Jpn. J. Cancer Res. **90**, 691–698, June 1999

A Dual Topoisomerase Inhibitor, TAS-103, Induces Apoptosis in Human Cancer Cells

Tomoko Ohyama,[1] Yin Li,[1] Teruhiro Utsugi,[2] Shinji Irie,[3] Yuji Yamada[2] and Taka-aki Sato[1, 3, 4]

[1]Departments of Otolaryngology/Head & Neck Surgery and Pathology, Columbia University, 630 W. 168th Street, P&S 11-451, New York, NY 10032, [2]Hanno Research Center, Taiho Pharmaceutical Co., Ltd., 1-27 Misugidai, Hanno, Saitama 357-8527 and [3]Molecular Oncology Laboratory, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074

TAS-103 (6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7*H*-indeno[2,1-*c*]quinolin-7-one dihydrochloride), a dual topoisomerase (topo) inhibitor, was developed as an anticancer agent by targeting topo I and topo II and has previously been shown to be effective against lung tumors. In this study, we investigated the cytotoxic activity of TAS-103 in various human cancer cell lines (including gastric, colon, squamous, lung, and breast cancer cells) and the induction of apoptosis by TAS-103. We next established stable transfectants of Bcl-2 in the gastric cancer cell line AZ521 and found that Bcl-2 blocked TAS-103-induced apoptosis. In addition, we demonstrated that the activities of ICE-like and CPP32-like proteases are involved in the signal transduction pathway of TAS-103-induced apoptosis. In summary, TAS-103 is a novel type of anticancer agent with a unique mechanism and could be useful as a lead compound for development of new drugs.

Key words: TAS-103 — Topoisomerase inhibitor — Apoptosis — Bcl-2 — Protease

Programmed cell death (apoptosis) is a genetically regulated mechanism that occurs during physiological processes, including normal cell turnover, immune system operation, embryonic development, and hormone-dependent atrophy, and also in chemical-induced cell death.[1–3] Morphologically, apoptosis is characterized by cell shrinkage, nuclear condensation, and DNA fragmentation.[4] Regulation of cell death is essential for normal development and is an important defence against viral infection and the emergence of cancer. The process of apoptosis is controlled through the expression of genes that are conserved in nematodes through to mammals. Some of the gene products are activators of apoptosis, whereas others are inhibitors, and the characterization of the function of these gene products will help to define the process of cell death at the molecular-biological level.

The *Bcl-2* gene was first identified as part of the most common translocation in human B cell follicular lymphoma, and overexpression of Bcl-2 in transgenic animal models mimicked human disease.[5, 6] Members of the Bcl-2 family are known to modulate apoptosis in different cell types in response to various stimuli.[7, 8] Bcl-2 and Bcl-X_L promote cell survival, while Bax enhances cell death. Overexpression of Bcl-2 often protects cells from apoptosis induced by various stimuli, including anticancer agents.[6, 9]

An emerging family of Ced/ICE-like cysteine proteases (caspases) has also been identified and cloned based on a conserved pentapeptide sequence possessing the active cysteine residue. The caspases are divided into three subfamilies based on their degrees of homology and phylogenetic analysis: the ICE-related family genes, the CPP32-related family including caspase-3, caspase-6, caspase-7, and caspase-10, and the third group including caspase-2, and caspase-9.[10–13] Recent studies have indicated that specific cysteine proteases (caspases) play a central role in the execution phase of apoptosis.[14]

Topoisomerases (topo) are enzymes that can change the topological state of DNA through the breaking and rejoining of DNA strands.[15] There are two classes of topo, known as type I and type II enzymes. Type I topo generate transient single-strand breaks in DNA, allowing controlled rotation about the nick. In contrast, type II topoisomerases produce transient double-strand breaks in DNA and pass a separate double-stranded molecule through the break.[16] Topo inhibitors have attracted much attention, as potential anticancer agents.

Chemotherapeutic drugs such as DNA topo I and II inhibitors trigger apoptosis in various cancer cell lines.[17–20] Etoposide, a topo II inhibitor, inhibits cell growth and induces apoptosis in leukemia, breast carcinoma, lung cancer and neuroblastoma.[21] TAS-103 (6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7*H*-indeno[2,1-*c*]quinolin-

[4] To whom correspondence should be addressed at the Departments of Otolaryngology/Head & Neck Surgery and Pathology, Columbia University.
E-mail: ts174@columbia.edu

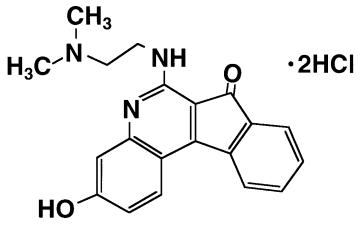

Fig. 1. The chemical structure of TAS-103 (6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7*H*-indeno[2,1-*c*]quinolin-7-one dihydrochloride) used in this study.

