Inhibition of Pancreatic Cancer Cell Proliferation by Propranolol Occurs Through Apoptosis Induction

The Study of β-Adrenoceptor Antagonist's Anticancer Effect in Pancreatic Cancer Cell

Dong Zhang, PhD, Qingyong Ma, PhD, Sugang Shen, PhD, and Hengtong Hu, PhD

Objectives: Propranolol inhibited pancreatic cancer cell proliferation by blocking signaling through the β-adrenoceptor. We hypothesized that propranolol may suppress pancreatic cancer cell growth through induction of apoptosis.

Methods: The β-adrenoceptor antagonist propranolol, β_1-adrenoceptor antagonist metoprolol, and β_2-adrenoceptor antagonist butoxamine were used to induce apoptosis in PC-2 cells. The mRNA and protein expression of β_1- and β_2-adrenoceptors was analyzed using reverse transcriptase–polymerase chain reaction and Western blot. The apoptotic index was determined using Hoechst 33342 fluorescent staining, TUNEL, and annexin V and fluorescein isothiocyanate/propidium iodide flow cytometry assay. The expression of caspase 3, caspase 9, and caspase 8 was analyzed using Western blotting.

Results: PC-2 cell line expressed mRNA and protein for both of β_1- and β_2-adrenoceptors. The Hoechst staining, TUNEL, and flow cytometry assay documented that the 3 drugs increased the number of apoptotic cells; the rate of apoptosis was the highest using butoxamine followed by propranolol, whereas the least was using metoprolol. β-Adrenoceptor antagonists therapy affected caspase 3 and caspase 9 expression.

Conclusions: The rate of apoptosis in PC-2 cells was higher after treatment with butoxamine than propranolol, suggesting that propranolol induces apoptosis in PC-2 cells via the β_2-adrenoceptors principally. Our data could be useful for developing β-adrenoceptor antagonists for inducing apoptosis in pancreatic cancer cells.

Key Words: propranolol, pancreatic cancer cell, apoptosis

(*Pancreas* 2009;38: 94–100)

Worldwide, more than 200,000 people die annually from pancreatic cancer.[1] Pancreatic cancer is typically diagnosed in late stages because there are few early indicators of illness and no screening tests.[2,3] The incidence of pancreatic cancer is almost equal to its mortality rate. No substantial treatment improvements have been made, and the treatments have little effect on prolonging survival time. The most common type of pancreatic cancer is ductal adenocarcinoma, comprising 75% of all exocrine pancreatic cancers.[4] Recently, considerable efforts have been directed to design drugs to selectively target the apoptotic pathways, with the hope that they will have a significant impact on the therapeutic response while minimizing toxicity.

β-Adrenoceptors are members of the superfamily of G protein–coupled adrenergic receptors, which mediate the actions of endogenous catecholamines (norepinephrine and epinephrine) in a variety of target cells. Adrenoceptors modulate diverse intracellular processes, such as DNA synthesis through activation of mitogen-activated protein kinases (MAPKs). Currently, 2 native β-adrenoceptor subtypes have been identified, β_1 and β_2. β_2-Adrenoceptors are expressed in the BXPC-3 and Panc-1 cell lines and are functionally important in mediating pancreatic ductal adenocarcinoma cells.[5,6]

Recent studies demonstrated that β-adrenoceptor antagonists may inhibit pancreatic cancer cell proliferation by blocking the Ras and MAPK pathway.[7] In addition, β-adrenoceptor antagonists may decrease the release of arachidonic acid (AA), which is a lipid signaling molecule that regulates cell proliferation.[7,8] In the Panc-1 and BxPC-3 cell lines, proliferation inhibition via β_2-adrenoceptor antagonists occurs more than β_1-adrenoceptor antagonists.[8] The observed tumor-promoting effects were mediated by β-adrenergic agents with postinitiation events such as cell proliferation or apoptosis.[9] The broad-spectrum β-adrenergic antagonist propranolol has been widely used for hypertension and atherosclerosis therapy,[10,11] and we hypothesize that the drug may inhibit pancreatic cancer cell proliferation by inducing cell apoptosis.

To test our hypothesis, we examined the effects of the β-adrenoceptor antagonist propranolol in human pancreatic tumor epithelial cells. Our findings showed that propranolol can induce apoptosis in human pancreatic cancer cells, and these results may have significant therapeutic implications for developing β-adrenoceptor antagonists for pancreatic cancer treatment.

