

Histamine Inhibits Cell Proliferation and Modulates the Expression of Bcl-2 Family Proteins *via* the H₂ Receptor in Human Pancreatic Cancer Cells

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Abstract. *In this study, the mechanisms involved in the inhibitory effect of histamine (HA) on PANC-1 cell proliferation were investigated. The action of HA on cell growth was evaluated by determining the cell doubling time from experimental growth curves and analysing the cell cycle using a flow cytometer. The expression of proteins related to cell death and proliferation (PCNA, p53, c-Fos and Bcl-2 family proteins) was studied using Western blot, immunocytochemistry and flow cytometric analysis. The results indicated that HA produced an accumulation of PANC-1 cells in G0/G1-phase and increased the doubling time via H₂HA (H₂R) stimulation. Expression of p53, c-Fos and Bcl-2 were not modulated by HA. However, HA decreased PCNA and Bax expression, while it increased the Bcl-x level. In summary, the antiproliferative effect exerted by HA was associated with a G0/G1-phase arrest and a modulation of the Bcl-2 family proteins.*

Pancreatic adenocarcinoma is a leading cause of cancer death worldwide. This lethal disease is characterised by an extremely poor prognosis because of its aggressive nature and lack of response to conventional therapy. On this basis, new therapeutic modalities for pancreatic cancer are currently being evaluated.

As an approach to the understanding of the molecular biology of this neoplasm, the human pancreatic carcinoma cell

line PANC-1 was employed to investigate the *in vitro* effects of histamine (HA) on cell growth. The PANC-1 cell line was derived from a human pancreatic carcinoma of ductal origin. This poorly differentiated epithelioid cell line, which harbours mutated p53, was originally established by Lieber *et al.* (1).

HA, a biogenic amine widely distributed throughout the body, is involved in the regulation of diverse physiological and pathological processes and exerts its actions through the activation of four classes of membrane G protein-coupled receptors (H₁R, H₂R, H₃R and H₄R). HA receptors have also been characterised in a variety of cell lines and human neoplasias. Several studies have shown that HA may regulate cell growth *via* these receptors (2).

We previously reported that PANC-1 cells overexpress H₁R and H₂R. HA concentrations higher than 1 μM activate the adenylate cyclase pathway by H₂R stimulation, increase 3',5'-cyclic adenosine monophosphate (cAMP) production and exert an inhibitory effect on cell proliferation (3). Apoptosis is not significantly induced by HA in this cell line. However, a partial cell differentiation is associated with the inhibitory action of HA (4).

Bax, Bcl-2 and Bcl-x are members of a large family of intracellular proteins described as important positive and negative regulators of apoptosis. It is well-known that the antiapoptotic Bcl-2 members bind the proapoptotic proteins to counteract one another's function, suggesting that their relative concentration may act as a rheostat for cell death programme (5). Moreover, there is general agreement that Bax is needed to induce apoptosis (6, 7). Recent reports have confirmed that apoptosis of cancer cells induced by several chemotherapy agents is dependent on Bax (8) and that epithelial cancer cells lacking Bax are resistant to apoptosis (9).

The aim of this study was to investigate the mechanisms involved in the inhibitory, not the apoptotic, effect of HA on PANC-1 cell growth by the analysis of cell cycle and the study of proteins related to cell death and proliferation.

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Materials and Methods

Materials. Histamine dihydrochloride, mouse monoclonal antibody anti β -actin (clone AC-15), mouse monoclonal anti-rabbit IgG (Gamma) peroxidase conjugate clone RG-96, rabbit anti-mouse IgG (whole molecule) peroxidase conjugate, goat anti-rabbit IgG (whole molecule) and goat anti-mouse both conjugated to fluorescein isothiocyanate (FITC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The H_2 agonist dimaprit dihydrochloride (Dim) was from Tocris Cookson, Inc. (MO, USA). Forskolin (Fk) was from Calbiochem (Merck Biosciences, USA). RPMI 1640 medium and fetal bovine serum (FBS) were obtained from GIBCO, Invitrogen (CA, USA). Rabbit polyclonal antibodies against human Bax (N-20), Bcl-2 (Δ 21), Bcl-x_{S/L} (S-18) and c-Fos (4-G) were from Santa Cruz Biotechnology, Inc. (CA, USA). Mouse monoclonal antibodies against p53 (DO-1 clone) and the proliferating cellular nuclear antigen (PCNA) were from Santa Cruz Biotechnology and DakoCytomation (CA, USA), respectively. The enhanced chemiluminescence system (ECL) was from Amersham Biosciences Argentina S.A.

Cell culture. PANC-1 (ATCC CRL 1649) cells were grown in monolayer in RPMI medium supplemented with 10% FBS, 0.3 g/l L-glutamine and 40 mg/l gentamycin, at 37°C in a 5% CO₂ humidified atmosphere.

Cell population doubling time. PANC-1 cells were seeded on plates at a density of 1×10^5 , starved for 24 h and then incubated with or without 10 μ M HA or 10 μ M Dim in RPMI medium containing 10% FBS. After 24, 48 and 72 h the cells were harvested by trypsinisation. Viable cells were counted using a hemocytometer. All experiments were performed in the logarithmic phase of cell growth. Triplicate plates were analysed for each treatment and time. Experimental curves were adjusted to the equation: $N^0 \text{cells}_t = N^0 \text{cells}_{t=0} \times e^{kt}$, using the GraphPad Prism 4.0 software (GraphPad Software Inc, Philadelphia, USA). Cell doubling time (T) was determined as: $T = \ln 2/k$.