7-one dihydrochloride) (Fig. 1) was developed as an anticancer agent targeting topo I and II, and has a strong cytotoxic effect on leukemia cell lines and various lung tumors.[22] Several research groups have reported that caspase activation is also involved in topo inhibitor-induced apoptosis in some tumors.[23–25] However, the mechanisms of action of topo inhibitors, including TAS-103, remain unclear.

In this study, we investigated the cytotoxic activity of TAS-103 in various human cancer cell lines and the induction of apoptosis by TAS-103. To analyze the mechanism of the growth inhibition and apoptosis induction by TAS-103, we established a stable transfectant of Bcl-2 in gastric cancer cells and found that Bcl-2 blocked TAS-103-induced apoptosis. In addition, we demonstrated that TAS-103 can activate both ICE-like and CPP32-like proteases and that their activities are inhibited by Bcl-2. Our data suggest that TAS-103 is a novel type of anticancer agent with a unique mechanism of action.

MATERIALS AND METHODS

Chemicals TAS-103 was synthesized by Taiho Pharmaceutical Co., Ltd. (Hanno, Saitama). Etoposide, YVAD-CHO and DEVD-CHO were obtained from Calbiochem (San Diego, CA). YVAD-AFC and DEVD-AFC were obtained from BIOMOL (Plymouth Meeting, PA).

Cell culture The cancer cell lines were obtained from American Type Culture Collection (ATCC). AZ521, FaDu, and A549 were maintained in minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS). TMK-1, HCT116, HT-29, COLO 320DM, LoVo, and MDA-MB-435S were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS.

Growth inhibition assay (MTT assay) Viable-cell number was determined by using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously.[26] Cells were seeded (5,000 per well) in 96-well plates and treated with various concentrations of drugs for different periods of time. The absorbance was read at 570 nm in a microplate reader.

Apoptosis analysis For morphological analysis, cells were treated with TAS-103, trypsinized, washed with phosphate-buffered saline (PBS), fixed with 5% glutaraldehyde and stained with Hoechst 33342 (final concentration: 5 mg/ml) (Sigma). Stained cells were observed by fluorescence microscopy. For the DNA fragmentation assay, cells were treated with TAS-103 24 h later, trypsinized, washed with PBS, and incubated with the lysis buffer (0.01 *M* EDTA, 1% sodium dodecyl sulfate (SDS), 1 *M* NaCl) at 4°C for 30 min. The lysate was centrifuged at 15,000*g* for 30 min, and the supernatant was incubated with RNase (1 mg/ml), and then with proteinase K (0.2 mg/ml), both at 45°C for 60 min. The purified DNA was dissolved in dH_2O and electrophoresed on a 1.2% agarose gel. For ELISA, cells were treated with 2.5 μM of TAS-103 for 18 h. DNA fragmentation assay was done using a Cell Death Detection ELISA kit (Boehringer-Mannheim, Mannheim, Germany) following the manufacturer's protocol.

Stable transfection cDNA for the human *Bcl-2* gene was cloned into the pRC/CMV expression vector. The plasmid DNA was transfected into AZ521 by electroporation at 300 V and 960 μF using a Gene Pulser II (Bio-Rad Labs., Hercules, CA). The post-transfected cells were selected with G418 (250 μg/ml). The insertion of the Bcl-2 cDNA was confirmed by western blot analysis.

Western blot analysis Cells were lysed in a solution containing 150 m*M* NaCl, 10 m*M* Tris-HCl (pH 8.0), 0.15 *M* NaCl, 1 m*M* EDTA, 1% NP40 and protease inhibitors (phenylmethylsulfonyl fluoride (PMSF), aprotinin, leupeptin, pepstatin and benzamide). Aliquots of lysates (40 μg) were boiled in SDS sample buffer, resolved by 12% SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose filters. After transfer, the membranes were blocked in TBST (50 m*M* Tris-HCl [pH 8.0], 150 m*M* NaCl, 0.1% Tween 20) containing antibody. Immunoreactive products were detected by chemiluminescence with an enhanced chemiluminescence system (ECL; Amersham Pharmacia Bio., Amersham). Anti-α-tubulin was used as a control.

ICE/CPP32 protease assay Preparation of cell extracts and enzyme assay were performed as previously described.[27] Cells were lysed in a solution containing 25 m*M* *N*-2-hydroxyethylpiperazine-*N*′-3-propanesulfonic acid (HEPES; pH 7.5), 5 m*M* EDTA, 5 m*M* dithiothreitol, protease inhibitors pepstatin, leupeptin, and PMSF for 20 min on ice and then were sonicated on ice. The lysates were centrifuged at 160,000*g* for 20 min and the protein concentrations were quantified using the Bio-Rad Protein Assay kit. For enzyme assay, lysates (25 μg) were incubated with the substrate, 25 μM YVAD-AFC or 15 μM DEVD-AFC (BIOMOL). Cleavage of the substrates generated fluorescence that was quantified in an SLM 8000 fluorometer (excitation, 400 nm and emission, 505 nm).