MATERIALS AND METHODS

Preparation of Chemicals

Dulbecco's modified Eagle medium and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY). The TUNEL kit was obtained from Roche Diagnostic (Mannheim, Germany). Trizol was purchased from Invitrogen (Carlsbad, Calif). The β-adrenoceptor antagonists, propranolol, metoprolol, and butoxamine, and Hoechst 33342, were purchased from Sigma Chemical (St Louis, Mo). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was obtained from Sigma Chemical. The reverse transcriptase–polymerase chain reaction (RT-PCR) kit was purchased from Fermentas Life Sciences (Vilnius, Lithuania). The annexin V–fluorescein isothiocyanate (FITC) and propidium iodide (PI) kit was obtained from BD Biosciences (San Jose, Calif). The primary antibodies of β1-adrenergic receptor, β_2-adrenergic receptor, caspase 3,

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Received December 4, 2007.
Accepted for publication June 25, 2008.
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ISSN: 0885-3177
DOI: 10.1097/MPA.0b013e318184f50c

caspase 9, and caspase 8 were purchased from Abcam (Cambridge, Mass). The nitrocellulose membrane was purchased from Millipore (Bedford, Mass). The BCA assay kit and the chemiluminescence kit were purchased from Pierce (Rockford, Ill).

Cell Lines and Treatment

The human ductal pancreatic adenocarcinoma cell line PC-2 was obtained from the Peking Union Medical College (Beijing, China), and the BxPC-3 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, Va). The cells were cultured in Dulbecco's modified Eagle medium with 10% (vol/vol) heat-inactivated FBS, penicillin (100 U/mL), and streptomycin (100 mg/mL) and grown at 37°C, 5% CO_2, 95% relative humidity. Before each experiment, cells were seeded at a density of 5×10^4 cells/cm^2.

Analysis of β-Adrenoceptor Expression Using RT-PCR and Western Blot

Total RNA was isolated from PC-2 and BxPC-3 cells using Trizol reagent, and the concentration of the RNA was determined by absorbance at 260 nm. For the RT reaction, 0.5 μg of oligo (dT) 18 primers in nuclease-free water was heated to 70°C for 5 minutes and then placed on ice. A total of 10 mM dNTPs, 20 units of RiboLock ribonuclease inhibitor, and 5× reaction buffer (250 mM Tris-HCl [pH 8.3], 250 mM KCl, and 20 mM $MgCl_2$, 50 mM DTT) were added, and the reaction was incubated at 37°C for 5 minutes. Next, 200 units of M-MuLV RT was added, and the reaction was incubated at 42°C for 1 hour, followed by heat inactivation for 10 minutes at 70°C.

Polymerase chain reaction was performed with 5 μL of the RT reaction, which was mixed with 1 μL of 10 mM dNTPs, 5 μL of 10× PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl, and 15 mM $MgCl_2$), 1.5 μL of Taq polymerase, 1 μL primers for the human β_1 or β_2- adrenoceptor, and nuclease-free water in a final volume of 50 μL. The β_1-adrenoceptor primers (forward, 5-GGGAGAAGCATTAGGAGGG-3; and reverse, 5-CAAGGAAAGCAAGGTGGG-3) amplified a 270–base pair (bp) fragment. The PCR conditions for the β_1 primers were as follows: 1 cycle of 2 minutes at 94°C; 35 cycles of 94°C for 30 seconds, 62°C for 60 seconds, and 70°C for 2 minutes; and a final extension for 7 minutes at 70°C. The β_2-adrenoceptor primers (forward, 5-CAGCAAAGGGACGAGGTG-3; and reverse, 5-AAGTAATGGCAAAGTAGCG-3) amplified a 334-bp fragment. The PCR conditions for the β_2 primers were as follows: 1 cycle of 2 minutes at 94°C; 35 cycles of 94°C for 30 seconds, 62°C for 60 seconds, and 70°C for 2 minutes; and a final extension for 7 minutes at 70°C. Reactions were run on an MJ Research PTC-200 thermal cycler. The PCR products and a 100-bp DNA ladder were run on a 1.5% agarose gel for 2 hours at 90 V. The gel was imaged by ethidium bromide staining using a UVP GDS 7500 or an Ultra Lum TUI-5000 gel documentation system. The sequences using the forward primers were compared with the sequence of human β_1-adrenoceptor (GenBank accession no. NM000684) or β_2-adrenoceptor (GenBank accession no. NM000024).

Protein was extracted from cultured cells in radioimmunoprecipitation assay lysis buffer on ice for 20 minutes. Lysates were cleared by centrifugation at 12,000 rpm for 12 minutes at 4°C. Supernatant was collected, and total protein concentration was measured using the BCA assay kit according to the manufacturer's instructions. Cell lysates containing equal amounts of protein were separated on 12% Tris-glycine polyacrylamide gels, and the protein was transferred electrically onto nitrocellulose membrane. Membranes were incubated in β_1-adrenergic receptor (1:500) or β_2-adrenergic receptor (1:500) primary antibodies (a minimum of 2 hours) and then secondary antibodies for 30 minutes. The protein bands specific for antibody were visualized by enhanced chemiluminescence associated fluorography.

