

Cytotoxic Activity of Selected Trifluoromethyl Ketones Against Oral Tumor Cells

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Abstract. Several trifluoromethyl ketones (**TF1-4**) and related non-fluorinated ketones (**TF5** and **6**) were tested for their relative cytotoxicity on four human tumor cell lines (oral squamous cell carcinoma HSC-2, HSC-3, HSC-4 and promyelocytic leukemia HL-60) and three normal human cells [gingival fibroblasts (HGF), pulp cells (HPC) and periodontal ligament fibroblasts (HPLF)]. Trifluoromethylated α -diketone (**TF1**, $CF_3COCOPh$) and α -hydroxy ketones (**TF2**, $CF_3CH(OH)COPh$; **TF3**, $CF_3CH(OH)COCH_2Ph$) showed higher tumor-specific cytotoxic activity than the corresponding non-fluorinated analogs (**TF5**, $CH_3COCOPh$; **TF6**, $CH_3CH(OH)COPh$), while the anti-tumor potency of trifluoromethyl ketone (**TF4**, CF_3COCH_2Ph) was lower. Among four tumor cell lines, HL-60 cells were the most sensitive to **TF1-4**, followed by HSC-2 and HSC-3 cells. HSC-4 cells were the most resistant in most cases. Agarose gel electrophoresis showed that **TF1-3** did not induce internucleosomal DNA fragmentation nor activated caspase-3. The cytotoxic activities of **TF1-3** were not significantly affected by $FeCl_3$. Electron microscopy of **TF2**- or **3**-treated HL-60 cells showed the development of autophagosomes in HL-60 cells, without the production of an apoptotic body, or affecting the mitochondria and cell surface microvilli. The autophagy inhibitor, 3-methyladenine (3-MA), partially inhibited the **TF2**- or **3**-induced cytotoxicity. These data suggest the induction of non-apoptotic cell death by **TF2** or **3**.

Trifluoromethyl ketones (TFs) have been shown to be inhibitors of a variety of proteases (1). The strong electron-

withdrawing character of the trifluoromethyl group alters the properties of the carbonyl group and increases their electrophilicity (2). Recent efforts have been devoted to the discovery of new biological activities, such as anti-*Helicobacter pylori* (3), antibacterial (4), cyclooxygenase-2 inhibitory (5) and histone deacetylase inhibitory (6) activities. We reported that two α -trifluoromethyl acyloins (**TF2** and **3**) (Figure 1) induced apoptotic cell death, characterized by DNA fragmentation (as identified by the TUNEL method) and activation of caspases (as identified by degradation products of cytokeratin 18 using M30 monoclonal antibody), in human submandibular gland carcinoma (HSG) cells (7). On the other hand, the structurally-related α -trifluoromethyl diketone (**TF1**) (Figure 1) inhibited K^+ deprivation-induced apoptosis in cerebellar granule neurons (CGNs) and induced internucleosomal DNA fragmentation in CGNs (8, 9). However, the induction of apoptosis by **TF1** was not mediated by the activation of caspase-3. Thus, two structurally-related TFs, $CF_3COCOPh$ (**TF1**) and $CF_3CH(OH)COPh$ (**TF2**), differed in their apoptosis-modulating activity. With the aim of providing further insight into the mode of action of the cytotoxicity of TF compounds (**TF1-4**) and the related non-fluorinated ketones (**TF5** and **6**) (Figure 1), their tumor-specific cytotoxic activity was investigated against four human tumor cell lines (oral squamous cell carcinoma HSC-2, HSC-3, HSC-4 and promyelocytic leukemia HL-60) and three normal human cells [gingival fibroblast (HGF), pulp cell (HPC) and periodontal ligament fibroblast (HPLF)].

Materials and Methods

Chemicals. The following chemicals and reagents were obtained from the companies indicated: Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL, Gland Island, NY, USA); fetal bovine serum (FBS; JRH, Bioscience, Lenexa, KS, USA), RPMI1640, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chem Co., St. Louis, MO, USA); dimethyl sulfoxide (DMSO; Wako Pure Chem. Ind., Osaka, Japan). **TF1**,