RESULTS

Cytotoxic activity of TAS-103 towards various human cancer cell lines To determine the cytotoxic activity of TAS-103 by MTT assay, two gastric cancer cell lines (AZ521 and TMK-1), four colon cancer cell lines (HCT116, HT-29, COLO 320DM and LoVo), one squamous cancer cell line (FaDu), one lung cancer cell line (A549) and one breast cancer cell line (MDA-MB-435S) were employed (Table I, Fig. 2, A and B). TAS-103 is more effective than etoposide in inhibiting the growth of various human cancer cell lines (Table I). The dose-response data are presented in Fig. 2A. TAS-103 reduced the number of growing cells with a 50% inhibitory value of approximately 0.2 μM, about 20 times lower than that of etoposide. As shown in Fig. 2B, AZ521 cells were treated with 0.2 μM and 1.0 μM TAS-103 or etoposide for different periods of time. TAS-103 at 1.0 μM completely inhibited cell growth after 3 days. However, under the same condition, 40% of these cells survived in the media containing 1.0 μM etoposide. These results clearly indicate that TAS-103 has strong cytotoxic activity and is more effective than etoposide against several human cancer cell lines.

Induction of apoptosis by TAS-103 in the gastric cancer cell line AZ521 To investigate whether the growth-inhibitory effect of TAS-103 is caused by apoptosis, the nuclear morphology of TAS-103-treated or non-treated gastric cancer cells AZ521 was studied with Hoechst 33342 staining. Treatment of AZ521 cells with 1.0 μM TAS-103 for 2 days triggered typical morphological characteristics of apoptosis, such as nuclear condensation (Fig. 3A). Furthermore, TAS-103-induced apoptosis was confirmed by observation of DNA fragmentation in a dose-dependent manner after 24 h treatment with TAS-103 (Fig. 3B). These results clearly demonstrate that TAS-103 induces apoptosis in AZ521 gastric cancer cells.

Table I. Cytotoxic Activity of TAS-103 in Various Human Cancer Cell Lines

Cell line	Origin	IC_{50} (μM)	
		TAS-103	Etoposide
AZ521	Gastric cancer	0.2	4.0
TMK-1	Gastric cancer	0.9	5.2
HCT116	Colon cancer	0.3	2.9
HT-29	Colon cancer	1.4	9.0
COLO 320DM	Colon cancer	1.1	3.9
LoVo	Colon cancer	0.7	3.3
FaDu	Squamous cancer	0.1	0.9
A549	Lung cancer	0.4	6.4
MDA-MB-435S	Breast cancer	1.4	10.9

Cells were treated with TAS-103 or etoposide for 3 days. Each IC_{50} value is the mean of three independent experiments by MTT assay.

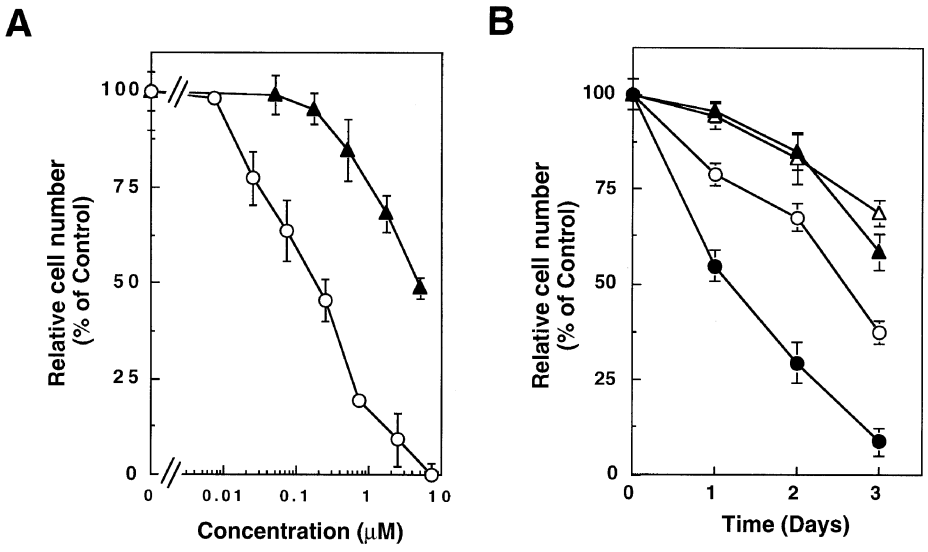

Fig. 2. Cytotoxic activity of TAS-103 in the gastric cancer cell line AZ521. (A) Effect of TAS-103 concentration on AZ521 cell growth. Cells were treated with the indicated concentration of TAS-103 (○) or etoposide (▲) for 3 days, then cell viability was determined by the MTT assay. (B) Time course. Cells were seeded at a cell density of 2,000 cells per well in 96-well plates. The cells were then treated with drugs for the indicated times, and cell viability was determined by the MTT assay. ● TAS-103 1.0 μM, ○ TAS-103 0.2 μM, ▲ etoposide 1.0 μM, △ etoposide 0.2 μM.

