BPB Reports

Report

Anti-proliferative effects of (–)-isostemonamine on highly aggressive human breast cancer MDA-MB-231 cells

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Received October 18, 2018; Accepted November 7, 2018

Stemona **alkaloids such as (±)-stemonamine/ (±)-isostemonamine, have a unique structure, possibly coupled with potential biological activities. The establishment of effective total synthesis protocols for stemonamine alkaloids has been a challenge for synthetic chemists so far. These stemonamine alkaloids are isolated as racemates and there is no report concerning their asymmetric total synthesis. It is generally understood that any pair of enantiomers have physically and chemically indistinguishable properties. However, stereochemistry is a critical point in biological systems because most biological reactions, such as those mediated by enzymes and receptors, are stereospecific. We have successfully established the methods of asymmetric total synthesis of the stemonamine alkaloids, (–)-stemonamine/(–)-isostemonamine. We studied the potential application of these** ***Stemona*** **alkaloids as anti-proliferative agents. Experiments were conducted by using two representative human breast cancer cell lines, MCF-7 and MDA-MB-231, and our results indicated that i) (–)-isostemonamine displays strong cytotoxic effects on the highly aggressive estrogen receptor α (ERα)-negative MDA-MB-231 cell line, but not on the ERα-positive MCF-7 cells, with an IC_{50} value (9.3 ± 1.9 μM), which is comparable to that of etoposide (IC_{50} = 7.1 μM ± 1.4 μM), and ii) the thioamide derivative of (–)-isostemonamine does not suppress the growth of MDA-MB-231 cells.**

Key words (–)-isostemonamine, (–)-stemonamine, MDA-MB-231 cells, *Stemona* alkaloids

INTRODUCTION

It is generally considered that natural products produced by plants are an optically pure form of only one enantiomer. *Stemona* alkaloids (stemonamine and isostemonamine), are isolated from the root of *Stemona japonica* Miq., which has been used for centuries in Chinese and Japanese traditional medicine for the treatment of respiratory diseases and also as an insecticide. Only stemonamine and its diastereomer, isostemonamine, have been isolated as racemates; *i.e.*, (±)-stemonamine and (±)-isostemonamine.[1)] Considering this enigma, it may be suggested that racemization of stemonamines can occur either naturally (although in rare cases), or during the isolation process, even though the compounds are biologically synthesized in their optically pure forms, (–) or (+).[2)] Recently, we have successfully established asymmetric total synthesis of (–)-stemonamine and (–)-isostemonamine and demonstrated that the racemization and epimerization of stemonamine/isostemonamine can take place under some experimental conditions,[2)] supporting the latter possibility.

Although *Stemona* alkaloids including stemonamine and isostemonamine have been effective in the treatment of some disease conditions as indicated above,[1)] less attention has been paid to biological aspects (activities) of these *Stemona* alkaloids, especially in terms of cancer biology. Furthermore, an attempt to obtain stemonamine/isostemonamine as individual enantiomers, but not as racemates, has not been performed. This is important because most biological reactions, such as those mediated by enzymes and receptors, are known to be stereospecific.

In this report, we utilized (–)-stemonamine/(–)-isostemonamine, obtained from an asymmetric total synthesis, as previously reported by us,[2)] for their anti-proliferative properties. Plant-derived alkaloids, such as etoposide and paclitaxel, can be used as anti-breast cancer agents. Here we sought to investigate the effects of two *Stemona* alkaloids, (–)-stemonamine and (–)-isostemonamine, on the proliferation of breast cancer cells together with the established anti-cancer alkaloids; experiments were conducted by using two representative human breast cancer cell lines, MCF-7 and MDA-MB-231. The results obtained here indicated that i) (–)-isostemonamine displays strong anti-proliferative effects on the highly aggressive estrogen receptor α (ERα)-negative MDA-MB-231 cell line, but not on the ERα-positive MCF-7, with an IC_{50} value (9.3 ± 1.9 μM), comparable to that of etoposide (IC_{50} = 7.1 ± 1.4 μM) (although paclitaxel exhibited the highest inhibitory activity; IC_{50} = 230 ± 37.1 nM), and ii) the thioamide derivative of (–)-isostemonamine does not suppress the growth of MDA-MB-231 cells. In this study, it was shown for the first time that (–)-isostemonamine is “biologically active”.

