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In Vitro Antitumor Activity of Triple-Modified Colchicine Derivatives

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Abstract

Colchicine, an alkaloid extracted from the Colchicum autumnale plant, is a potent microtubule polymerization inhibitor both in vitro and in vivo. Although the parent molecule colchicine exhibits greatly high anticancer activity in vitro and in vivo, due to the high toxicity of colchicine, the use of colchicine has been limited. Previous studies have indicated that different modifications of colchicine enhance its anti-proliferative activity against several types of cancer cells. Thus, I study a series of triple-modified colchicine derivatives antitumor activity against a variety of cancer cells in vitro. There are three main possible results: (1) Triple-modified colchicine derivatives have better anti-proliferative activity against all cell lines than compound 1; (2) Triple-modified colchicine derivatives have better anti-proliferative activity against cancer cell lines than compound 1; (3) Triple-modified colchicine derivatives have better anti-proliferative activity against normal cell lines than compound Regardless of these results, further studies are going to focus on doing similar experiments in vivo and finding more effective drug inhibitors, as well as exploring the specific regulation mechanism of colchicine precisely.

Keywords

Triple-Modified Colchicine Derivatives; ALL-5; Antitumor Activity.

1. Introduction

Chinese medicine plays an important role in the treatment of human diseases and is a foundation for research and development of new drugs [1]. Alkaloid has antibacterial and anti-inflammatory properties and is often an effective component of many Chinese herbal medicines and medicinal plants. For example, ephedrine in ephedra has antiasthmatic effects and morphine in opium has a strong analgesic effect. Colchicine, an alkaloid extracted from the Colchicum autumnale plant, is one of the oldest drugs known to people. Its structure consists of three rings, namely, ring A, a benzene ring with a trimethoxy group; ring B, a heptameric ring with an acetamide group; and ring C, a tropolonic ring (Figure 1) [2].

Colchicine can cause cells to accumulate in mitotic arrest during cell cycle powerfully and inhibit the growth of cancer cells [3]. It’s used clinically to treat different diseases such as fibrotic disorders, acute gout, and Behcet's disease [4-7]. Although the parent molecule colchicine exhibits very high anticancer activity in vitro [1], due to the high toxicity of colchicine [8], the use of colchicine as anticancer medicines has been restricted. Thus, in order to further improve anticancer activity and reduce toxicity to normal cells, we must pour our efforts into the development of clinically applicable colchicine derivatives [9-14]. Over the past decade, scientists have made a number of achievements in this research area and synthesized several derivatives exhibited strong antitumor activities [15]. Chiba University researchers published a study of 4-Halocolchicines in 2011 [15]. Also modified colchicine derivatives in the C-10 position were reported by Lihong Shen [3]. 4-Halocolchicines and

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C-10 modified derivatives showed strong antitumor activity. However, little information has been done on triple-modified colchicine derivatives and data about it is still scarce, so further studies are necessary. View of this, In the work, the aim of this paper is to study their antitumor activity against normal cells and a variety of cancer cells. It is hoped that the seven triple-modified colchicine derivatives have a better effect on the anti-proliferation than the parent colchicine against cancer cells *in vitro*.

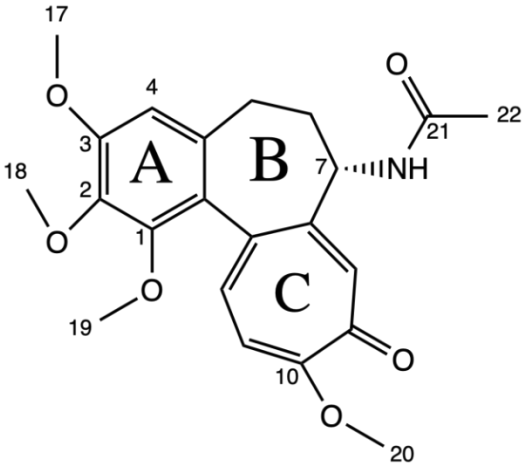

Fig 1. Colchicine structure.