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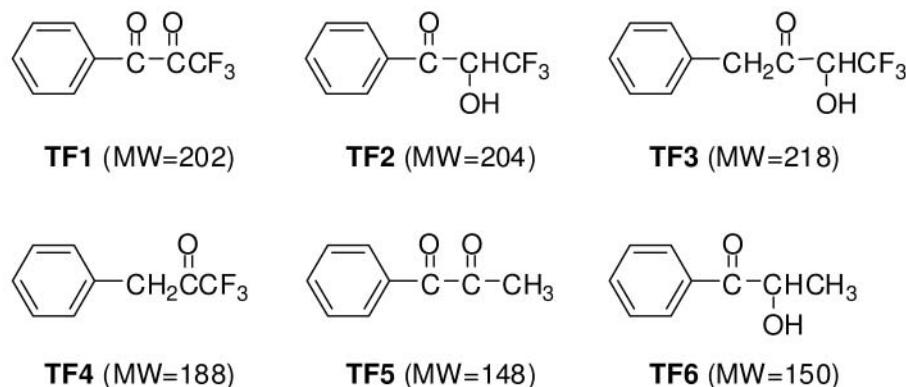


Figure 1. Structures of TF compounds studied.

TF2, TF3, TF4, TF5 and TF6 (Figure 1) were prepared, as described previously (2-4), dissolved in DMSO at 80 mM and added to the cell cultures at the indicated final concentrations. The final DMSO concentration in the culture medium was below 0.5%, a concentration that does not affect the growth of the cells.

Cell culture. HL-60 cells were obtained from Prof. K. Nakaya, Showa University, Japan. HSC-2 and HSC-4 cells were obtained from Prof. M. Nagumo, Showa University. HSC-3 cells were obtained from Prof. Y. Ohmori, Meikai University, Japan. Normal oral cells (HGF, HPC, HPLF) were prepared from periodontal tissues, according to the guideline of Meikai University Ethics Committee (No. 0206), after obtaining the informed consent from the patients. Since normal oral cells have a limited lifespan, ceasing proliferation at 20 population doubling level (PDL) (10), these cells were used at 5-9 PDL in the present study. HL-60 cells were cultured in RPMI 1640 medium supplemented with 10% FBS in a humidified 5% CO₂ atmosphere. Other adherent cells (three normal cells and three tumor cells) were cultured in DMEM medium supplemented with 10% heat-inactivated FBS. Normal cells were washed with phosphate-buffered saline without Mg and Ca (PBS(-)), detached with 0.25% trypsin-0.025% EDTA-2Na in PBS(-) and subcultured at a 1:4 split ratio once a week, with one medium change in between. Five adherent tumor cell lines were similarly trypsinized and subcultured twice a week.

Cytotoxic activity. The relative viable cell number of adherent cells was determined using the MTT method, while that of non-adherent cells (HL-60 cells) was determined using trypan blue dye exclusion. For the MTT assay, near confluent cells (1-2x10⁴/well in 96-well microplate) were treated for 24 h without (control) or with various concentrations of test samples. The cells were washed once with PBS(-) and further incubated for 4 h with 0.2 mg/mL MTT in DMEM + 10% FBS. After removal of the medium, the cells were lysed with 0.1 mL of DMSO. The absorbance at 540 nm of the solubilized formazan pellet (which reflects the relative viable cell number) was then determined using a plate reader (Labsystems Multiskan, Biochromatic LabSystem, Osaka, Japan) with a Star/DOT Matrix printer JL-10. For the trypan blue dye exclusion assay, the number of cells which do not incorporate the trypan blue dye was determined using a hemocytometer. From the dose-

response curve, the 50% cytotoxic concentration (CC₅₀) was determined (11). The tumor-specificity index (TS) was defined by equation [1]:

$$TS = \{[CC_{50}(\text{HGF}) + CC_{50}(\text{HPC}) + CC_{50}(\text{HPLF})]/[CC_{50}(\text{HSC-2}) + CC_{50}(\text{HSC-3}) + CC_{50}(\text{HSC-4}) + CC_{50}(\text{HL-60})]\} \times (4/3) \quad (\text{equation [1]})$$

Assay for DNA fragmentation. Cells were lysed with 50 μL lysate buffer [50 mM Tris-HCl (pH 7.8), 10 mM EDTA, 0.5% (w/v) sodium N-lauroylsarcosinate]. The lysate was incubated with 0.4 mg/ml RNase A and 0.8 mg/mL proteinase K for 1-2 h at 50°C, mixed with 50 μL NaI solution [7.6 M NaI, 20 mM EDTA-2Na, 40 mM Tris-HCl, pH 8.0] and 100 μL of ethanol. After centrifugation for 20 min at 20,000 xg, the precipitate was washed with 1 mL of 70% ethanol and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Sample (10-20 μL) was applied to 2% agarose gel electrophoresis in TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA, pH 8.0). DNA molecular weight marker (Takara), and the DNA from apoptotic HL-60 cells induced by UV irradiation (6 J/m²/min, 1 min) followed by 6 h incubation in regular culture medium (12), were run in parallel as positive controls. After staining with ethidium bromide, DNA was visualized using UV irradiation and photographed with a CCD camera (Bio Doc Inc, UVP) (12).