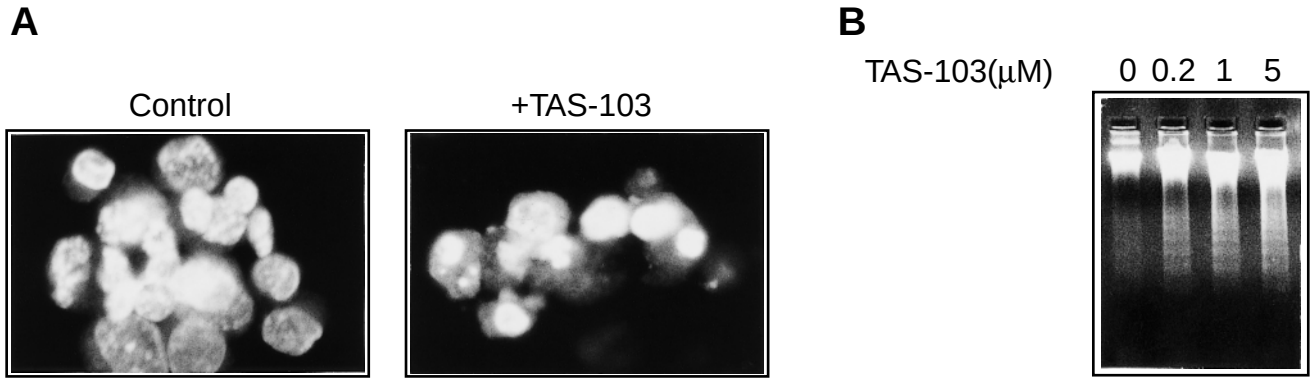

Fig. 3. Induction of apoptosis by TAS-103 in AZ521 cells. (A) Morphological analysis of apoptotic cells. Cells were treated with 1.0 μM TAS-103 for 2 days, and nuclear morphology was analyzed by Hoechst 33342 staining. (B) Cells were treated with various concentrations of TAS-103 for 24 h. The cell lysates were extracted and electrophoresed on 1.2% agarose gel.

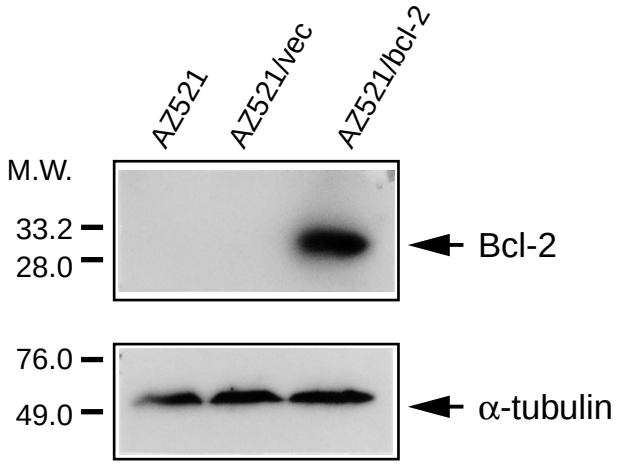

Fig. 4. Western blot analysis. Cell lysates of AZ521, AZ521/vec, and AZ521/bcl-2 were analyzed by western blot for Bcl-2 expression. α-Tubulin was used as a control.

Bcl-2 blocks growth inhibition and apoptosis induction by TAS-103 in AZ521 cells In order to study the mechanism by which TAS-103 inhibits cell growth and induces apoptosis, we examined the expression level of Bcl-2 family proteins, such as Bcl-2 and Bax (data not shown). Using the gastric cancer cell line AZ521, which does not express Bcl-2, we established a stable transfectant of Bcl-2 and measured the Bcl-2 protein content by western blot analysis (Fig. 4). In this experiment, one Bcl-2 transfectant and one vector-transfected clone were subjected to the MTT assay and Hoechst 33342 staining. The results of the MTT assay are shown in Fig. 5, A and B. In contrast to the parental and vector-transfected AZ521 cells (AZ521/vec), the viability of Bcl-2-transfected cells (AZ521/bcl-2) was over 70% even after the addition of 20 μM TAS-103 for 24 h (Fig. 5A). In a time course experiment, more than 50% of AZ521/bcl-2 cells were viable after 48 h of incubation with 10 μM TAS-103 (Fig. 5B).

A stable clone with a high level of Bcl-2 was also examined by Hoechst 33342 staining and ELISA assay (Fig. 6, A and B). TAS-103 induced over 20% apoptotic cells at 2.5 μM for 2 days in AZ521/vec cells (Fig. 6A). However, apoptotic cells amounted to only about 5% when AZ521/bcl-2 cells were similarly treated. Fig. 6B shows the DNA fragmentation based on Cell Death Detection ELISA. We observed a similar induction pattern by TAS-103 in AZ521/vec and AZ521/bcl-2. These data suggest that Bcl-2 blocks growth inhibition and apoptosis induction by TAS-103 in the gastric cancer cell line AZ521.