Cell cycle analysis. After synchronization, the cells were incubated, as previously described. At 24, 48 and 72 h they were fixed with 1 ml ice-cold 70% ethanol, centrifuged and resuspended in 100 μ l Dnase-free RNase A (0.2 mg/ml). The cells were stained with propidium iodide (50 μ g/ml), stored at 4°C in the dark and analysed by flow cytometry on a FACSCalibur instrument (Becton-Dickinson, CA, USA) within 2 h. Red fluorescence was measured using a fluorescence detector 2 (FL2) on the X-axis. Cell cycle phase distribution, *i.e.*, the percentage of G0/G1, S and G2/M nuclei of the analysed cell population, was determined by applying Cylchred 1.0.2 cell cycle analysis software (Cardiff University, Cardiff, UK) on the DNA histograms.

Western blotting. Synchronised cells were cultured with or without 10 μ M HA, 10 μ M Dim or 10 μ M Fk. Bcl-2 family proteins, PCNA and p53 expression were studied at 24 and 48 h using Western blot, while c-Fos was studied at 2, 4, 12 and 24 h. Cells were scraped into lysis buffer (0.1 mM sodium fluoride, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM ethylene glycol tetra acetic acid (EGTA), 1 mM sodium vanadate, 0.02 mg/ml leupeptin and 100 mM Tris (pH 8.8) at 4°C. After centrifugation, supernatants were boiled for 3 min in reducing conditions. Protein concentration

was determined using the Bradford method (10). Fifty μ g of protein were separated on a 12% sodium dodecylsulfate polyacrylamide gel by electrophoresis and transblotted onto nitrocellulose membranes. Membranes were blocked in Tris-buffered saline/Tween (25 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween-20) containing 5% (w/v) non-fat dried milk. Blots were probed overnight at 4°C with the rabbit antibodies (1: 200) against Fos, Bcl-2, Bcl-x and Bax or with the mouse antibodies anti PCNA (1: 500) and anti p53 (1: 200). Immunoreactivity was developed using anti-mouse or anti-rabbit IgG horse-radish peroxidase-conjugate antibody, as appropriate, and detected by the enhanced chemiluminescence reagents following the manufacturer's instructions. Blots were re-probed with mouse antibody against β -actin (1:1000) to control for equal protein loading of the gel. The relative expression level of proteins was determined by comparing signal intensity to β -actin bands and was quantitated by densitometry employing the image J 1.32 software (NIH, USA).

Flow cytometric analysis. PANC-1 cells were cultured as previously described for Bcl-2, Bax, PCNA and c-Fos detection. The nonspecific background staining was controlled with a rabbit or mouse IgG isotype control as appropriate. Cells were fixed in 4% formaldehyde in phosphate-buffered saline (PBS), centrifuged and permeabilised with 0.1% saponin in PBS. After centrifugation, the first appropriate antibody (1:100) was added for 60 min at room temperature. Cells were washed with 0.1% saponin, treated with the anti-mouse or anti-rabbit FITC conjugate antibody (1:200) and incubated in darkness. They were then washed and resuspended in 2% formaldehyde in PBS, ready to be analysed by flow cytometry on a FACSCalibur instrument using WindMI 2.8 software (Scripps Institute, La Jolla, CA, USA).

Immunocytochemistry. Cells were cultured on glass slides for 24 and 48 h with or without 10 μ M HA or Dim. They were then fixed with 4% formaldehyde in PBS, permeabilised with 0.25% Triton and incubated overnight at 4°C with a rabbit polyclonal antibody against Bcl-2 or Bax followed by the appropriate FITC-labelled antibody for 1-h at room temperature in the darkness. The non-specific background staining was controlled, as previously indicated. Stained cells were examined with a fluorescence microscope (Karl Zeiss, Germany) bearing a digital camera.

Statistical analysis. Statistical analysis performed is indicated in the legends. Data were analysed by One-way or Two-way ANOVA and Bonferroni or Dunnett post-test, using the GraphPad Prism Version 4.00 software (GraphPad Software Inc., Philadelphia, USA). *P*-values less than 0.05 were considered statistically significant.

Results

HA enlarges the cell doubling time and induces G0/G1 cell cycle arrest. To investigate the antiproliferative effect induced by HA *via* H₂R on PANC-1 clonogenic growth (3), we analysed the population doubling time from experimental growth curves when cells were cultured in monolayers with or without HA or Dim. As shown in Figure 1, the cell number at 72 h in cultures exposed to HA or Dim was

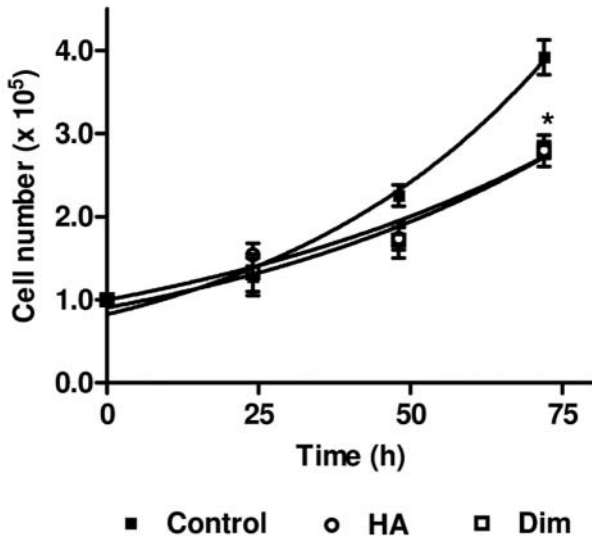


Figure 1. Experimental growth curves. Cells were untreated (■) or treated with Dim (□) or HA (○). Each point represents the mean \pm SD of one representative experiment of the three performed. * $p < 0.01$ vs. control, Two-way ANOVA and Bonferroni post-test.

significantly lower than in the control. This would appear to be a time-dependent effect since there were no significant differences up to 24 h. Doubling time was determined from the experimental growth curves as described in Materials and Methods. An important increase in the T-value was observed when cells were cultured in the presence of 10 μ M HA or Dim (1.6-fold higher than untreated cells) indicating that HA and Dim slowed down the cell cycle (Table I).