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MATERIALS AND METHODS

Reagents (–)-Stemonamine, (–)-isostemonamine, ST-3 (thioamide), and ST-4 (thioamide) (Fig. 1) were synthesized using our established methods.[2] The purities of these compounds were found to be ≥95% by HPLC or column chromatography. Etoposide and paclitaxel were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) (purity: ≥98% each, HPLC).

Cell culture Human breast cancer cell lines, MCF-7 and MDA-MB-231, were purchased from the American Type Culture Collection (ATCC, HTB-22, and HTB-2, respectively) (Rockville, MD, USA). The cell culture conditions were based on previously reported procedures.[3] Cell counting was performed by using TC10™ Automated Cell Counter (Bio-Rad, Hercules, CA, USA).

Cell proliferation analysis (MTS assay) The MTS assay was performed as described previously.[3] Briefly, the MCF-7/MDA-MB-231 cells were seeded into 96-well plates at a density of 5×10^3 cells/well. After chemical treatments and incubation, the degree of cell proliferation was analyzed by using CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS reagent; Promega, Madison, WI, USA). The chemicals used in this study were prepared in cell culture grade dimethyl sulfoxide (DMSO). Control cells were incubated with equivalent concentrations of DMSO.

Data analysis IC_{50} values were obtained using SigmaPlot 11® software (Systat Software, Inc., San Jose, CA). Differences were considered significant when the P value was calculated as < 0.05. Dunnett's test was used to compare the control group and other treatment groups. These calculations were performed using Statview 5.0J software (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

When comparing proliferation potential between human breast cancer cells, MCF-7 and MDA-MB-231, as expected, the latter (MDA-MB-231 cells) exhibited more aggressive proliferation than the former (MCF-7 cells) (Fig. 2A and B).[4] In the following study, we utilized these two breast cancer cell lines to investigate the effects of (–)-stemonamine and (–)-isostemonamine (Fig. 1), obtained from the method of asymmetric total synthesis.[2] As shown in Figure 2C–F, it was revealed that (–)-stemonamine and (–)-isostemonamine had anti-proliferative effects on MDA-MB-231 cells, but not on MCF-7 cells. (–)-Isostemonamine was found to be a more potent abrogator of cancer cell proliferation than (–)-stemonamine (*i.e.*, IC_{50} values: 9.3 ± 1.9 μM versus >25 μM) (panels C and E). The (–)-isostemonamine's IC_{50} value of 9.3 μM on MDA-MB-231 cells was comparable to that of etoposide (*i.e.*, 7.1 ± 1.4 μM). However, paclitaxel, another anti-cancer alkaloid used in this study, displayed extremely strong anti-proliferative effects (*i.e.*, 230 ± 37.1 nM) (Table 1).

We next studied the effects of ST-3 (thioamide) and ST-4 (thioamide) (Fig. 1), which are two thioamide derivatives of (–)-stemonamine and (–)-isostemonamine, respectively, on the proliferation of MDA-MB-231 cells. No anti-prolifera-

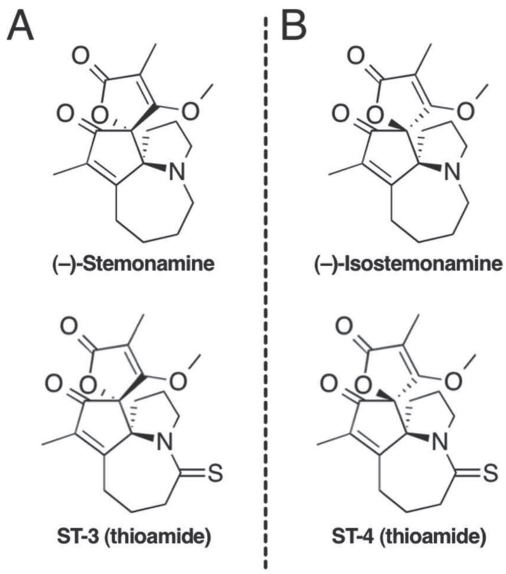

Fig. 1. Chemical structures of (–)-stemonamine/(–)-isostemonamine and their respective thioamide derivatives