Assay for caspase activation. Cells were washed with PBS(-) and lysed in lysis solution (MBL, Nagoya, Japan). After standing for 10 min on ice and centrifugation for 5 min at 10,000 xg, the supernatant was collected. Lysate (50 μL, equivalent to 200 μg protein) was mixed with 50 μL 2x reaction buffer (MBL) containing substrates for caspase-3 [DEVD-pNA (*p*-nitroanilide)], caspase-8 (IETD-pNA) or caspase-9 (LEHD-pNA). After incubation for 2 h at 37°C, the absorbance at 405 nm of the liberated chromophore pNA was measured using a microplate reader (13, 14).

Electron microscopy. Cells were washed with PBS(-), fixed for 1 h with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C, post-fixed for 1 h with 1% osmium tetroxide-0.1 M cacodylate buffer (pH 7.4) at 4°C, dehydrated and then embedded in Araldite 502 (CIBA-GEIGY, Switzerland; NISSHIN EM Co., Ltd., Tokyo, Japan). Fine sections were stained with uranyl acetate and lead citrate, then observed under a JOEL-1210 transmission electron microscope at an accelerating voltage of 100 kV (15). Mitochondrial

Table I. Tumor-specificity of trifluoromethylketones.

50% cytotoxic concentration (CC ₅₀ : μM)							
	Human normal cells			Human tumor cell lines			
	HGF	HPC	HPLF	HSC-2	HSC-3	HSC-4	HL-60
TF1	>400	301±41	398±4	107±47	223±112	175±36	30±6
TF2	341±68	128±63	337±48	43±9	46±12	119±82	21±5
TF3	347±67	181±37	326±51	61±15	76±10	108±56	33±4
TF4	>353±32	>366±31	350±12	221±51	277±19	279±20	90±36
TF5	>400	>400	>400	>400	>400	>400	239±70
TF6	>400	>400	>400	>400	>400	>400	>400

Each value represents the mean ± S.D. of three independent experiments.

area (S) was calculated using the following equation $S=\pi ab$, where a and b are the shorter and longer radiiuses of mitochondria, respectively.

Results

Tumor-specificity. The α-trifluoromethyl acyloins (**TF1-3**) showed higher cytotoxic activity against tumor cell lines (CC₅₀=30-223, 21-119 and 33-108 μM, respectively) and tumor-specificity (TS=≥3.5, 4.5 and 4.0, respectively) than the corresponding non-fluorinated analogs (**TF5, TF6**) (CC₅₀=239->400 and >400 μM, respectively; TS=>1.0 and >1.0, respectively). On the other hand, the anti-tumor potency of **TF4** was much less in comparison (CC₅₀=90-279 μM; TS=>1.6) (Table I). Of the four tumor cell lines, HL-60 cells were the most sensitive to **TF1-4**, followed by HSC-2 and HSC-3 cells. HSC-4 cells were the most resistant in most cases (Table I).

As acyloins generally have metal ion chelating ability, the cytotoxicity of **TF1-3** was examined in the presence of FeCl₃. The activities of **TF1-3** against HSC-2 and HL 60 were not influenced by Fe³⁺ (Table II).

Induction of non-apoptotic cell death. Agarose gel electrophoresis showed that **TF1-3** induced large DNA fragments, but not internucleosomal DNA fragmentation in HL-60 cells, in contrast to UV irradiation that induced a ladder pattern of DNA fragmentation typical for apoptotic cell death (Figure 2). The fluorometric protease assay showed that **TF2** and **TF3** did not activate, but slightly inhibited caspase-3, in contrast to actinomycin D (a positive control) that activated caspase-3, approximately, 13-fold (Figure 3). On the other hand, **TF2** and **TF3** only marginally activated caspase-8 and -9 (Figure 3).

Possible induction of autophagy. EM study shows that **TF2** and **TF3** did not induce the apoptotic body, nor affected

Table II. Effect of Fe³⁺ on the cytotoxicity of **TF1-3** against HSC-2 and HL-60 cells.

Compound	0.25 mM FeCl ₃	CC ₅₀ (μM)	
		HSC-2	HL-60
TF1	-	107	30
	+	137	29
TF2	-	43	21
	+	49	23
TF3	-	61	33
	+	58	33

Each value represents the mean from three determinations.

Table III. Effect of **TF2** and **TF3** on the mitochondrial area in HL-60 cells.