In order to determine whether HA might induce cell cycle arrest *via* H₂R, we carried out the cell cycle analysis of PANC-1 cells cultured with or without 10 μ M HA or Dim. Results revealed that HA changed the cell cycle distribution. At 48 h and 72 h there was an increased accumulation of G₀/G₁-phase cells for HA-treated cultures compared to control cultures (Figure 2A). Similar results were obtained when cells were grown in the presence of 10 μ M Dim (Figure 2B), indicating a retardation of cell cycle progression coincident with the larger T-value mentioned above.

Effects of HA on the expression of proteins related to cell death and proliferation. Seeking HA-induced factors potentially influencing the balance between cell survival and cell death, we evaluated the expression pattern of PCNA, c-Fos, p53 and the Bcl-2 family proteins in PANC-1 cell cultures. In view of the known stimulatory action of H₂R on adenylate cyclase/cAMP, we examined whether modulation of these protein levels was *via* the cAMP signaling pathway. For this purpose, cells were exposed to HA, the H₂ agonist Dim, or the direct activator of adenylate cyclase, Fk.

Table I. Cell doubling time.

Treatment	T (h)
Control	34.2 \pm 2.9
10 μ M HA	50.1 \pm 1.2*
10 μ M Dim	46.9 \pm 1.0*

Synchronized cells were treated, harvested and counted at 24, 48 and 72 h. T values are the mean \pm SEM of three independent experiments ran in triplicate (* $p < 0.05$ vs. Control, One-way ANOVA and Dunnett post-test).

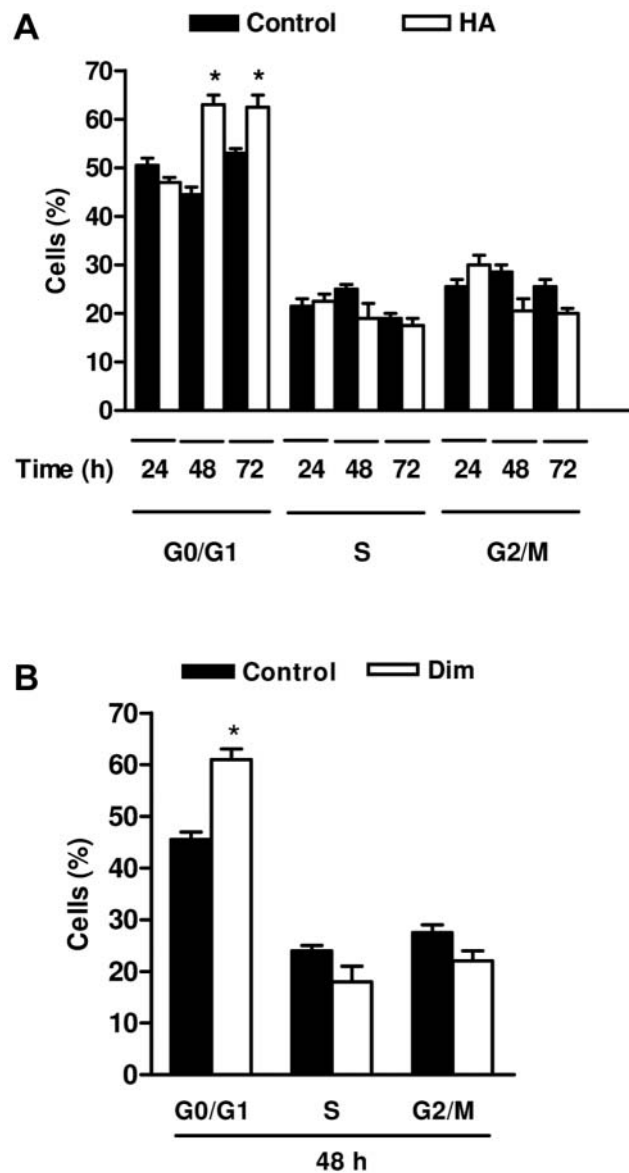


Figure 2. Effect of HA (A) and Dim (B) on cell cycle progression. Cells were stained with PI and measured by flow cytometry. Bars are the mean \pm SD of three independent experiments. Two-way ANOVA and Bonferroni post-test, * $p < 0.05$ vs. control.