(A) (–)-Stemonamine and its thioamide derivative, ST-3 (thioamide), are shown (B) (–)-Isostemonamine and its thioamide derivative, ST-4 (thioamide), are shown.

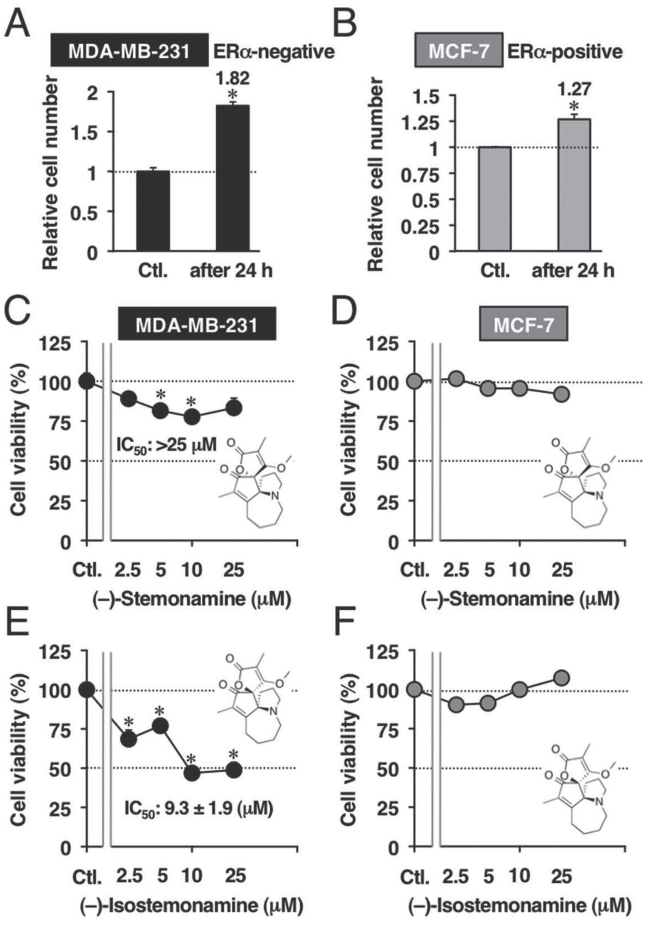

Fig. 2. Effects of (–)-stemonamine and (–)-isostemonamine on the proliferation of MDA-MB-231 cells (ERα-negative) and MCF-7 cells (ERα-positive)

(A: MDA-MB-231 cells) (B: MCF-7 cells) Cell counting was done 24 h after incubation (indicated as "after 24 h"). Each cell line was seeded at a density of 3×10^5 cells/well (control, Ctl.) in the cell-counting assay. Data represent means ± S.E. from three independent experiments in triplicate, and are expressed as fold increase from the control. *, $P < 0.05$ versus the control group. (C –F) MDA-MB-231 cells and MCF-7 cells were exposed 48 h to (–)-stemonamine (2.5 μM–25 μM) (C and E) or (–)-isostemonamine (2.5 μM–25 μM) (D and F) and then cell proliferation was measured by using MTS assay. The control group (indicated as Ctl.) was treated with vehicle (DMSO) alone. Data represent means ± S.E. from six independent experiments in duplicate, and are expressed as a percentage of the control. *, $P < 0.05$ versus the control group.