MTT Assay

The effects of propranolol on cell proliferation were assessed using the colorimetric MTT assay. The MTT test is based on the nicotinamide adenine dinucleotide–dependent enzymatic reduction of the tetrazolium salt MTT in metabolically active cells, but not in dead cells. Cells were seeded in basal medium at a concentration of 1×10^4 cells/well in 96-well plates and allowed to grow and adhere in complete media at 37°C with 5% CO_2 for 20 to 24 hours. The cells were then switched to basal media with 0.05% FBS. The cells were incubated for 24 hours with (*a*) control, no treatment, (*b*) epinephrine (25-200 μM), (*c*) propranolol (25–200 μM), and (d) 5-fluorouracil (25–200 μM). After incubation, 20 μL MTT (5 mg/mL) was added to each well, and the cells grew in complete media at 37°C with 5% CO_2 for 4 hours. Supernatant was removed, and 150 μL DMSO was added to each well of the 96-well plate and then was swung for 10 minutes. Optical density at 490 nm was determined using an enzyme-linked immunosorbent assay reader.

Determination of Cell Apoptosis by Hoechst 33342 Fluorescent Staining

Cellular morphology consistent with apoptosis was assessed using Hoechst staining, as described previously. Briefly, cells were seeded in 24-well plates at 5×10^4 cells/well. Subconfluent cells were treated with propranolol (100 μM) for 24 hours. After treatment, cells were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) and stained with 10 μg/mL Hoechst 33342 dye. Fixed cells were incubated overnight at 4°C and visualized under a 365-nm UV light microscope. Quantitative analysis was performed by counting the blue fluorescent (apoptosis positive) cells from 3 independent fields at 400× magnification. Values were expressed as the percentage of apoptotic cells relative to the total number of cells per field (the average number of cells that were apoptosis-positive varied from 2–40 per field, whereas the total number of cells counted per field was 50–100).[12]

Apoptosis Evaluation Using TUNEL Assay

Apoptotic cells were detected using the TUNEL assay. Cells were seeded in 24-well plates at 5×10^4 cells/well. When cells were subconfluent, they were treated with propranolol (100 μM), metoprolol (100 μM), and butoxamine (100 μM) for 24 hours. Cell monolayers were washed twice with washing buffer, and then 10 mM proteinase K (1:200 dilution) was added for 15 seconds. Cells were then washed twice in TBS, followed by incubation with 1 μL terminal deoxynucleotidyl transferase (1:10 dilution) and 1 μL DIG-UTP (1:10 dilution) in a humidified box at 37°C for 2 hours. Cells were then washed twice in TBS and covered with remaining liquid at 37°C for 30 minutes in a humidified box. The cells were then incubated with a biotinylated anti-DIG antibody (1:20 dilution) and incubated for an additional 30 minutes with SABC (1:100 dilution) at room temperature. Cells were then washed twice in TBS, and 50 μL of a DAB solution modified as described earlier for immunohistochemical staining was applied to each section after TUNEL incubation. Sections were subsequently rinsed in distilled water, counterstained with hematoxylin,