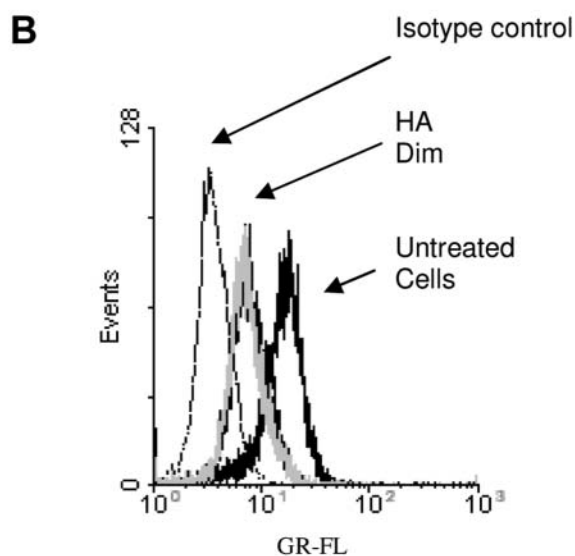
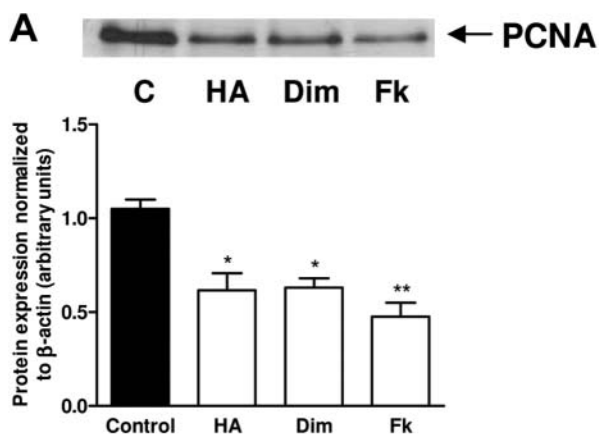


Figure 3. PCNA expression in PANC-1 cells after 24 h of treatment. (A) Immunoblotting of PCNA protein. A representative gel of three performed. Bars represent protein expression normalized to β -actin (mean \pm SE, n=3). One-way ANOVA and Bonferroni post-test, * p <0.05 and ** p <0.01 vs. control. (B) Flow cytometric analysis. Peaks represent the mean fluorescence intensity.

At 48 h, a significant decrease in PCNA expression, a marker of cell proliferative activity, was observed using Western blot (Figure 3A) and flow cytometry (Figure 3B). The PCNA level was down-modulated by HA, Dim and Fk concomitantly with a reduction in the rate of population growth in cultures treated with HA or Dim, as shown in Figure 1.

At 24 h, the expression of mutated p53 protein was not modified by treatment (Figure 4). In addition, no change in c-Fos level was detected by Western blot when cells were treated with HA for 2, 4, 12 and 24 h or when cells were cultured with HA, Dim or Fk for 4 h (Figure 5). Similar

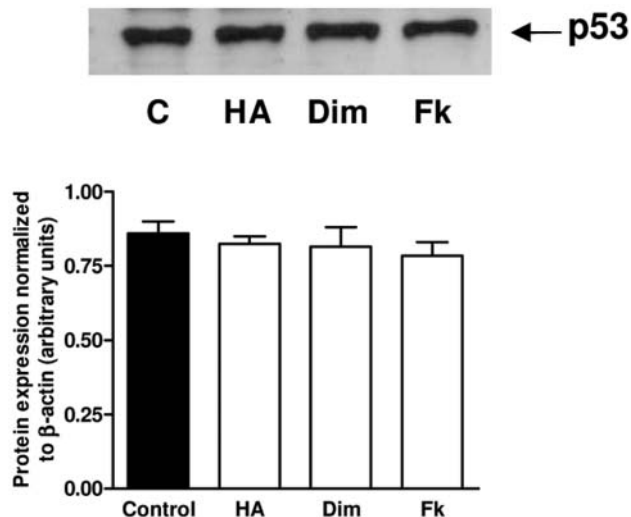


Figure 4. Representative immunoblotting of p53 in PANC-1 cells after 24 h of treatment. One gel of three performed. Bars represent protein expression normalized to β -actin (mean \pm SE, n=3). Treatments did not significantly modify p53 expression.

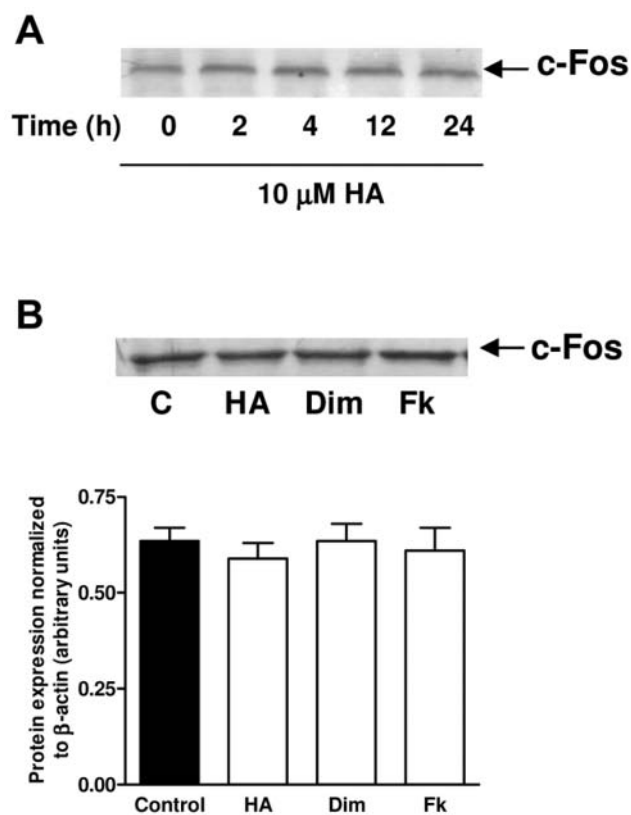


Figure 5. Representative immunoblottings of c-Fos. PANC-1 cells were exposed to HA during 2, 4, 12 and 24 h (A); cells were treated with HA, Dim or Fk during 4 h (B). Bars represent protein expression normalized to β -actin (mean \pm SE, n=3).