Treatment (μM)	Mitochondrial area (μm ²)		
Control	0	0.128±0.089	(n=114)
TF2	20	0.125±0.092	(n=101)
	40	0.163±0.102	(n=165)
	80	0.130±0.088	(n=157)
TF3	30	0.173±0.144	(n=131)
	60	0.145±0.094	(n=121)
	120	0.131±0.095	(n=143)

Each value represents the mean ± S.D. from the indicated number of samples.

mitochondrial integrity or size (Figure 4A, C, E, Table III). **TF2** and **TF3** induced vacuolization and the production of autophagosome in which organelles were engulfed (Figure 4D, F). This result suggests the occurrence of autophagy. To

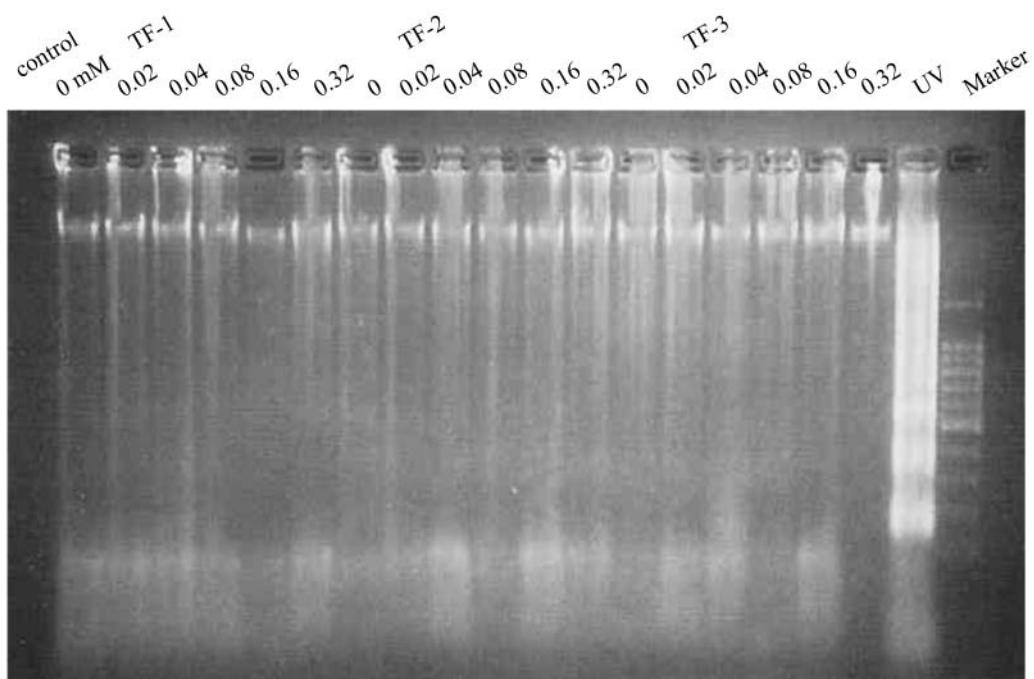


Figure 2. Induction of internucleosomal DNA fragmentation of HL-60 cells by TF compounds (TF1-3). HL-60 cells were incubated for 6 h with the indicated concentrations of TF1, TF2 or TF3, and the DNA was extracted for the assay of DNA fragmentation using agarose gel electrophoresis. UV: DNA extracted from apoptotic HL-60 cells induced by UV irradiation; marker: DNA molecular weight marker.