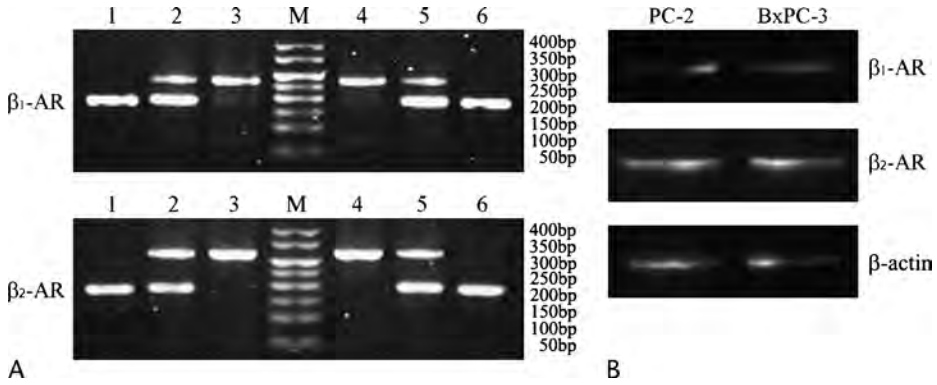

FIGURE 1. Expression of mRNA and protein for β_1- and β_2-adrenoceptor in the human ductal pancreatic adenocarcinoma cell line PC-2 by RT-PCR and Western blotting. In RT-PCR, the β_1 primers amplified a 270-bp fragment, whereas the β_2 primers amplified a 334-bp fragment (A). β_1-AR: lane 1, β-actin; lane 2, PC-2 amplified with β_1 primers and β-actin; lane 3, PC-2 amplified with β_1 primers; lane 4, BxPC-3 amplified with β_1; lane 5, BxPC-3 amplified with β_1 primers and β-actin; lane 6, β-actin; lane M, 50-bp DNA ladder. β_2-AR: lane 1, β-actin; lane 2, PC-2 amplified with β_2 primers and β-actin; lane 3, PC-2 amplified with β_2 primers; lane 4, BxPC-3 amplified with β_2; lane 5, BxPC-3 amplified with β_2 primers and β-actin; lane 6, β-actin; lane M, 50-bp DNA ladder. In Western blot, β_1- and β_2-adrenergic receptor immunoreactivity was visualized as a single band that migrates at approximately 64 and 95 kd, respectively.

washed and dehydrated through graded ethanols, and cleared in xylene. Sections were coverslipped, and the results were documented using an Olympus PM-6 microscope equipped with an Olympus digital camera. Quantitative apoptosis analysis of cells treated with β-adrenoceptor antagonists was performed by counting the TUNEL-positive cells within a field of 200 cells at 400× magnification.[13]

Analysis of the Apoptosis Rate by Annexin V and FITC/PI Flow Cytometry

The apoptosis rate was measured by flow cytometry (FCM) according to the instructions provided by the annexin V–FITC kit. In brief, after treatment with propranolol (100 μM), metoprolol (100 μM), and butoxamine (100 μM) for 24 hours, cells were harvested by centrifugation, washed once with ice-cold PBS, and resuspended in binding buffer at a concentration of 1×10^6 cells/mL, in which 100 mL of cell suspension was added in a 5-mL FCM tube. A total of 5 mL of annexin V–FITC and 10 mL of 20 mg/mL PI were added and incubated for 15 minutes in the dark before further addition of 400 mL PBS. Quantitative analysis of apoptotic level was performed using a flow cytometer (BD Biosystems, Calif). The apoptotic percentage of 10,000 cells was determined, and all the experiments reported in this study were performed in triplicate.

Analysis of Caspase 3, Caspase 9, and Caspase 8 Expression Using Western Blot

The PC-2 cells were treated with propranolol (100 μM), metoprolol (100 μM), and butoxamine (100 μM) for 24 hours, then protein was extracted from the treated cells and was subjected to Western blotting analysis using specific antibodies to caspase 3, caspase 9, and caspase 8. The cells were harvested and rinsed twice with PBS. Protein was extracted from cultured cells in radioimmunoprecipitation lysis buffer on ice for 20 minutes and cleared by centrifugation at 12,000 rpm for 30 minutes at 4°C. Supernatant was collected, and total protein concentration was measured using the BCA assay kit according to the manufacturer's instructions. Cellular extract