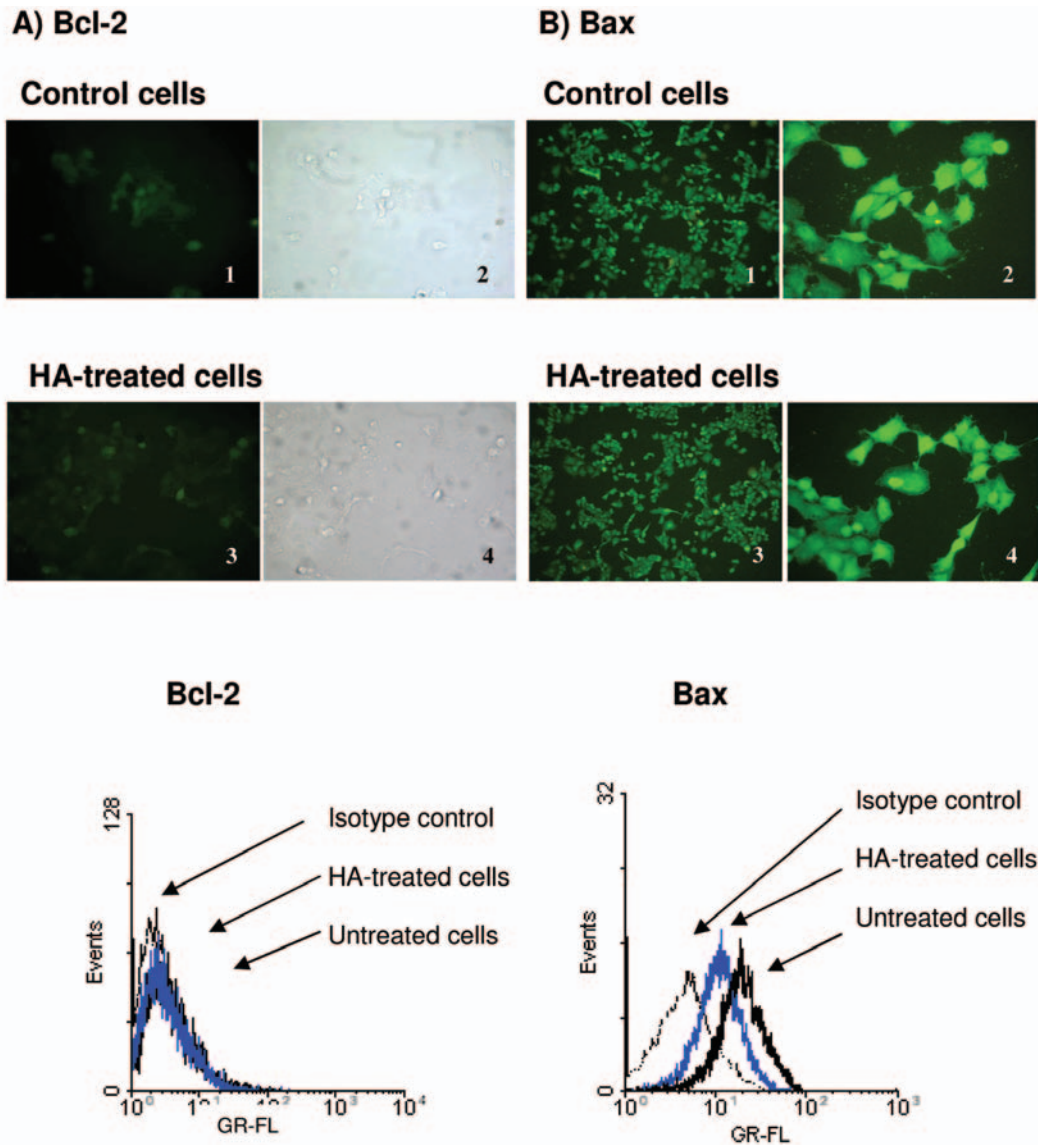


Figure 6. Immunodetection of Bcl-2 (A) and Bax (B) after 24 h of treatment. Upper panel, fluorescence microscopy, (A) with excitation light: A-1, A-3 (x400); without excitation light: A-2, A-4, (x400). (B) with excitation light: B-1, B-3 (x100); B-2, B-4 (x1000). Lower panel, flow cytometry: Bcl-2 (left); Bax (right).

results were obtained at 2, 12 and 24 h and were corroborated by flow cytometric analysis (data not shown).

At 24 h, Bcl-2 could not be detected by the assayed techniques in the control, nor the treated cells. Immunocytochemical studies displayed no fluorescence staining for Bcl-2 when treated and control cells were irradiated with the excitation light (Figures 6A-1 and 6A-3). Observation of the same fields without excitation light confirmed that cells were present on the slides (Figures 6A-2 and 6A-4). These data were corroborated by flow cytometric analysis (Figure 6, lower panel). Similar results were obtained at 48 h.

Bax protein expression was detected by immunocytochemistry in both treated and untreated cultures (Figure 6B). However, a lower level of Bax protein expression in HA-exposed cells compared to control cells was evidenced by flow cytometric analysis (Figure 6, lower panel). These results were also observed by Western blotting (Figure 7). In addition, two isoforms of Bcl-x protein, the antiapoptotic Bcl-x_L and the proapoptotic Bcl-x_S were also visualised using the Western blot technique (Figure 7). The expression of both isoforms was augmented significantly in cells receiving HA, Dim or Fk at 24 h, the enhancement of Bcl-x_L being larger than Bcl-x_S for each treatment. Similar data were acquired at 48 h (data not shown).