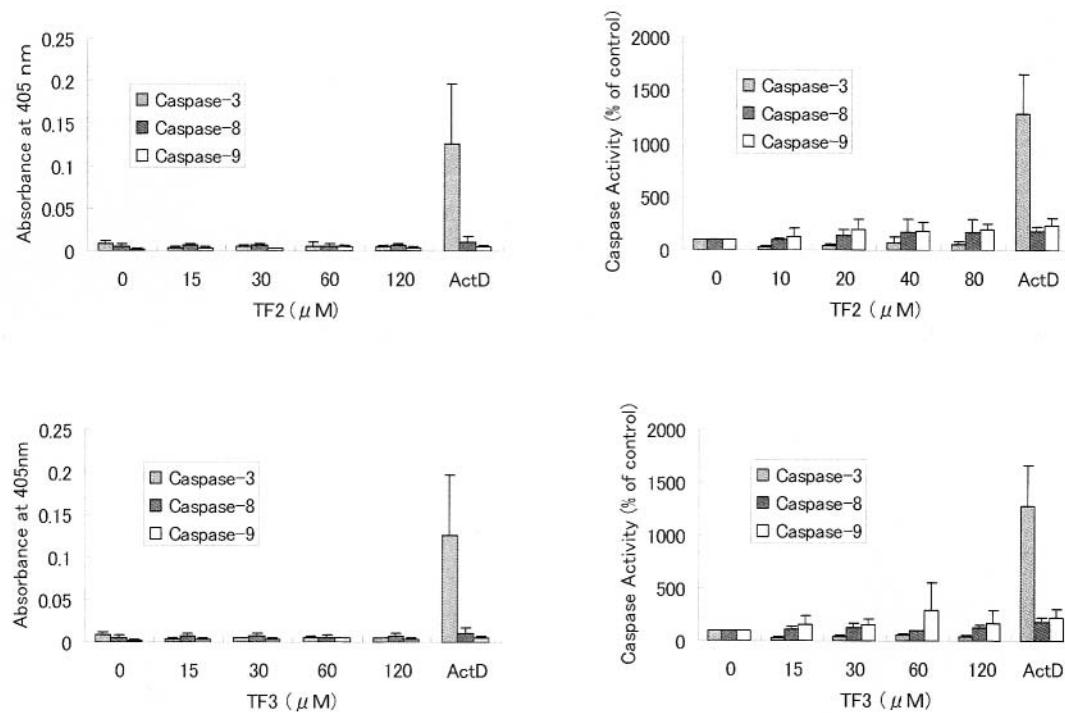


Figure 3. Effect of TF compound on the caspase-3, -8 and -9 activity in HL-60 cells. HL-60 cells were incubated for 4 h without (control) or with the indicated concentrations of TF2 or TF3 or 1 μg/mL actinomycin D (ActD, a positive control), and the activities of caspase-3, -8 and -9 were assayed using a substrate cleavage assay. Caspase activity was either expressed as the absorbance at 405 nm of cleaved product (pNA from the substrate of caspase-3, -8 and -9, left panels) or by % of control (right panels).