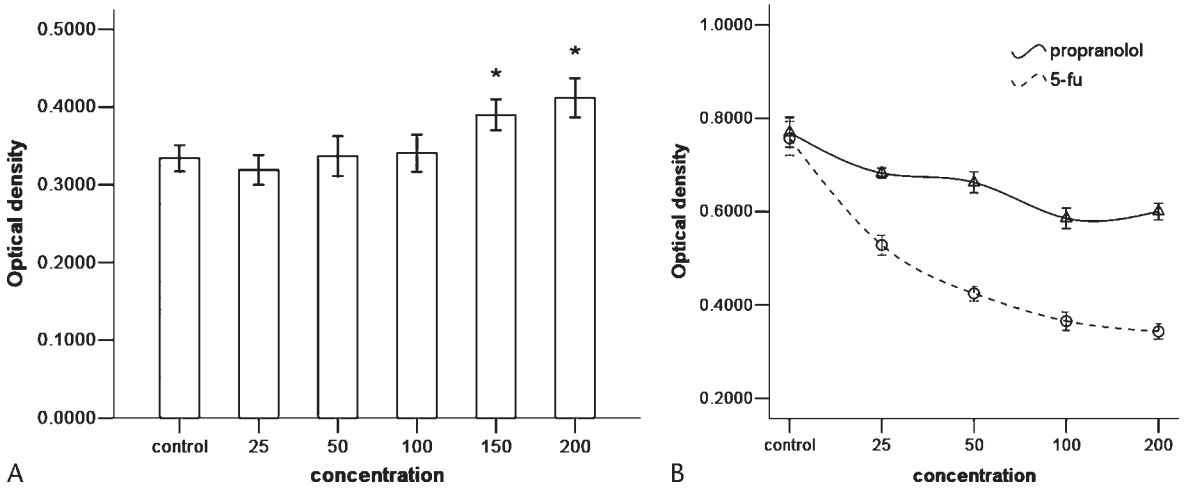

FIGURE 2. PC-2 cells treated with epinephrine for 24 hours (A) showed increased cellular proliferation in a concentration dependent manner. *$P < 0.05$ when compared with controls. Effect of β-adrenoceptor antagonist propranolol on cell proliferation of PC-2 cells (B). PC-2 cells were exposed to increasing concentrations of propranolol, and cell proliferation was determined using the MTT assay.

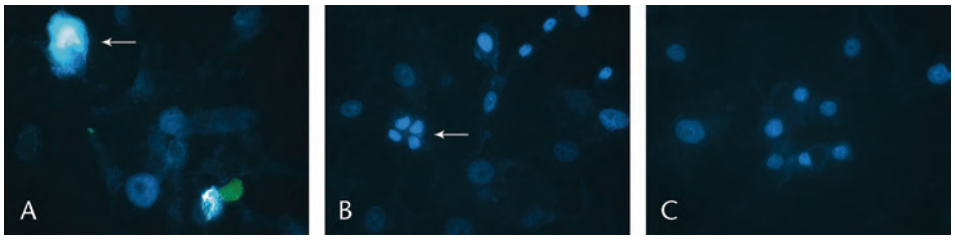

FIGURE 3. Hoechst staining to detect apoptotic PC-2 cells after propranolol treatments. PC-2 cells were treated with 100 μM propranolol (A and B) for 24 hours or left untreated as control (C). Cells were fixed and stained with Hoechst 33342. Arrows indicate apoptotic cells.

containing 30 μg of total protein was separated using 12% sodium dodecyl sulfate –polyacrylamide gel electrophoresis, and the protein was transferred electrically onto nitrocellulose membrane. The membrane was then blocked with TBST (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween-20) containing 5% (wt/vol) nonfat dry milk and incubated with primary antibody in TBST at 4°C overnight. The membrane was washed 3 times and then was incubated in secondary antibody (dilution factor, 1:2000) for 2 hours at room temperature. After washing 3 times for 10 minutes each with 15 mL TBST, protein bands specific for antibody were visualized by enhanced chemiluminescence-associated fluorography.

Statistical Analysis

In vitro data were analyzed by 1-way analysis of variance and by the nonparametric test, Kruskal-Wallis. Values were expressed as the mean ± SEM, and differences were considered statistically significant at $P < 0.05$.

RESULTS

mRNA and Protein Expression of β-Adrenoceptors in PC-2 Cells

In accordance with recent studies, our results showed the human ductal pancreatic adenocarcinoma cell line PC-2 expressed mRNA and protein for both of the β_1- and β_2-adrenoceptors same as BxPC-3 cell line, with the β_2 mRNA and protein yielding the more prominent band (Fig. 1).[8] The PCR fragments amplified by the human β_1 primers in the PC-2 cells were 100% identical to the published sequence (GenBank accession no. NM000684). The PCR fragments amplified by the human β_2 primers in PC-2 cells were also 100% identical to the published sequence (GenBank accession no. NM000024). Western blot showed β_1-adrenergic receptor immunoreactivity was visualized as a single band that migrates at approximately 64 kd, whereas β_2-adrenergic receptor migrates at 95 kd.

Cell Proliferation in Propranolol-Treated Cells

To determine the optimal epinephrine concentrations for pancreatic cancer cell proliferation, PC-2 cells were treated with various concentrations of epinephrine for 24 hours. Exposure to epinephrine increased cell proliferation compared with the control group, with optimal cellular proliferation at 150 to 200 μM ($P < 0.05$; Fig. 2A). In contrast, cellular proliferation was significantly inhibited upon treatment with the broad-spectrum β-adrenergic antagonist propranolol for 24 hours compared with the control group. The rate of suppression by propranolol was lower than the 5-fluorouracil treatment used as a positive control ($P < 0.05$; Fig. 2B). To determine the optimum propranolol concentration for inhibiting cellular proliferation, PC-2 cells were treated with various concentrations of propranolol for 24 hours. The maximal response was obtained with 100 μM propranolol.