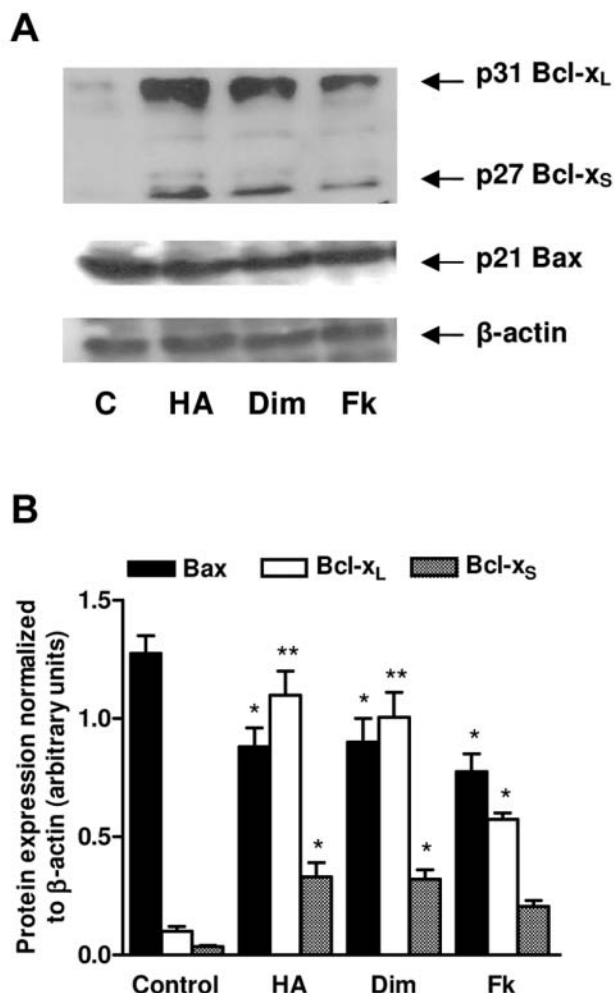


Figure 7. Immunoblottings of Bcl-2 family proteins. PANC-1 cells were treated during 24 h. A) A representative gel of three performed is shown. Bcl-x gel was re-stripped and probed twice for Bax and β-actin detection. B) Bars represent protein expression normalized to β-actin (mean±SE, n=3). *p<0.05 and **p<0.01, treatments vs. control for each protein. One-way ANOVA and Dunnett post-test.

Discussion

HA has been proposed as a potential modulator of exocrine pancreatic function acting through H₁R and H₂R in the normal pancreas (11). However, the role of HA and these receptors in pancreatic cancer have not yet been elucidated.

There is a large amount of data available regarding the effects of HA on normal and neoplastic cell proliferation and differentiation (2, 12). Histamine receptors have been characterised in several malignant cell types with the additional finding that they can activate multiple signaling pathways (13). Moreover, HA has been demonstrated to act as an autocrine/paracrine growth factor both *in vivo* and *in vitro* (14-16).

We have previously reported that HA stimulates the cAMP production *via* H₂R inhibiting the clonogenic growth of PANC-1 cells. This antiproliferative effect is evoked by H₂ agonists or by Fk, a direct activator of adenylate cyclase (3). We have also demonstrated that HA induces a partial cellular differentiation through H₂R stimulation in PANC-1 cells (4). HA-induced differentiation of tumour cells through H₂R activation has been reported (17, 18). Proteins related to cell proliferation and differentiation, such as c-Fos and c-Jun, have also been described as being regulated by HA *via* H₂R in a differential manner in neoplastic cell lines (19, 20). However, our present results showed that the c-Fos level was not modulated by HA, the H₂-agonist Dim or Fk suggesting that a potential linkage between c-Fos protein expression and cAMP metabolic pathway was not present.

In our current work, the antiproliferative effect induced by HA through H₂R and evoked by the specific H₂-agonist Dim on PANC-1 cells was clearly corroborated by a significant time-dependent decrease of the cell population growth rate. These results correlated with a lower expression of PCNA protein when cells were exposed to HA, Dim or Fk. To get insights into the mechanisms involved in this inhibitory action exerted by HA *via* H₂R on PANC-1 cells, we studied the cell cycle and the population doubling time. Cell cycle distribution analysis revealed an increased accumulation of G₀/G₁-phase cells at 48 h, indicating a retardation of cell cycle progression in coincidence with a larger T-value in treated cells.

In order to study the HA-mediated G₀/G₁ cell arrest, we evaluated p53 protein in the PANC-1 cell line bearing mutated p53. Several lines of evidence show that the cell cycle arrest and apoptotic activities of the wild-type p53 protein are separable functions in the suppression of tumour cell growth. In addition, a series of p53 mutants retaining these activities has been recently characterized (21). Our results indicated that mutated p53 expression is not related to the G₀/G₁ cell accumulation induced by HA in PANC-1 cells.

