REPORTS

Hereditary Pancreatitis and the Risk of Pancreatic Cancer

*Albert B. Lowenfels, Patrick Maisonneuve, Eugene P. DiMagno, Yoram Elitsur, Lawrence K. Gates, Jr., Jean Perrault, David C. Whitcomb, and the International Hereditary Pancreatitis Study Group**

Background: **Hereditary pancreatitis is an autosomal-dominant disease, with a variable expression and an estimated penetrance of 80%. The gene for this disease has recently been mapped to chromosome 7q35, and the defect is believed to be caused by a mutation in the cationic trypsinogen gene. Acute attacks of abdominal pain begin early in life and the disease often progresses to chronic pancreatitis. Although the risk of pancreatic cancer is thought to be increased in more common types of chronic pancreatitis, the frequency of pancreatic cancer in the inherited type of pancreatitis is uncertain.** ***Purpose:*** **The aim of this study was to assess the frequency of pancreatic cancer and other tumors in patients with hereditary form of pancreatitis.** ***Methods:*** **To determine the natural history of hereditary pancreatitis, we invited all members of the American Pancreatic Association and the International Association of Pancreatology to participate in a longitudinal study of this rare form of pancreatitis. The initial criteria for patient eligibility were as follows: early age (⩽30 years) at onset of symptoms, positive family history, and absence of other causes. From April 1995 through February 1996, 37 physicians from 10 countries contributed medical records of 246 (125 males and 121 females) patients thought to have hereditary pancreatitis as the most likely diagnosis. This group included 218 patients where the diagnosis appeared to be highly probable and 28 additional patients where the diagnosis of hereditary pancreatitis was less certain: 25 patients who had relatively late onset of disease and a positive family history and three patients with onset of disease before age 30 years but with an uncertain family history. We reviewed all causes of death and compared the observed to the expected frequency of cancer in this historical cohort of patients with hereditary pancreatitis. The strength of the association between pancreatitis and pancreatic cancer was estimated by the standardized incidence ratio (SIR), which is the ratio of observed pancreatic cancer cases in the cohort to the expected pancreatic cancers in the background population, adjusted for age, sex, and country.** ***Results:*** **The mean age (± standard deviation [SD]) at onset of symptoms of pancreatitis was 13.9 ± 12.2 years. Compared with an expected number of 0.150, eight pancreatic adenocarcinomas developed (mean age ± SD at diagnosis of pancreatic cancer: 56.9 ± 11.2 years) during 8531 person-years of follow-up, yielding an SIR of 53 (95% confidence interval [CI] = 23-105). The frequency of other tumors was not increased: SIR = 0.7 (95% CI = 0.3-1.6). Eight of 20 reported deaths in the cohort were from pancreatic cancer. Thirty members of the cohort have already been tested for the defective hereditary pancreatitis gene: all 30 carry a mutated copy of the trypsinogen gene. The transmission pattern of hereditary pancreatitis was known for 168 of 238 patients without pancreatic cancer and six of eight with pancreatic cancer. Ninety-nine of the 238 patients without pancreatic cancer and six of the patients with pancreatic cancer inherited the disease through the paternal side of the family. The estimated cumulative risk of pancreatic cancer to age 70 years in patients with hereditary pancreatitis approaches 40%. For patients with a paternal inheritance pattern, the cumulative risk of pancreatic cancer is approximately 75%.** ***Conclusions:*** **Patients with hereditary pancreatitis have a high risk of pancreatic cancer several decades after the initial onset of pancreatitis. A paternal inheritance pattern increases the probability of developing pancreatic cancer. [J Natl Cancer Inst 1997;89:442-6]**

Hereditary pancreatitis is characterized by recurrent attacks of abdominal pain beginning early in life and affects several family members in different generations. The inheritance pattern is believed to be autosomal dominant, with an estimated penetrance of 80% (*1*). Many afflicted individuals eventually develop chronic pancreatitis. The gene for this disorder, which has been mapped to chromosome 7q35, has recently been cloned (*2-4*).

Other more common adult types of chronic pancreatitis have been associated with an increased risk of pancreatic cancer (*5-8*). Although hereditary pancreatitis is rare, there are anecdotal case reports of pancreatic cancer developing several decades after the onset of symptoms of ab-

**Affiliations of authors:* A. B. Lowenfels, Departments of Surgery and Community and Preventive Medicine, New York Medical College, Valhalla, and Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy; P. Maisonneuve, Division of Epidemiology and Biostatistics, European Institute of Oncology; E. P. DiMagno, Mayo Medical School, Gastrointestinal Research Unit, Mayo Clinic and Foundation, Rochester, MN; Y. Elitsur, Pediatric Gastroenterology Division, Marshall University School of Medicine, Huntington, WV; L. K. Gates, Jr., University of Kentucky Medical Center and Lexington Veterans Affairs Medical Center, KY; J. Perrault, Internal Medicine and Pediatrics, Mayo Medical School, Rochester; D. C. Whitcomb, University of Pittsburgh, PA, and Gastroenterology and Hepatology Section, Pittsburgh Veterans Affairs Medical Center.