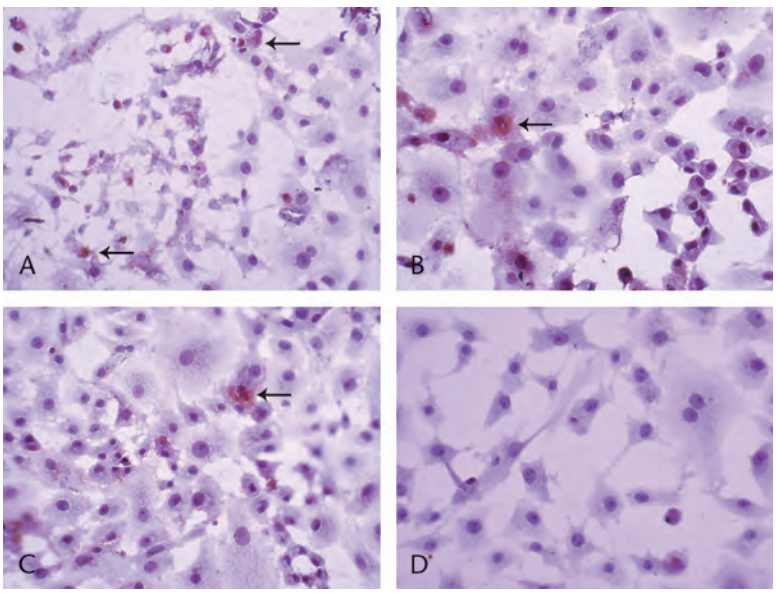

FIGURE 4. Apoptosis detection by TUNEL assay. PC-2 cells were treated with β-adrenoceptor antagonists: 100 μM butoxamine (A), 100 μM propranolol (B), and 100 μM metoprolol (C) for 24 hours or untreated as control (D). TUNEL-positive cells were counted using an Olympus PM-6 microscope.

TABLE 1. β-Adrenoceptor Antagonist–Induced Apoptosis in Human Pancreatic Cancer Cells

Treatment	PC-2 (% of TUNEL-Positive Cells)
Control	2.27 ± 0.77
Propranolol	11.25 ± 1.95*
Metoprolol	7.62 ± 1.46*
Butoxamine	15.4 ± 1.99*

Values represent the mean percentages of the number of apoptotic cells in 3 different 400× magnification fields ± SEM.

*$P < 0.05$ when compared with controls.

Apoptosis of Propranolol-Treated PC-2 Cells Measured by Hoechst Staining

We have previously demonstrated that PC-2 cells underwent apoptosis in response to treatment with propranolol. In this experiment, the effect of propranolol was examined in pancreatic cancer cell PC-2 (Fig. 3). Figure 3 shows the characteristic appearance of the apoptotic PC-2 cells in control (untreated cultures) and after treatment with propranolol for 24 hours. The propranolol treatment resulted in a significant increase in the number of apoptotic cells (10.2% ± 2.6%), compared with basal apoptosis levels in untreated controls (2.3% ± 1.2%). The percentage of apoptotic cells in the propranolol-treated group was statistically different from the untreated control cells ($P < 0.05$).

Apoptosis of Propranolol-Treated PC-2 Cells Measured by TUNEL Assay

Induction of apoptosis by propranolol in PC-2 cells was assessed by TUNEL assay (Fig. 4). Table 1 summarizes the results from the quantitative evaluation of apoptosis induction in response to β-adrenoceptor antagonist treatment. There was a significant increase in the number of TUNEL-positive pancreatic cancer cells after 24 hours of treatment with propranolol compared with the untreated control cells, metoprolol, and butoxamine as a positive control. The effect of the broad-spectrum β-adrenoceptor antagonist propranolol on the rate of apoptosis of pancreatic cancer cells was lower than the β_2-adrenoceptor antagonist butoxamine, but higher than β_1-adrenoceptor antagonist metoprolol.

Evaluation of the Apoptosis Rate by Annexin V and FITC/PI FCM

Apoptosis was quantitatively confirmed by analyzing the percentage of early apoptotic cells using annexin V–FITC/PI double staining. A marker of early apoptosis, measured by annexin V, is phosphatidylserine, which is released as a result of redistribution of the plasma membrane of the cells. Three main populations of cells were distributed in dot plots: viable cells ($FITC^-/PI^-$), early apoptotic cells ($FITC^+/PI^-$), and secondary necrotic cells ($FITC^+/PI^+$). The percentages of early apoptotic cells increased significantly after the treatment of propranolol, metoprolol, and butoxamine in PC-2, which were 14.7%, 6.29%, and 16.2%, respectively (Fig. 5).