The action of HA on programmed cell death is still controversial. In this regard, pro- and anti-apoptotic effects of HA and HA-antagonists in both normal and cancer cells have been described (22, 23). We recently reported the resistance to apoptosis induced by HA *via* H₂R in PANC-1 cells (4). However, the molecular mechanism that renders pancreatic cancer cells resistant to HA-mediated apoptosis still remains unknown. In this work, we evaluated the alteration of the apoptotic pathway by the study of Bcl-2 proteins. It is well-known that Bcl-2 family proteins are the main regulators of the apoptotic process acting either to inhibit or to promote it. There has been much discussion on a model in which the ratio of Bcl-2 to its heterodimerisation partner Bax determines survival or death, following a cell-death stimulus in normal cells and cancer cell lines carrying wild-type p53 (5, 24). Though overexpression of Bcl-2 is

common in many types of human cancer, we could not detect Bcl-2 in treated and untreated PANC-1 cells. In this sense, our results are similar to other reports of absence or low expression of Bcl-2 protein in pancreatic ductal adenocarcinomas (25, 26).

It is known that Bcl-x_L and Bcl-x_S are isoforms encoded by two splice variants of the *bcl-x* gene. Bcl-x_L acts as a potent inhibitor of apoptosis, while Bcl-x_S is thought to antagonize Bcl-2 and Bcl-x_L by binding and inactivating these apoptotic inhibitors (24). Though our findings showed an enhanced expression of Bcl-x_L and Bcl-x_S isoforms in PANC-1 cells receiving HA, Dim or Fk, the level of Bcl-x_L expression was higher than that of Bcl-x_S for each treatment. Recent reports show the ability of Bcl-2 and Bcl-x_L to inhibit the entry into the cell cycle through elevation of p27 (27). A similar function of Bcl-x_L in delaying HA-induced cell cycle progression *via* H₂R might be suggested in PANC-1 cells.

Bax is a transcriptional target of the tumour suppressor *p53*, which is mutated in the majority of human cancers. It has been reported that Bax protein may be modulated in presence of wild-type or mutated *p53* after apoptotic stimuli (28). Results from our current study demonstrated that HA, Dim and Fk diminished Bax expression and that Bax modulation was not related to the p53 level in PANC-1 cells. Taken all together, these findings indicate that cAMP levels are involved in Bcl-2 protein modulation. The relative levels of Bax, Bcl-x_L and Bcl-x_S might define the HA-induced resistance to apoptosis in PANC-1 cells, since the balance between the proapoptotic Bax and the antiapoptotic Bcl-x_L protein was altered in HA-exposed cells. In this sense, our findings are also in agreement with other reports about the protective role of high levels of Bcl-x_L protein from the induction of apoptosis by different agents in pancreatic cancer cells (28, 29).

The growth-inhibitory action of cAMP in mesenchymal and epithelial cells has been known for more than 25 years. Nevertheless, recent data suggest a role for cAMP in cell protection against apoptosis. In concordance with our results, Boucher and coworkers have reported that increased intracellular cAMP concentrations inhibit cell growth, cell cycle progression and apoptosis in the pancreatic tumour cells Mia-PaCa (30). Our findings suggest that the antiproliferative, not apoptotic, effect of HA-induced cAMP production *via* H₂R on PANC-1 cells may involve a modulation of the Bcl-2 protein family. The alteration of this balance may allow sensitisation to the differentiating effect of HA, opening the perspectives for the potential application of HA in pancreatic cancer therapy. At present, HA is being tested in clinical trials as an adjuvant in metastatic melanoma therapy due to its well-known effect on immune response (31).

In conclusion, we clearly demonstrated that HA exerts an inhibitory effect on PANC-1 cell growth *via* H₂R activation, induces a G0/G1 cell cycle arrest slowing down the cell cycle progression and also modulates the expression of Bcl-2 family proteins.

Acknowledgements

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References

- Lieber M, Mazzeta J, Nelson-Rees W, Kaplan M and Todaro G: Establishment of a continuous tumor-cell line (PANC-1) from a human carcinoma of the exocrine pancreas: *Int J Cancer* 15: 741-747, 1975.
- Pós Z, Hegyesi H and Rivera E: Histamine and cell proliferation. *In: Histamine: Biology and Medical Aspects*. Falus A (ed.). Budapest, SpringMed Publishing, pp. 199-217, 2004.
- Cricco G, Martín G, Labombarda F, Cocca C, Bergoc R and Rivera E: Human pancreatic carcinoma cell line PANC-1 and the role of histamine on cell growth. *Inflamm Res* 49: S68-69, 2000.
- Martín G, Cricco G, Darvas Z, Croci M, Núñez M, Bergoc R, Falus A and Rivera E: Histamine inhibits proliferation of a pancreatic carcinoma cell line without inducing apoptosis significantly. *Inflamm Res* 51: S67-68, 2002.
- Oltvai Z, Milliman C and Korsmeyer S: Bcl-2 heterodimerizes *in vivo* with a conserved homolog Bax, that accelerates programmed cell death. *Cell* 74: 609-619, 1993.
- Hersey P and Zhang X: Overcoming resistance of cancer cells to apoptosis. *J Cell Physiol* 196: 9-18, 2003.
- Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB and Korsmeyer SJ: Pro-apoptotic Bax and Bak are a requisite gateway to mitochondrial dysfunction and death. *Science* 292: 727-730, 2001.
- Mohamad N, Cocca C, Núñez M, Gutiérrez A, Martín G, Cricco G, Medina V, Rivera E and Bergoc R: Mitochondrial apoptotic pathways. *Biocell* 29: 149-161, 2005.
- Theodorakis P, Lomonosova E and Chinnadurai G: Critical requirement of Bax for manifestation of apoptosis induced by multiple stimuli in human epithelial cancer cells. *Cancer Res* 62: 3373-3376, 2002.
- Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254, 1976.
- Singh J, Pariente J and Salido G: The physiological role of histamine in the exocrine pancreas. *Inflamm Res* 46: 159-165, 1997.
- Hegyesi H, Darvas Z, Laszlo V, Pos Z, Pallinger E, Hirschberg K, Kovacs P and Falus A: Retinoic acid enhances histamine content and H₁ receptor expression in human neuroblastoma cell line Paju. *Anticancer Res* 24: 1657-1664, 2004.
- Leurs R, Smit M and Timmerman H: Molecular pharmacological aspects of histamine receptors. *Pharmacol Ther* 66: 413-463, 1995.