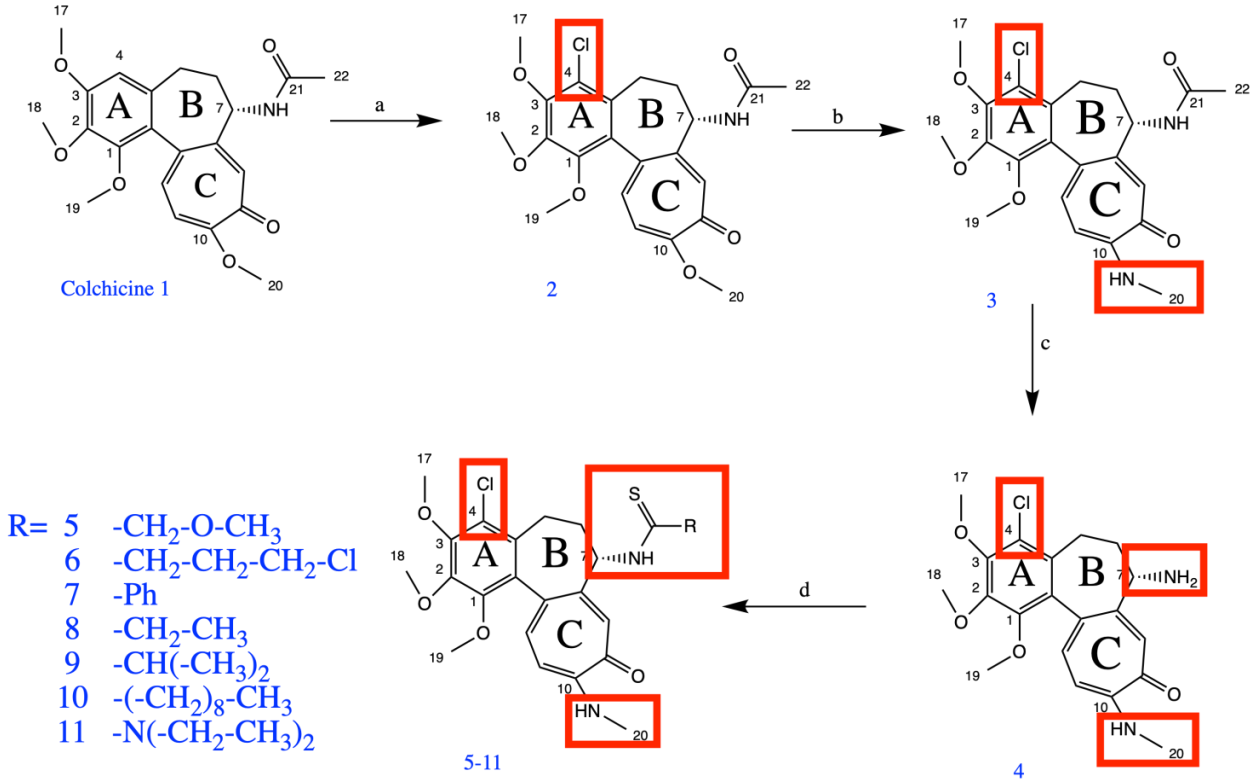

Fig 2. colchicine derivatives (2-11).

2. Methods

2.1 Materials

2.1.1 Chemicals

The structures and purity of all new compounds 2–11 (Fig. 2) are determined by Infrared Spectroscopy (IR), Mass Spectrometry (MS), Proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR).

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2.1.2 Cell lines and culture

In this study, we use six types of cell lines: ALL-5, a primary acute lymphoblastic leukemia cell; A549, a human lung adenocarcinoma cell; MCF-7, a human breast cancer cell; LoVo, a human colon adenocarcinoma cell; MCF-7/ADR, MCF-7's adriamycin-resistant subline [1]; and BALB/3T3, a murine embryonic fibroblast cell [15] .