The Relationship of β-Adrenoceptor Antagonists Treatment With Expression of Caspase 3, Caspase 9, and Caspase 8

Caspase family proteases have been reported to be activated in apoptosis. In our experiment, caspase 3, caspase 9, and caspase 8 were detected by Western blot in PC-2 after treatment with propranolol (100 μM), metoprolol (100 μM), and butoxamine (100 μM) for 24 hours. As shown in Figure 6, cleavages of caspase 3 and caspase 9 increased with treatment with β-adrenoceptor antagonists, with the butoxamine and propranolol yielding the more prominent band than metoprolol, whereas procaspase 8 had no cleavage fragments. These results suggested that β-adrenoceptor antagonist therapy affects caspase 3 and caspase 9 expression in PC-2 cells.

DISCUSSION

The broad-spectrum β-adrenoceptor antagonist propranolol is widely used for hypertension and atherosclerosis therapy.[10,11] The pharmacology of the drug has been well characterized in humans, and their safety profiles have been established. Overall, the adverse-effect profile of these β-adrenoceptor antagonists is acceptable.[14]

Our data showed that β-adrenoceptor agonist epinephrine–induced pancreatic cancer proliferation is dose-dependent, because it could make MAPKs and PKA/AA pathway active in pancreatic cancer cells.[7,8] However, β-adrenoceptor antagonist propranolol inhibited the PC-2 cell proliferation; the effect of inhibition by propranolol occurs through apoptosis induction in our study.

The effect of the broad-spectrum β-adrenoceptor antagonist propranolol on the rate of apoptosis of pancreatic cancer

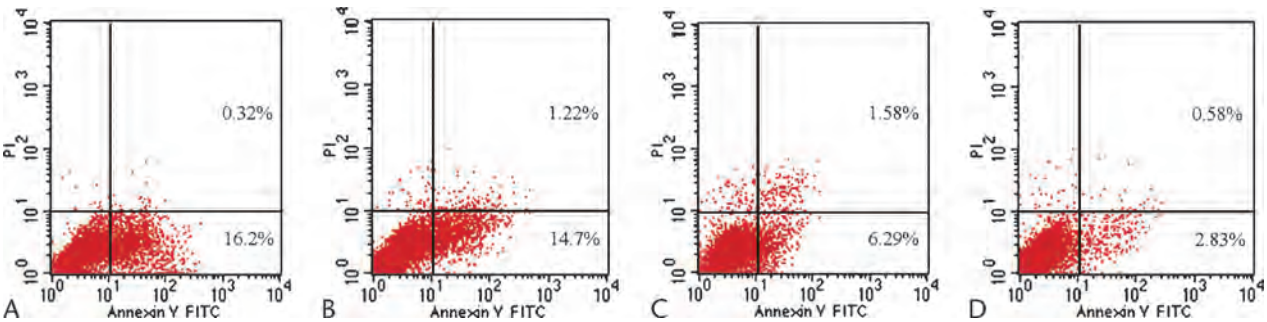

FIGURE 5. Apoptotic PC-2 cells after treatment with β-adrenoceptor antagonists for 24 hours were detected by FCM. PC-2 cells were treated with β-adrenoceptor antagonists: 100 μM butoxamine (A), 100 μM propranolol (B), and 100 μM metoprolol (C), or untreated as control (D). The percentages of early apoptotic cells increased significantly after the treatment of propranolol, metoprolol, and butoxamine in PC-2, which were 14.7%, 6.29%, and 16.2%, respectively.

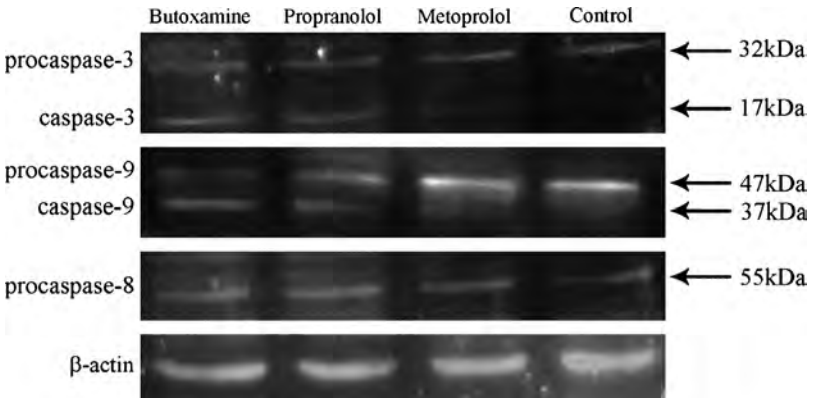

FIGURE 6. Expression of caspase 3, caspase 9, and caspase 8 in PC-2 cells treated with propranolol (100 μM), metoprolol (100 μM), and butoxamine (100 μM) for 24 hours as well as negative control group. The expression of the respective proteins in whole cell lysate was examined by Western blot. β-Adrenoceptor antagonists can increase the level of caspase 3 and caspase 9, whereas they have no effect in cleavage of caspase 8.

