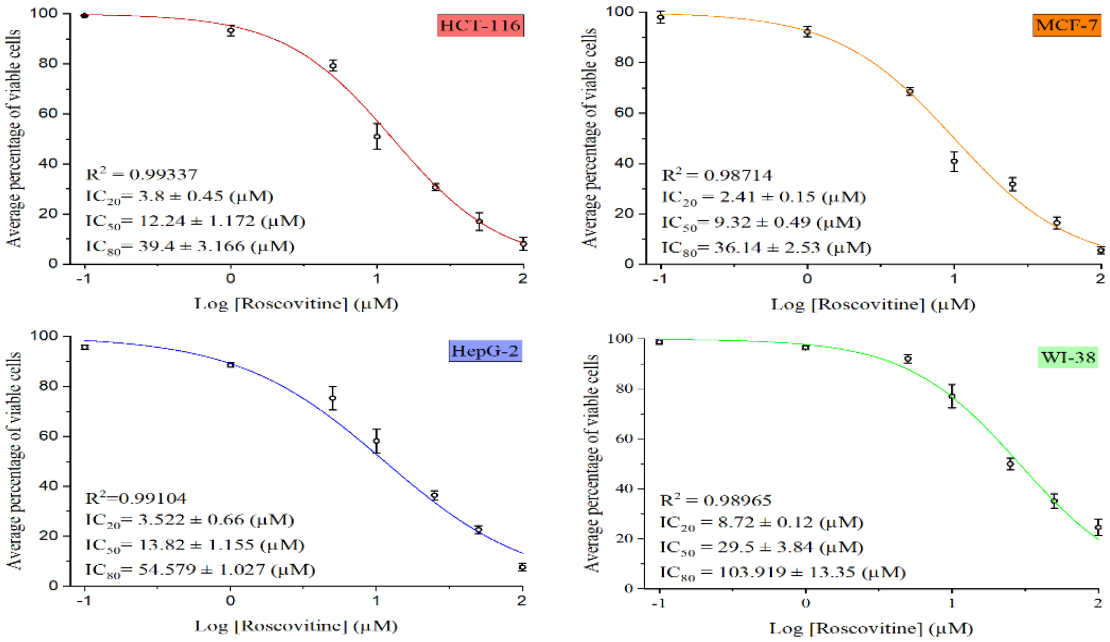


Figure 1. Dose response curves of roscovitine on HCT-116, MCF-7, HepG-2 and WI-38 cell lines.

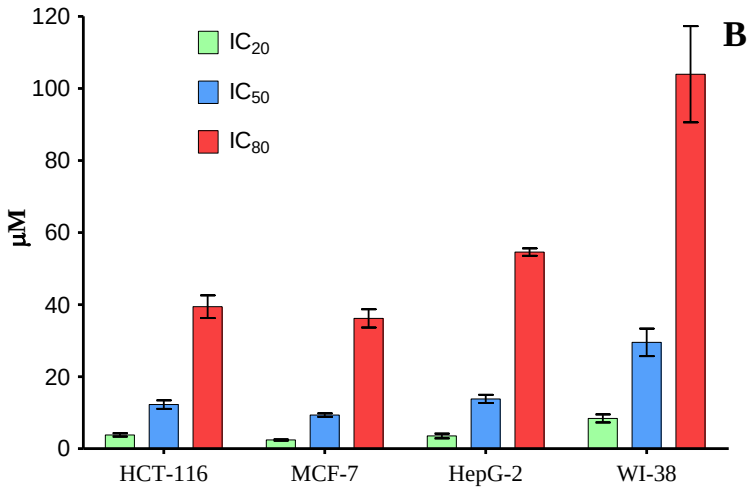


Figure 2. IC_{20}, IC_{50} and IC_{80} ± S.D values of roscovitine on HCT-116, MCF-7, HepG2 and WI-38 cell lines. The results are the mean of three independent experiments.

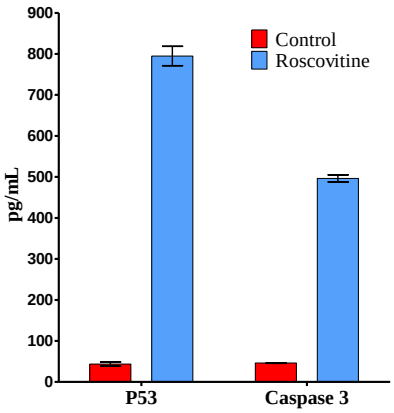


Figure 3. The effect of roscovitine on level of expression of both p53 and caspase 3 in HCT-116 cells versus control untreated cells. Results are the mean of three independent experiments ± S.D values.

Conclusion

From the results, it could be concluded that roscovitine cytotoxic effect is more selective to cancerous cells over normal cells, and it exerts a potent pro-apoptotic effect through p53-dependent pathways. Moreover, roscovitine could be used as a reference compound for studying novel anticancer agents that are designed to trigger apoptosis in cancer cells.

Conflicts of interest

The authors declare no conflict of interest.

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