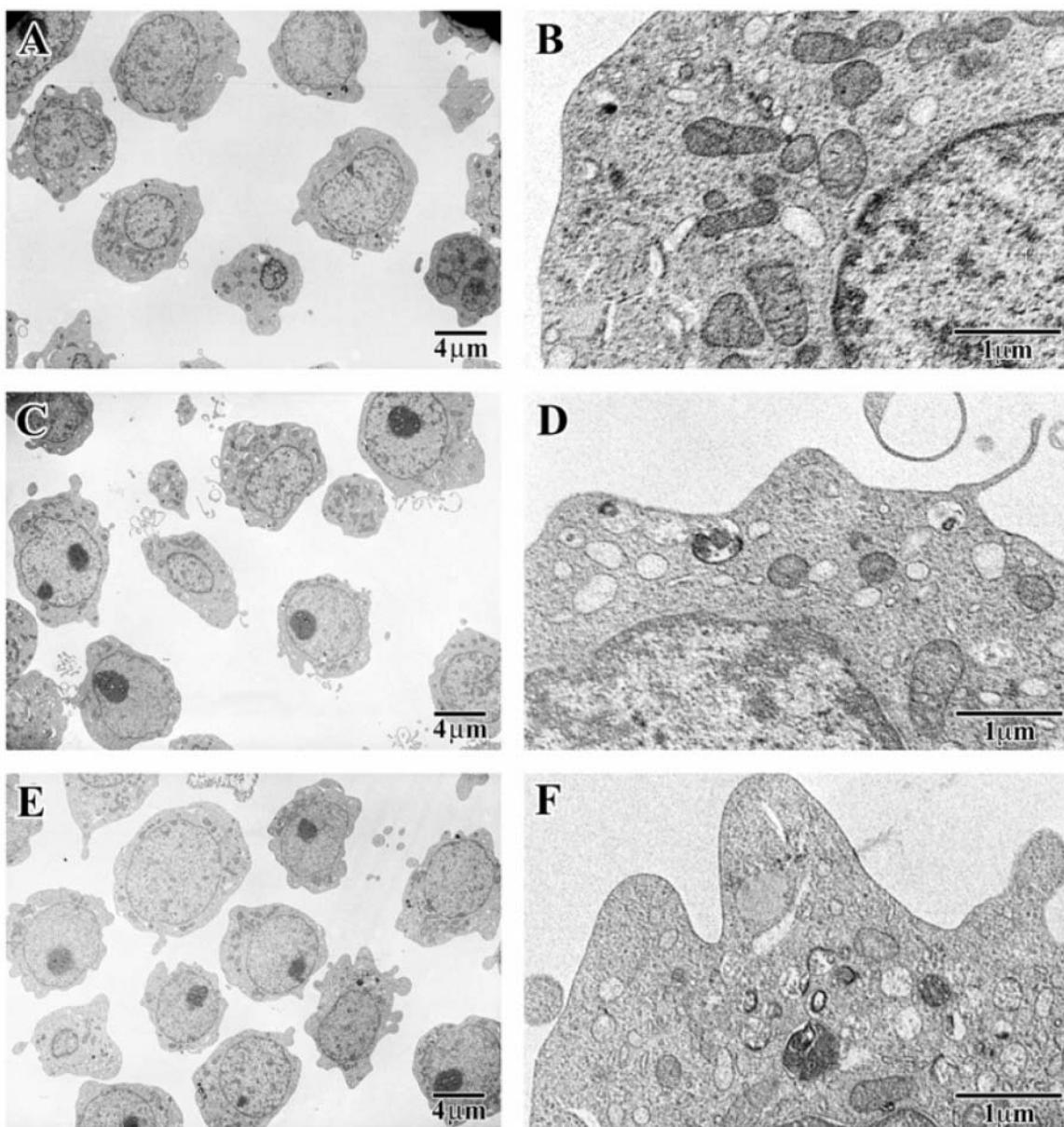


Figure 4. Transmission electron microscopy of TF compound-treated HL-60 cells. HL-60 cells were incubated for 6 h without (control) (A, B) or with 40 μ M TF2 (C, D), or 120 mM TF3 (E, F), and then processed for electron microscopy. Bar: 4 μ m (A, C, E), 1 μ m (B, D, F).

test this possibility, the effect of 3-methyladenine (3-MA), an inhibitor of autophagy, on the **TF2** or **3**-induced cell death was investigated (Figure 5). HL-60 cells were highly sensitive to 3-MA; the viability of HL-60 cells was reduced by 45% at 10 mM (left panels in Figure 5), a concentration that has been used to inhibit autophagy (16). When corrected for 3-MA cytotoxicity, the **TF2**- or **3**-induced cell death was found to be slightly inhibited by 3-MA (right panels in Figure 5).

Discussion

A series of structurally-related TF compounds (**TF1-6**) were examined in order to define the importance of the trifluoromethylated α -hydroxy ketone (acyloin) moiety. The potency of cytotoxic activity was in the order of $\text{CF}_3\text{CH}(\text{OH})\text{COPh}$ (**TF2**)> $\text{CF}_3\text{CH}(\text{OH})\text{COCH}_2\text{Ph}$ (**TF3**)> CF_3COCOPh (**TF1**)> $\text{CF}_3\text{COCH}_2\text{Ph}$ (**TF4**)>> CH_3COCOPh (**TF5**), $\text{CH}_3\text{CH}(\text{OH})\text{COPh}$ (**TF6**). The non-

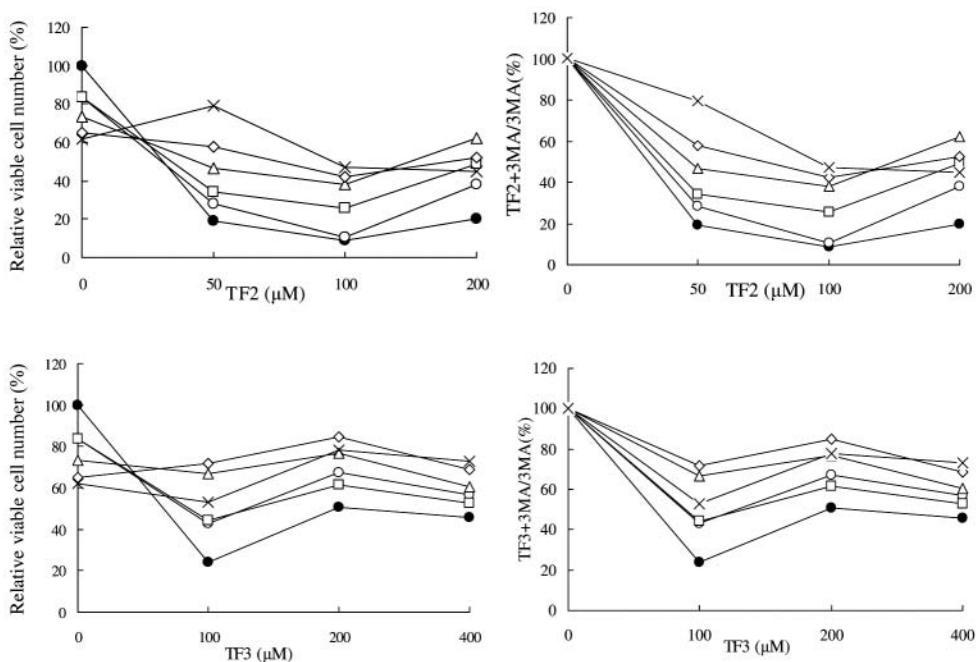


Figure 5. Effect of 3-methyladenine (3-MA) on TF compound-induced cytotoxicity. HL-60 cells were incubated for 24 h with the indicated concentrations of TF2 or TF3 in the presence of 0 (●), 0.3 (○), 0.6 (□), 1.3 (△), 2.5 (◇) or 5 mM (×) 3-MA, and the viable cell number was determined using trypan blue dye exclusion. Data was corrected for 3-MA cytotoxicity (right panels). Each value represents the mean from four independent experiments. S.D. < 10%.