cells was lower than the β_2-adrenoceptor antagonist butoxamine, but higher than β_1-adrenoceptor antagonist metoprolol. These findings suggest that blockage of β_2-adrenoceptor produces more effective suppression than β_1-adrenoceptor; propranolol induces apoptosis via the β_2-adrenoceptor principally. Three points of evidence from the study support that propranolol induces apoptosis via the β_2-adrenoceptor principally. (1) The PC-2 cell line expressed mRNA and protein for both β_1- and β_2-adrenoceptors; the expression of β_2-adrenoceptor was predominant in Western blotting. Our findings are in accordance with a recent report showing that the expression of β_2-adrenoceptor was distinguished in the Panc-1 and BxPC-3 pancreatic cell lines and that β_2-adrenoceptor antagonists significantly reduced pancreatic cancer cell proliferation.[7,8] (2) Agonists of β_2-adrenoceptors stimulated the activation of Ras and Src tyrosine kinase–dependent MAPK pathway in fibroblasts.[15,16] Activating point mutations in the *Ki-ras* gene are common in ductal pancreatic carcinomas; the metabolites of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) function as β-adrenergic agonists; because of activating point mutations in the *ras* gene, it is conceivable that binding of NNK to β_2-adrenoceptors may further stimulate this pathway via activation of Src tyrosine kinase and Ras.[7,8] β-Adrenergic antagonists can inhibit Src tyrosine kinase and Ras pathway, including inhibiting MAPKs, and also stimulate dephosphorylation of Akt.[17,18] (3) The NNK stimulates proliferation of immortalized human pancreatic duct epithelia through β-adrenergic transactivation of epidermal growth factor receptors, especially through β_2-adrenoceptors.[7,8,19] The activation of these growth factor receptors results in the stimulation of antiapoptotic signaling cascades, including MAPK, phosphatidylinositol 3' kinase (PI_3K/Akt), and nuclear factor κB, which participate in the sustained proliferation, antiapoptosis, survival, and metastasis of pancreatic cancer epithelial cells.[20–23] For this reason, the broad-spectrum β-adrenergic antagonist propranolol can inhibit β_2-adrenoceptors principally, which suppresses pancreatic cancer cell proliferation and induces apoptosis of PC-2 cells through disengaging the inhibition of the apoptosis pathway.

Our results demonstrated that propranolol and other β-adrenergic antagonists induced apoptosis in PC-2 cells. At the apoptosis-inducing concentration, propranolol stimulated proteolytic activities of caspase 3 and caspase 9, whereas procaspase 8 had no cleavage fragments; this result indicates that β-adrenergic antagonists induced apoptosis of PC-2 cells via intrinsic apoptotic pathway. β_2-Adrenergic agonists might stimulate the pathway via activation of Src tyrosine kinase and Ras, whereas β-adrenergic antagonists could inhibit Src tyrosine kinase and Ras, and also stimulated dephosphorylation of Akt.[17,18] Akt is a protein kinase that promotes cell survival by inhibiting apoptosis, although precisely how it exerts its antiapoptotic effects is not clear. It has recently been reported to directly phosphorylate and inactivate procaspase 9 as well as to block caspase 9–mediated apoptosis induced by cytochrome C via Apaf-1 in response to an early apoptotic stimuli.[17] Akt can also phosphorylate Bad, resulting in the release of Bcl-2 and inhibition of the apoptotic process. Members of the Bcl-2 family of proteins have been demonstrated to be associated with the mitochondrial membrane and regulate its integrity.[17,18] Propranolol has several different molecular targets including suppression of Ras/Akt, MAPKs, and PKA/AA signaling pathways that could contribute to inhibition of proliferation and induction of apoptosis, down-regulation of antiapoptotic proteins, and activation of caspase 3.

In conclusion, β-adrenoceptor antagonists have been used as therapies for hypertension and the prevention of heart attacks for many years. Increasing evidence suggests that patients with pancreatic adenocarcinomas share many risk factors, such as smoking and a high-fat diet, with cardiovascular disease patients.[2,24,25] These patients may therefore benefit from strategies that have been successfully used to treat cardiovascular diseases. Epidemiological and experimental evidence indicates that the β-adrenergic pathways are promising targets for the prevention and treatment of pancreatic adenocarcinomas.[26] The data presented here may promote the development of β-adrenoceptor antagonists designed for targeting pancreatic cancer cell apoptosis.

ACKNOWLEDGMENTS

The authors thank the staff of the Institution of Endemic Disease Research, Xi'an Jiaotong University for their technical assistance.

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