- 14 Tilly BC, Tertoolen LG, Remorie R, Ladoux A, Verlaan I, de Laat SW and Moolenaar WH: Histamine as a growth factor and chemoattractant for human carcinoma and melanoma cells: action through Ca²(+)-mobilizing H₁ receptors. *J Cell Biol* 110: 1211-1215, 1990.
- 15 Rivera E, Cricco G, Engel N, Fitzsimons C, Martín G and Bergoc, R: Histamine as an autocrine growth factor: an unusual role for a widespread mediator. *Semin Cancer Biol* 10: 15-23, 2000.
- 16 Lázár-Molnár E, Hegyesi H, Pállinger E, Kovács P, Tóth S, Fitzsimons C, Cricco G, Martín G, Bergoc R, Darvas Z, Rivera E and Falus A: Inhibition of human primary melanoma cell proliferation by histamine is enhanced by interleukin-6. *Eur J Clin Invest* 32: 743-749, 2002.
- 17 Ucar K: The effects of histamine H₂ receptor antagonists on melanogenesis and cellular proliferation in melanoma cells in culture. *Biochem Biophys Res Commun* 177: 545-550, 1991.
- 18 Shimada K, Abe T, Zhao Q, Mio M and Kamei C: Effects of histamine and related compounds on the differentiation of HL-60-Eo cells into eosinophils. *Exp Clin Pharmacol* 23: 383-388, 2001.
- 19 Shayo C, Davio C, Brodsky A, Mladovan AG, Legnazzi BL, Rivera E and Baldi A: Histamine modulates the expression of *c-fos* through cyclic AMP production *via* the H₂ receptor in the human promonocytic cell line U937. *Mol Pharmacol* 51: 983-990, 1997.
- 20 Wang L, Wang M, Todisco A, Grand E and del Valle J: The human histamine H₂ receptor regulates *c-jun* and *c-fos* in a differential manner. *Am J Physiol Cell Physiol* 278: C1246-C1255, 2000.
- 21 Ryan KM and Vousden KH: Characterization of structural *p53* mutants which show selective defects in apoptosis but not cell cycle arrest. *Mol Cell Biol* 18: 3692-3698, 1998.
- 22 Jangi SM, Asumendi A, Arlucea J, Nieto N, Perez-Yarza G, Morales MC, de la Fuente-Pinedo M and Boyano MD: Apoptosis of human T-cell acute lymphoblastic leukemia cells by diphenhydramine, an H₁ histamine receptor antagonist. *Oncol Res* 14: 363-372, 2004.
- 23 Hur J, Kang MK, Park JY, Lee SY, Bae YS, Lee SH, Park YM and Kwak JY: Pro-apoptotic effect of high concentrations of histamine on human neutrophils. *Int Immunopharmacol* 3: 1491-1502, 2005.
- 24 Kirkin V, Joos S and Zörnig M: The role of Bcl-2 family members in tumorigenesis. *Biochem Biophys Acta* 1644: 229-249, 2004.
- 25 Evans J, Cornford P, Dodson A, Greenhalf W, Foster C and Neoptolemos J: Detailed tissue expression of bcl-2, bax, bak and bcl-x in the normal pancreas and in chronic pancreatitis, ampullary and pancreatic ductal adenocarcinomas. *Pancreatology* 1: 254-262, 2001.
- 26 Campani D, Esposito I and Boggi U: Bcl-2 expression in pancreas development and pancreatic cancer progression. *J Pathol* 194: 444-450, 2001.
- 27 Greider C, Chattopadhyay A, Parkhurst C and Yang E: Bcl-x_L and Bcl-2 delay Myc-induced cell cycle entry through elevation of p27 and inhibition of G1 cyclin-dependent kinases. *Oncogene* 21: 7765-7775, 2002.
- 28 Hinz S, Trauzold A, Boenicke L, Sandberg C, Beckmann S, Bayer E, Walczak H, Kalthoff H and Ungefroren H: Bcl-x_L protects pancreatic adenocarcinoma against CD95 and TRAIL receptor-mediated apoptosis. *Oncogene* 19: 5477-5486, 2000.
- 29 Lee J, Hosotani R and Wada M: Role of Bcl-2 family proteins (Bax, Bcl-2 and Bcl-X) on cellular susceptibility to radiation in pancreatic cancer cells. *Eur J Cancer* 35: 1374-1380, 1999.
- 30 Boucher M, Duchesne C, Laine J, Morisset J and Rivard N: cAMP protection of pancreatic cancer cells against apoptosis induced by ERK inhibition. *Biochem Biophys Res Commun* 285: 207-216, 2001.
- 31 Lindner P, Rizell M, Mattsson J, Hellstrand K, Naredi P: Combined treatment with histamine dihydrochloride, interleukin-2 and interferon-alpha in patients with metastatic melanoma. *Anticancer Res* 24: 1837-1842, 2004.

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