fluorinated compounds (**TF5** and **6**) significantly diminished the cytotoxic activity. On the other hand, the anti-tumor potency of trifluoromethyl ketone (**TF4**) was lower, whereas the α -diketone (**TF1**) exhibited cytotoxicity comparable to those of α -hydroxy ketones (**TF2** and **3**). These data suggest the importance of α -hydroxy ketone moiety for cytotoxicity.

We demonstrated that the α -trifluoromethyl acyloins (**TF2** and **3**) and the structurally related α -trifluoromethyl diketone (**TF1**) did not induce apoptosis (type I programmed cell death) in HL-60 cells, based on their inability to induce apoptosis markers, such as internucleosomal DNA fragmentation and caspase-3 activation. This finding is quite different from the previous report that **TF1** and **2** induced DNA fragmentation (as identified using the TUNEL method) and activation of caspases (as identified by degradation products of cytokeratin 18 using M30 monoclonal antibody) in human submandibular gland carcinoma HSG cells (7). Although the TUNEL method is a popular method for detecting apoptosis on an individual cell basis, this method cannot distinguish between internucleosomal DNA fragmentation and large DNA fragments. Furthermore, the detection of degradation products with M30 monoclonal antibody is an indirect method for the evaluation of caspase activation. Therefore, further criteria for apoptosis should be assayed in HSG cells.

Autophagy (type II programmed cell death) is a pathway

for the bulk degradation of subcellular constituents through the creation of autophagosomes / autolysosomes in response to stresses, such as nutrient deprivation (16). In general, autophagy is utilized so that cells can survive, but constitutive activation of autophagy may induce cell death. We found that **TF2** and **TF3** induced the production of autophagosomes in HL-60 cells, suggesting the induction of autophagy. This possibility was tested, using 3-MA, which is known to inhibit autophagy (17). We found that this inhibitor was very cytotoxic to HL-60 cells, and when the cell death induced by **TF2** or **3** was corrected for 3-MA cytotoxicity, it was considerably reduced. However, more rigorous investigations of autophagy-specific markers, such as LC3 expression in the autophagosome, should be performed for any final conclusions of autophagy induction to be drawn. It has been reported that brain tumor cell lines are easily susceptible to undergo autophagy upon treatment with various chemotherapeutic agents (18). This suggests that the type of the cell may be one of the factors that determines the type of cell death (either apoptosis or autophagy).

The other factor that may determine the type of cell death may be the compound itself. For example, as previously reported, α,β -unsaturated ketones, such as 2-cyclohexen-1-one, 2-cyclopenten-1-one, 4,4-dimethyl-2-cyclopenten-1-one, 2-cyclohepten-1-one, α -methylene- γ -butyrolactone, 5,6-dihydro-2H-pyran-2-one, methyl 2-oxo-2H-pyran-3-carboxylate (13), codeinone (oxidative product of codeine)

(16) and morphinone (an oxidative metabolite of morphine) (19) very weakly activated caspases-3, -8 and -9, and that morphinone also induced the vacuolization and production of autophagosome and the loss of cell surface microvilli, without destruction of the cell surface and nuclear membranes in HL-60 cells. **TF2** and **3** belong to the α -hydroxy ketone group, whereas **TF1** belongs to the α -diketones. This suggests that not only α -hydroxy ketones but also α -diketones may induce non-apoptotic cell death.