In vitro cell culture:

ALL-5 cells are isolated from the bone marrow of a patient, they are maintained in IMD modified medium, kept at 37°C with 5 % CO_2, and supplemented with cholesterol, gluta-mine and human serum albumin [1].

MCF-7 cells are derived from the pleural effusion of a breast cancer patient, they are maintained in Dulbecco's Modified Eagle's Medium, kept at 37°C with 5 % CO_2, and supplemented with heat-inactivated fetal bovine serum [15].

A549 cells are established through a lung cancer tissue transplant, which they are maintained in McCoy's 5A Medium, kept at 37°C with 5% CO_2, and supplemented with fetal bovine serum.

LoVo cells are isolated from the ascites of a patient with colon cancer, they are maintained in RPMI-1640 medium, kept at 37°C with 5 % CO_2, and supplemented with 10 % qualified fetal bovine serum.

MCF-7/ADR cells are derived from the pleural effusion of a breast cancer patient, they are maintained in RPMI-1640 medium, kept at 37°C with 5 % CO_2, and supplemented with 10 % fetal bovine serum and 500ng/ml ADR.

BALB/3T3 cells are established from mouse embryos, they are maintained in Dulbecco's Modified Eagle's Medium, kept at 37°C with 5 % CO_2, and supplemented with 10 % fetal bovine serum.

2.2 Cell in vitro anti-proliferation assays

2.2.1 MTT assay

MTT assay is a method for detecting the effect of drugs on the viability of in vitro cultured cells [13]. Using detector to measure the absorbance at 570nm wavelength, and the number of living cells was judged according to the measured absorbance value (OD value). The greater the OD value, the stronger the cell activity and the less the drug toxicity.

Preparation of MTT solution (0.5%):

Weigh 0.5 g of MTT, dissolve in 100 ml of phosphate buffer (PBS), filter with 0.22μm filter membrane to remove bacteria from the solution, store at 4°C and away from light. In the process of preparation and preservation, the container is wrapped in aluminum foil.

2.2.2 Procedures:

Cells (10^3/well-10^4/well) in 100μL of medium containing 10 % fetal bovine serum (FBC) are injected into 96-hole plates. Five concentration gradients of drugs are added and five replicate groups are set up for each same concentration of drug. Control cells receive 150μL DMSO only. The plates are incubated at 37°C for 48h with humidified 5 % CO_2. After 48 hours treatment, 20μL of MTT solution (0.5 %) is added to each well, continuing cultivating 4h. Then centrifuge and remove the supernatant, add 150μL of dimethyl sulfoxide to each well except for control cells. The plates oscillate at a low speed for 10 minutes. Absorbance of each hole is recorded at 570nm using the MicroplateReader. Repeat experiments 3 times.

When the binding rate is 50 %, the concentration of the corresponding inhibitor is called IC50. IC50 values are determined by non-linear regression analysis using GraphPad software [1]. Selectivity index (SI) is the safety range to judge the drug effect, it is calculated using the IC50 values of BALB/3T3 cells divided by the IC50 values of individual cancer cell lines [13]. Resistance index (RI) is calculated using the IC50 values of MCF-7/ADR cells divided by the IC50 values of MCF-7 cells, that is, the ratio of LD50 of drug-resistant cells to LD50 of sensitive cells.