Involvement of ICE-like and CPP32-like proteases in TAS-103-induced apoptosis We next assessed whether TAS-103 activates the caspase cascade by the upregulation of ICE-like and CPP32-like proteases. Using the MTT assay to assess the viability of AZ521 cells, we found that YVAD-CHO (ICE inhibitor) and DEVD-CHO (CPP32 inhibitor) partially prevented TAS-103-inhibition of cell growth (data not shown). The fluorogenic substrates YVAD-AFC and DEVD-AFC, which mimic the cleavage sites at which ICE cleaves pIL-1β and CPP32 cleaves PARP, were used to confirm the activities of ICE-like and CPP32-like proteases in AZ521/vec and AZ521/bcl-2 cells treated with TAS-103 (Fig. 7). YVAD-AFC cleavage activity was observed in AZ521/vec cells treated with 25 μM TAS-103 for the indicated times. However, this activity was not seen in AZ521/bcl-2 cells (Fig. 7A). Similar results were obtained with DEVD-AFC treatment under the same conditions (Fig. 7B). Our data indicated that the activities of ICE-like and CPP32-like proteases are

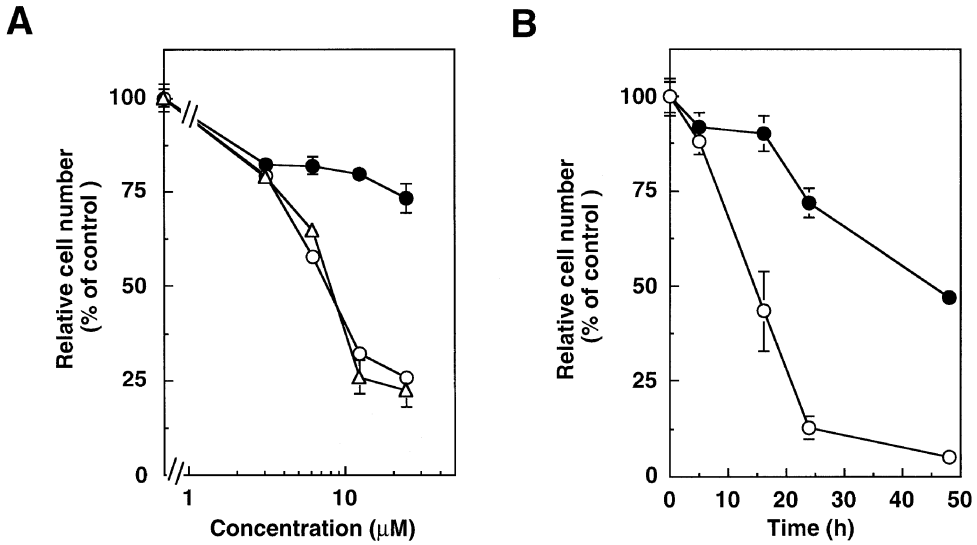

Fig. 5. Bcl-2 blocks the growth inhibition of TAS-103 in AZ521 cells. (A) Effect of TAS-103 on the growth inhibition in AZ521 (△), AZ521/vec (○), and AZ521/bcl-2 (●) cells. Cells were treated with the indicated concentrations of TAS-103 for 24 h, then the cell viability was determined by the MTT assay. (B) Time course. AZ521/vec (○) and AZ521/bcl-2 (●) cells were seeded at a cell density of 2,000 cells per well in 96-well plates. The cells were then treated with 12 *μM* TAS-103 for the indicated times, and the cell viability was determined by MTT assay.

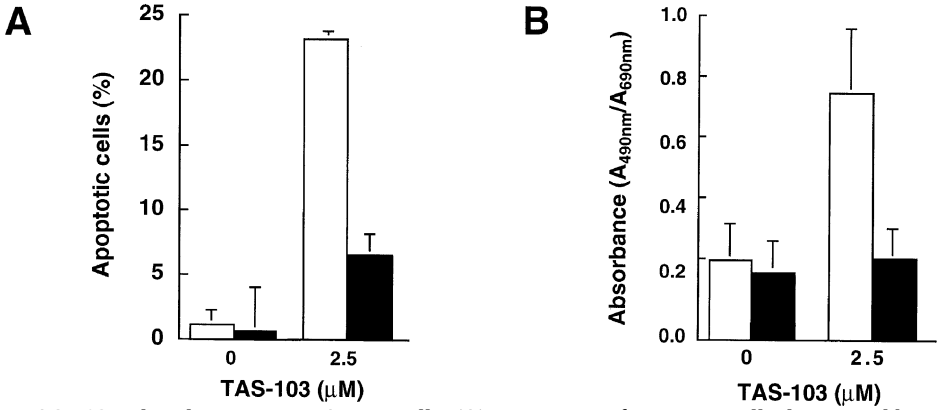

Fig. 6. Bcl-2 blocks TAS-103-induced apoptosis in AZ521 cells. (A) Percentage of apoptotic cells determined by Hoechst 33342 staining. The number of apoptotic cells with nuclear morphology typical of apoptosis was scored in at least 400 cells in each sample by using a fluorescence microscope. Cells were treated with 2.5 *μM* TAS-103 for 2 days. □ AZ521/vec, ■ AZ521/bcl-2. (B) Analysis of DNA fragmentation in AZ521/vec (□) and AZ521/bcl-2 (■) cells by ELISA. Cells were treated with 2.5 *μM* TAS-103 for 18 h and analyzed for DNA fragmentation using a Cell Death Detection ELISA kit. The results are expressed as mean±SD.

involved in the signal transduction in TAS-103-induced apoptosis.