References

- 1 Begue J-P and Bonnet-Delpon D: Preparation of trifluoromethyl ketones and related fluorinated ketones. *Tetrahedron* 47: 3207-3258, 1991.
- 2 Kawase M: Synthetic and medicinal chemistry of trifluoromethyl ketones. *J Synth Org Chem. Jpn* 59: 755-765, 2001.
- 3 Kawase M, Harada H, Saito S, Cui J and Tani S: *In vitro* susceptibility of *Helicobacter pylori* to trifluoromethyl ketones. *Bioorg Med Chem Lett* 9: 193-194, 1999.
- 4 Kawase M, Motohashi N, Sakagami H, Kanamoto T, Nakashima H, Ferenczy K, Wolfard K, Miskolci C and Molnar J: Antimicrobial activity of trifluoromethyl ketones and their synergism with promethazine. *Int J Antimicro Agents* 18: 161-165, 2001.
- 5 Khanna IK, Weier RM, Yu Y, Collins PW, Miyashiro JM, Koboldt CM, Veenhuizen AW, Currie JL, Seibert K and Isakson PC: 1,2-Diarylpyrroles as potent and selective inhibitors of cylooxygenase-2. *J Med Chem* 40: 1619-1633, 1997.
- 6 Frey RR, Wada CK, Garland RB, Curtin ML, Michaelides MR, Li J, Pease LJ, Glaser KB, Marcotte PA, Bouska JJ, Murphy SS and Davidson SK: Trifluoromethyl ketones as inhibitors of histone deacetylase. *Bioorg Med Chem Lett* 12: 3443-3447, 2002.
- 7 Kawase M, Sakagami H, Kusama K, Motohashi N and Saito S: α -Trifluoromethylated acyloins induce apoptosis in human oral tumor cell lines. *Bioorg Med Chem Lett* 9: 3113-3118, 1999.
- 8 Kawase M, Sunaga K, Tani S, Niwa M and Uematsu T: Trifluoromethyl ketone-based inhibitors of apoptosis in cerebellar granule neurons. *Biol Pharm Bull* 24: 1335-1337, 2001.
- 9 Sunaga K, Tanaka T, Tani S and Kawase M: Trifluoromethyl ketones show culture age-dependent inhibitory effects on K^+ -induced apoptosis in cerebellar granule neurons. *In Vivo* 16: 97-102, 2002.
- 10 Satoh R, Kishino K, Morshed SRM, Takayama F, Otsuki S, Suzuki F, Hashimoto K, Kikuchi H, Nishikawa H, Yasui T and Sakagami H: Changes in fluoride sensitivity during *in vitro* senescence of human normal oral cells. *Anticancer Res* 25: 2085-2090, 2005.
- 11 Sakagami H, Jiang Y, Kusama K, Atsumi T, Ueha T, Toguchi M, Iwakura I, Satoh K, Ito H, Hatano T and Yoshida T: Cytotoxic activity of hydrolysable tannins against human oral tumor cell lines – A possible mechanism. *Phytomedicine* 7: 39-47, 2000.
- 12 Yanagisawa-Shiota F, Sakagami H, Kurabayashi N, Iida M, Sakagami T and Takeda M: Endonuclease activity and induction of DNA fragmentation in human myelogenous leukemic cell lines. *Anticancer Res* 15: 259-266, 1995.
- 13 Nakayachi T, Yasumoto E, Nakano K, Morshed SRM, Hashimoto K, Kikuchi H, Nishikawa H, Kawase M and Sakagami H: Structure-activity relationships of α,β -unsaturated ketones as assessed by their cytotoxicity against oral tumor cells. *Anticancer Res* 24: 732-742, 2004.
- 14 Yasumoto E, Nakano K, Nakayachi T, Morshed SRM, Hashimoto K, Kikuchi H, Nishikawa H, Kawase M and Sakagami H: Cytotoxic activity of deferiprone, maltol and related hydroxyketones against human tumor cell lines. *Anticancer Res* 24: 755-762, 2004.
- 15 Satoh K, Ida Y, Hosaka M, Arakawa H, Maeda M, Ishihara M, Kunii S, Kanda Y, Toguchi M and Sakagami H: Induction of apoptosis by cooperative action of vitamins C and E. *Anticancer Res* 18: 4371-4376, 1998.
- 16 Broker LE, Krut FA and Giaccone G: Cell death independent of caspase: a review. *Clin Cancer Res* 11: 3155-3162, 2005
- 17 Shimizu S and Tsujimoto Y: Molecular mechanisms of autophagic cell death. (in Japanese) *SAIBO-KOGAKU* 24: 581-586, 2005
- 18 Daido S, Yamamoto A, Fujisawa K, Sawaya R, Kondo S and Kondo Y: Inhibition of the DNA-dependent protein kinase catalytic subunit radiosensitizes malignant glioma cells by inducing autophagy. *Cancer Res* 65: 4368-4375, 2005.
- 19 Takeuchi R, Hoshijima H, Nagasaka H, Chowdhury SA, Kikuchi H, Kanda Y, Kunii S, Kawase M and Sakagami H: Induction of non-apoptotic cell death by morphinone in human promyelocytic leukemia HL-60 cells. *Anticancer Res* 26: in press, 2006

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