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3. Results

Possible Result 1: Compounds 5-11 have better anti-proliferative activity against all cell lines than compound 1

Possible Result 2: Compounds 5-11 exhibit superior anti-proliferative activity only against ALL-5 cell lines than compound 1

Possible Result 3: Compounds 5-11 exhibit superior anti-proliferative activity only against MCF-7 and MCF-7/ADR cell lines than compound 1

Possible Result 4: Compounds 5-11 exhibit superior anti-proliferative activity only against A549 cell lines than compound 1

Possible Result 5: Compounds 5-11 exhibit better anti-proliferative activity only against LoVo cell lines than compound 1

Possible Result 6: Compounds 5-11 exhibit better anti-proliferative activity to compound 1 only against normal cell lines (BALB/3T3)

Possible Result 7: Compounds 5-11 exhibit better anti-proliferative activity to compound 1 against all cancer cell lines (ALL-5, A549, MCF-7, LoVo, MCF-7/ADR), but not the normal cell line (BALB/3T3)

4. Discussion

Previous studies have already reported that 4-Halocolchicines and C-10 modified derivatives showed antitumor activity in a superior way than parent colchicine, namely inhibiting the cell proliferation in several known cell lines. With a view to investigate the anticancer activity and the possibility of clinical application of other colchicine derivatives against diverse normal and cancer cell lines, this comparative study injects distinct cells from humans and mice, which are cultured in vitro, to different plates, processing and observe absorbance. The study directly uses in vitro cell lines culture and experiments to avoid the effects of various cellular responses in organisms on the results.

Possible Results 1 illustrates that these derivatives not only have a better anti-proliferation effect on cancer cells but also inhibit the growth of normal cells. Any adverse effects of derivatives on normal cells are not expected. The failure of the in vitro experiment described in Possible Result 1 is most likely to be caused by the toxicity of colchicine derivatives or derivatives maintain in cells too long. Furthermore, an analogous study about triple-modified colchicine derivatives done in the past also appears similar results [15]. Although all triple-modified derivatives exhibit an inhibitory effect against cancer cell lines in vitro experimental models, in vivo experiments should be done in the future to make the results more convincing.

Possible Result 2,3,4,5,7 strongly make evident that compound 5-11 have potentiality for clinical use. It is keeping abreast of the ideal hypothesis that triple-modified colchicine derivatives are more effective on the anti-proliferation than the parent colchicine against cancer cell lines. However, why such differences between cancer cells and normal cells are shown, there is a little structural distinction between cancer and normal cells. Alternatively, further investigations need to be done. It is vital to make sure that the toxicity of drugs to normal cells does not significantly affect the work of normal cells before clinical trials.

Only drugs inhibit the proliferation of cancer cells, drugs have potential therapeutic effects, and clinical trials should be carried out. Selectivity index is the safety range to judge the drug effect, higher SI values are desirable. Ideally, the drug should kill the patient's cancer cells without significantly affecting their healthy cells, that is, the drug is more effective than its toxicity [15]. Nevertheless, Possible Result 6 indicates that compound 5-11 are not adequate to be a universal treatment for many cancer and even irreparable damage to normal cells because the result is contradictory to our anticipate. Notwithstanding, only a limited amount in vitro studies have been

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done to explore the anti-proliferative activity on cancer and normal cell lines. Therefore, more studies should be conducted to evaluate the anti-proliferative activity in vivo.

The ability of the derivatives to overcome the drug resistance is investigated by comparing effects in drug-sensitive MCF-7 and drug-resistant MCF-7/ADR cells [1]. The tested compounds are able to efficiently overcome the drug resistance of the MCF-7/ADR cell line. It is clear that 4-chlorocolchicine is more potent than colchicine towards all the cancer cell lines, thus we must identify that substance 2 is a promising lead in our future studies.

5. Conclusion

In conclusion, this study investigates the in vitro antitumor activity of triple-modified colchicine derivatives on normal and cancer cells by analyzing cell proliferation. The result of the study will indicate whether or not these colchicine derivatives have better antitumor activity in vitro and determine whether they can access to clinical treatment. However, regardless of the experiment results, further investigations are required to study in vivo condition under the premise of clarifying the mechanism of colchicine. In view of people have not fully discovered and developed the drugs of colchicine derivatives with the best efficacy and there is a narrow number of studies on it, more specific structure and regulation mechanism of derivatives still needs to be explored in clearer details.

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