DISCUSSION

Topo inhibitors such as CPT-11 and topotecan (topo I inhibitors), etoposide and genistein (topo II inhibitors) were discovered by several research groups.[28–31] Interestingly, the expression levels of the two enzymes vary in different types of cancers,[32, 33] and further, topo I and II can each compensate for loss of function of the other.[34, 35] Therefore TAS-103 was developed as an anticancer agent targeting both topo I and topo II, and has been shown to be effective against lung tumors.[22] Here we have dis-

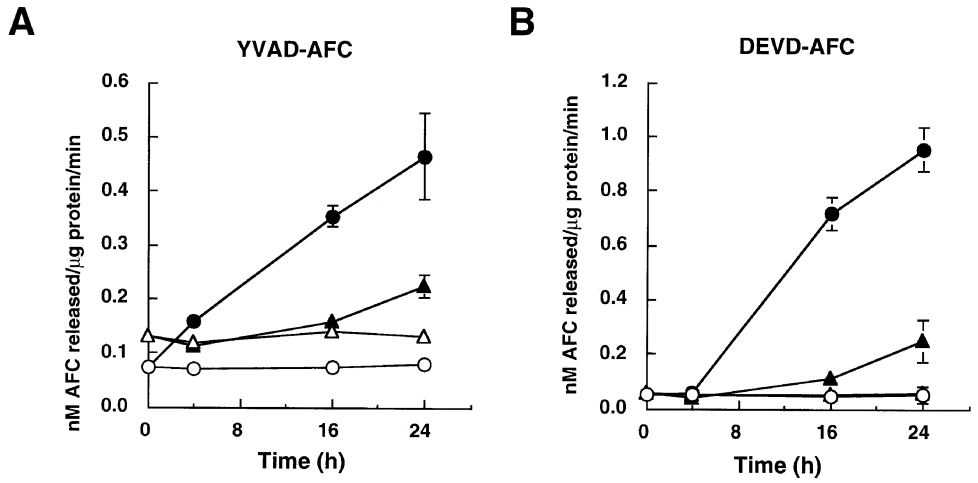

Fig. 7. Involvement of ICE-like and CPP32-like proteases in TAS-103-induced apoptosis. (A) Cell lysate of AZ521/vec or AZ521/bcl-2 was treated with 25 μM TAS-103 for the indicated times and incubated with 25 μM YVAD-AFC. Cleavage of the substrate produced a fluorescent signal that was quantified with an SLM 8000 fluorometer. ○ AZ521/vec, ● AZ521/vec+TAS-103, △ AZ521/bcl-2, ▲ AZ521/bcl-2+TAS-103. (B) Cell lysate with the same treatment as in (A) was incubated with 15 μM DEVD-AFC. ○ AZ521/vec, ● AZ521/vec+TAS-103, △ AZ521/bcl-2, ▲ AZ521/bcl-2+TAS-103. The results are expressed as mean±SD.

cussed the cytotoxic activity of TAS-103 in various human cancer cells (including gastric, colon, squamous, lung, and breast cancer cell lines), and our results indicate that TAS-103 is a more potent inhibitor than etoposide (Table I and Fig. 2, A and B). Furthermore, TAS-103-induced apoptosis was confirmed by the observation of typical morphological changes and DNA fragmentation in the gastric cancer cell line AZ521 (Fig. 3, A and B). All these results demonstrate that TAS-103 induces apoptosis in AZ521 gastric cancer cells.

Bcl-2 family proteins are known to be involved in the regulation of apoptotic signals.[7, 8] The induction of apoptosis by various stimuli involves a cytotoxic proteolytic cascade that is associated with a pathway of endonuclease activation, which leads to for DNA cleavage. In some studies, overexpression of Bcl-2 has been demonstrated to inhibit drug-induced apoptosis by preventing the activation of caspase-3, acting at points both upstream and downstream from cytochrome *c* release.[35, 36] Some results also indicated that cytochrome *c* release is not accompanied by changes in mitochondrial membrane potential and that Bcl-2 acts to inhibit cytochrome *c* translocation, thereby blocking the caspase activation step of the apoptotic step process.[37] It has been reported that Bcl-2 can inhibit the induction of apoptosis by drugs such as CPT-11 and etoposide.[38] In order to understand the mechanism of TAS-103-induced apoptosis, we established a stable transfectant of Bcl-2 in the gastric cancer cell line AZ521 and found that Bcl-2 blocks TAS-103-induced apoptosis in these cells (Fig. 6, A and B). Our results also demonstrated that TAS-103 induced the activities of both ICE-like and CPP32-like proteases, as judged from protease activity assay *in vitro*. However, overexpression of Bcl-2 inhibited TAS-103-induced activity of both proteases in AZ521 gastric cancer cells (Fig. 7, A and B). These data suggested that Bcl-2 antagonizes TAS-103-induced apoptosis by blocking the activity of a cysteine protease. But we did not observe cytochrome *c* release after treatment of AZ521 cells with TAS-103 in our experiment. Thus, further studies will be necessary to address the mechanisms by which TAS-103 induces protease activity.