Table 1. IC_{50} values of (–)-stemonamine/(–)-isostemonamine and the established anti-cancer agents in the abrogation of MDA-MB-231 cell viability

Compounds	IC_{50} values
(–)-Stemonamine	> 25 (μM)
(–)-Isostemonamine	9.3 ± 1.9 (μM)
Etoposide	7.1 ± 1.4 (μM)
Paclitaxel	230 ± 37.1 (nM)

As is the case with the experiments in Figure 2, cell proliferation assay was performed with etoposide and paclitaxel, and their IC_{50} values were summarized together with those of (–)-stemonamine/(–)-isostemonamine.

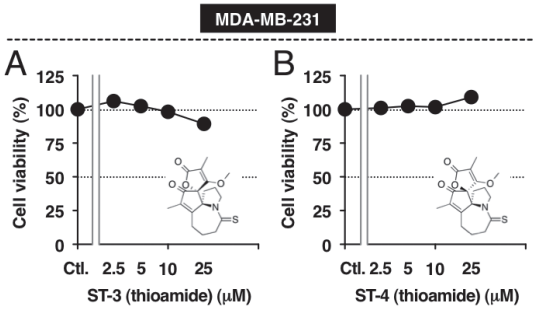

Fig. 3. Effect of thioamide derivatives of (–)-stemonamine and (–)-isostemonamine on the proliferation of MDA-MB-231 cells

(A and B) MDA-MB-231 cells were exposed for 48 h to the thioamide derivatives of (–)-stemonamine (2.5 μM–25 μM) (A) and (–)-isostemonamine (2.5 μM–25 μM) (B) and then cell proliferation was measured by using MTS assay. The control group (indicated as Ctl.) was treated with vehicle (DMSO) alone. ST-3 (thioamide) and ST-4 (thioamide) are the thioamide derivatives of (–)-stemonamine and (–)-isostemonamine, respectively. Data represent means ± S.E. from six independent experiments in duplicate, and are expressed as a percentage of the control.

tive effects of these thioamide derivatives were detected, even at the highest concentration (25 μM) tested (Fig. 3A and B), implicating that an introduction of thioamide on the azepane moiety (C ring) possibly interferes with parent stemonamines' action. Furthermore, the results suggested that an entire structure of (–)-stemonamine/(–)-isostemonamine may be important for the exertion of their respective anti-proliferative effects (Fig. 3, *see* also Fig. 2C and E).

In this study, it is demonstrated for the first time that (–)-stemonamine /(–)-isostemonamine have anti-proliferative effects on ERα-negative MDA-MB-231 cells, but not on ERα-positive MCF-7 cells. These results may be attributed to the differences in expression status of ERα. Furthermore, (–)-stemonamine and (–)-isostemonamine, especially (–)-isostemonamine, may be effectively entrapped by highly proliferative MDA-MB-231 cells.

We have reported previously, through experimental evidence, that (–)-stemonamine/(–)-isostemonamine can be racemized to (+)-stemonamine/(+)-isostemonamine in the presence of protic solvents.[2] We hypothesize that, if a similar racemization reaction takes place in a cell culture system used in this study, the resultant isomer molecules might also be active. The other possibility is that stemonamine can be epimerized to isostemonamine. However, this possibility may be very low, because stemonamine is shown to be thermodynamically more stable than isostemonamine.[2] Although we are currently unable to provide evidence for the mechanism of cytotoxicity of (–)-isostemonamine together with (–)-stemonamine, our results suggest that these stemonamines, at least in part, can be used as a therapeutic modality for ERα-negative cells. Clearly, further studies are needed to reveal the mechanism of action of these stemonamines.

Acknowledgments This work was performed under the Cooperative Research Program of the Network Joint Research Center for Materials and Devices [Research No. 20181308 (to S.T.)]. This study was also supported, in part, by JSPS KAKENHI Grant Numbers JP18H02557, JP18H04418, and JP18H04624 (to M.S.).

Conflict of interest The authors declare no conflict of interest.

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