In conclusion, TAS-103, a dual topo inhibitor, has strong cytotoxic activity in various human cancer cells and effectively induces apoptosis of AZ521 gastric cancer cells. Most importantly, our results not only suggest that Bcl-2 blocks TAS-103-induced apoptosis, but also indicate that ICE-like and CPP32-like proteases are involved in the signal transduction pathway leading to TAS-103-induced apoptosis. TAS-103 appears to be a novel type of anticancer agent with a unique mechanism of action. It could be an important lead compound for the development of new anticancer agents.

ACKNOWLEDGMENTS

This work was supported by grants from Taiho Pharmaceutical Co., Ltd., the Princess Takamatsu Cancer Research Fund and the Haraguchi Cancer Research Foundation.

(Received February 12, 1999/Revised April 8, 1999/Accepted April 12, 1999)

REFERENCES

1) Thompson, C. B. Apoptosis in the pathogenesis and treatment of disease. *Science*, **267**, 1456–1462 (1995).
2) Steller, H. Mechanisms and genes of cellular suicide. *Science*, **267**, 1445–1449 (1995).
3) Jacobson, M. D., Weil, M. and Raff, M. C. Programmed cell death in animal development. *Cell*, **88**, 347–354 (1997).
4) Kerr, J. F., Wyllie, A. H. and Currie, A. R. Apoptosis. A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br.J.Cancer*, **26**, 239–257 (1972).
5) Hengartner, M. O. and Horvitz, H. R. *C. elegans* cell survival gene ced-9 encodes a functional homolog of the mammalian proto-oncogene bcl-2. *Cell*, **76**, 665–676 (1994).
6) Korsmeyer, S. J. Regulators of cell death. *Trends Genet.*, **11**, 101–105 (1995).
7) Hockenbery, F. M., Oltvai, Z. N., Yin, X.-M., Milliman, C. L. and Korsmeyer, S. J. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell*, **75**, 241–251 (1993).
8) Kane, D. J., Sarafian, T. A., Anton, R., Hahn, H., Gralla, E. B., Valentine, F. S., Ordm, T. and Bredesen, D. E. Bcl-2 inhibition of neural death: decreased generation of reactive oxgen species. *Science*, **262**, 1274–1277 (1993).
9) Reed, J. C. Bcl-2 and regulation of programed cell death. *J. Cell Biol.*, **124**, 1–6 (1994).
10) Cohen, G. M. Caspases: the executioners of apoptosis. *Biochem. J.*, **326**, 1–16 (1997).
11) Salvesen, G. S. and Dixit, V. M. Caspases: intracellular signaling by proteolysis. *Cell*, **91**, 443–446 (1997).
12) Whyte, M. ICE/CED-3 proteases in apoptosis. *Trends Cell Biol.*, **6**, 245–248 (1996).
13) Cryns, V. and Yuan, J. Proteases to die for (review). *Genes Dev.*, **12**, 1551–1570 (1998).
14) Miura, M., Zhu, H., Rotello, R., Hartwieg, E. A. and Yuan, J. Induction of apoptosis in fibroblasts by IL-1b-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene ced-3. *Cell*, **75**, 653–660 (1993).
15) Berger, J. M. Structure of DNA topoisomerases (review). *Biochim. Biophys. Acta*, **1400**, 3–18 (1998).
16) Osheroff, N. DNA topoisomerases (review). *Biochim. Biophys. Acta*, **1400**, 1–2 (1998).
17) Benjamin, C. W., Hiebsch, R. R. and Jones, D. A. Caspase activation in MCF7 cells responding to etoposide treatment. *Mol. Pharmacol.*, **53**, 446–450 (1998).
18) Dubrez, L., Savoy, I., Hamman, A. and Solary, E. Pivotal role of a DEVD-sensitive step in etoposide-induced and FAS-mediated apoptotic pathways. *EMBO J.*, **15**, 5504–5512 (1996).
19) Shao, R. G., Cao, C. X. and Pommier, Y. Activation of PKCα downstream from caspases during apoptosis induced by 7-hydroxystaurosporine or the topoisomerase inhibitors, camptothecin and etoposide, in human myeloid leukemia HL60 cells. *J. Biol. Chem.*, **272**, 31321–31325 (1997).
20) Garcia-Bermejo, L., Perez, C., Vilaboa, N. E., Blas, E. and Aller, P. cAMP increasing agents attenuate the generation of apoptosis by etoposide in promyelocytic leukemia cells. *J. Cell Sci.*, **111**, 637–644 (1998).
21) Hande, K. R. Clinical applications of anticancer drugs targeted to topoisomerase II. *Biochim. Biophys. Acta*, **1400**, 173–184 (1998).
22) Utsugi, T., Aoyagi, K., Asao, T., Okazaki, S., Aoyagi, Y., Sano, M., Wierzba, K. and Yamada, Y. Antitumor activity of a novel quinoline derivative, TAS-103, with inhibitory effects on topoisomerases I and II. *Jpn. J. Cancer Res.*, **88**, 992–1002 (1997).
23) Martins, L. M., Mesner, P. W., Basi, K. G, Sinha, S., Tung, J. S., Svingen, P. A., Madden, B. J., Takahashi, A., McCormick, D. J., Earnshaw, W. C. and Kaufmann, S. H. Comparison of caspase activation and subcellular localization in HL-60 and K562 cells undergoing etoposide-induced apoptosis. *Blood*, **90**, 4283–4296 (1997).
24) Adjei, P. N., Kaufmann, S. H., Leung, W. Y., Mao, F. and Gores, G. J. Selective induction of apoptosis in Hep 3B cells by topoisomerase inhibitors: evidence for a protease-dependent pathway that does not activate cysteine protease CPP32. *J. Clin. Invest.*, **98**, 2588–2596 (1997).
25) Sarin, A., Williams, M. S., Alexander-Miller, M. A., Berzofsky, J. A., Zacharchuk, C. M. and Henkart, P. A. Target cell lysis by CTL granule exocytosis is independent of ICE/Ced-3 family proteases. *Immunity*, **6**, 209–215 (1997).
26) Hansen, M. B., Nielsen, S. E. and Berg, K. Re-examination and further development of a precise and rapid dye method for measuring cell growth and cell kill. *Immunol. Methods*, **119**, 203–210 (1989).
27) Stefanis, L., Park, D. S., Yan, C. Y. I., Farinelli, S. E., Troy, C. M., Shelanski, M. L. and Green, L. A. Induction of CPP32-like activity in PC12 cells by withdrawal of tropic support. *J. Biol. Chem.*, **271**, 30663–30671 (1996).
28) Fukuoka, M., Niitani, H., Suzuki, A., Motomiya, M., Hasegawa, K., Nishiwaki, Y., Kuriyama, T., Ariyoshi, Y., Negoro, S., Masuda, N., Nakajima, S. and Taguchi, T. A phage II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. *J. Clin. Oncol.*, **10**, 16–20 (1992).
29) Armand, J. P., Ducreux, M., Mahjoubi, M., Abigerges, D., Bugat, R., Chabot, G., Herait, P., de Forni, M. and Rougier, P. CPT-11 (irinotecan) in the treatment of colorectal cancer. *Eur. J. Cancer*, **31A**, 1283–1287 (1995).
30) O'Dwyer, P. J., Leyland-Jones, B., Alonso, M. T., Marsoni, S. and Wittes, R. E. Etoposide (VP-16-213). Current status of an active anticancer drug. *N. Engl. J. Med.*, **312**, 692–700 (1985).
31) Barnes, S., Peterson, T. G. and Coward, L. Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer. *J. Cell. Biochem. Suppl.*, **22**, 181–187 (1995).
32) Giovanella, B. C., Stehlin, J. S., Wall, M. E., Wani, M. C., Nicholas, A. W., Liu, L. F., Silber, R. and Potmesil, M.

DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science*, **246**, 1046–1048 (1989).
33) Holden, J. A., Rolfson, D. H. and Wittwer, C. T. Human DNA topoisomerase II: evaluation of enzyme activity in normal and neoplastic tissues. *Biochemistry*, **29**, 2127–2134 (1990).
34) Sugimoto, Y., Tsukahara, S., Oh-hara, T., Liu, L. F. and Tsuruo, T. Elevated expression of DNA topoisomerase II in camptothecin-resistant human tumor cell lines. *Cancer Res.*, **50**, 7962–7965 (1990).
35) Pommier, Y. and Shimizu, T. Camptothecin-induced apoptosis in p53-null leukemia HL60 cells and their isolated nuclei: effects of the protease inhibitors z-VAD-fmk and dichloroisocoumarin suggest an involvement of both caspases and serine proteases. *Leukemia*, **11**, 1238–1244 (1997).
36) Kluck, R. M., Martin, S. J., Hoffman, B. M., Zhou, J. S., Green, D. R. and Newmeyer, D. D. Cytochrome c activation of CPP32-like proteolysis plays a critical role in a *Xenopus* cell-free apoptosis system. *EMBO J.*, **16**, 4639–4649 (1997).
37) Kluck, R. M., Bossy-Wetzel, E., Green, D. R. and Newmeyer, D. D. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science*, **275**, 1132–1136 (1997).
38) Yang, E. and Korsmeyer, S. J. Molecular thanatopsis: a discourse on the Bcl-2 family and cell death. *Blood*, **88**, 386–401 (1996).