Correspondence to: Albert B. Lowenfels, M.D., Department of Surgery, New York Medical College, Valhalla, NY 10595.

See "Notes" following "References."

dominal pain in childhood (*9,10*). These reports suggest that the cumulative risk of pancreatic cancer might be substantial in patients with hereditary pancreatitis, because in many patients, the natural history of the disease is characterized by recurrent attacks of abdominal pain beginning in childhood, with eventual progression to chronic pancreatitis in adulthood. The aim of this report was to study the natural history of this disease and to assess the risk of pancreatic and other types of cancer.

Methods

Subjects

In April 1995, to identify patients with this rare type of pancreatitis, we wrote to 571 current members of the American Pancreatic Association and the International Association of Pancreatologists. Our initial letter invited all members to join a collaborative study aimed at investigating the natural history of this rare disease. Cancer was not mentioned in the original letter. We also asked for the approximate number of available patient records and the approximate number of kindreds. Those physicians who responded positively to the initial letter were sent data collection forms to record patient information. Thirty-nine physicians responded to our initial invitation. Three physicians who would have contributed a total of approximately six patients subsequently withdrew from the study: two who were unable to contact the families and one who had retired from active practice. Thirty-seven physicians in 10 countries contributed patients to this study aimed at clarifying the natural history of hereditary pancreatitis. The closure date for patient accrual was February 29, 1996. For each patient, we obtained information about demography, family history of pancreatitis, onset of disease, initial and most recent symptoms, medications, operations, vital status, and presence or absence of cancer. We excluded nine patients: three because the diagnosis was questionable and six with incomplete information. The final cohort consisted of 246 patients from 10 countries: United States—175 patients, Italy—19, Federal Republic of Germany—11, Japan—11, Sweden—8, France—7, Spain—6, Switzerland—4, U.K.—4, and Greece—1. All patients but three were born in the same country where the disease was initially diagnosed. After the closure date for the cohort, records for two additional patients, a father and a son, both of whom had hereditary pancreatitis and pancreatic cancer, were submitted to us from the United States. These two additional patients were not included in the calculation of person-years or in the analysis of cancer risk.

Calculation of Person-Years

Since hereditary pancreatitis is a genetic disease and since early symptoms of this disease are easily overlooked, we used the year of birth as the initial date for the accrual of person-years. The final date was the date of last contact or the date of death.

Diagnosis of Hereditary Pancreatitis

The initial criteria for entry in the cohort were as follows: early onset of pancreatitis, a positive family history of pancreatitis, and the absence of other known causes of pancreatitis. Two hundred thirty-seven (96%) patients gave a positive family history for one or more family members with pancreatitis: 213 (87%) patients had one or more relatives in a different generation who had been diagnosed as having pancreatitis; the other 24 (10%) patients had one or more siblings with pancreatitis but no other afflicted relatives. The reported age at onset of pancreatitis was as follows: less than or equal to 21 years, 207 (84%) patients; less than or equal to 30 years, 221 (90%) patients; and greater than 30 years, 25 (10%) patients. On the basis of onset and family history, we classified 218 patients as the most likely to have hereditary pancreatitis and 28 patients as possible cases of hereditary pancreatitis. The 28 patients classified as possible cases included 25 patients who were somewhat older at onset of symptoms (mean age, 34.8 ± 8.4 years) but who had one or more afflicted family members in another generation and three additional patients with an uncertain family history, with onset of symptoms at ages 25, 26, and 28 years, respectively. These 28 patients contributed 1518 person-years to the cohort; none developed pancreatic cancer during the follow-up period. No other cause for pancreatitis could be demonstrated for any of the 246 members of the final cohort. Four patients in this report who did not develop pancreatic cancer had been included in our previous study of pancreatitis and pancreatic cancer (*5*).

The diagnosis of pancreatitis was based on a combination of several different findings: recurrent attacks of upper abdominal pain (225 [95%] of 238 patients); hyperamylasemia (123 [53%] of 234 patients); calcification of the pancreas (49 [21%] of 233 patients); computed tomography or ultrasound findings (40 [17%] of 238 patients); diabetes (24 [10%] of 238 patients); and jaundice (16 [7%] of 238 patients).

Diagnosis of Pancreatic Cancer

All pancreatic cancers were confirmed histologically and occurred between 1977 and 1993. The mean calendar year for the diagnosis of pancreatic cancer was 1987.

Parent of Origin Analysis

For each patient in the cohort, we analyzed available parent of origin data. If the father or any paternal relative (uncle, aunt, or grandparent) had pancreatitis, the disease was considered paternal in origin; similar criteria were used to assign maternal origin. For this analysis, we included a father and son with both hereditary pancreatitis and biopsy-proven pancreatic cancer who had been excluded from the main analysis because their data forms arrived shortly after closure of data collection in March 1996. These additional patients belonged to a large U.S. kindred with well-documented hereditary pancreatitis.

Statistical Analysis

We used published age-stratified data according to 5-year age groups (0-4, 5-9, . . . 80-84, ⩾85), sex-specific data, and country-specific cancer incidence data to determine the expected number of cancers (*11*). For countries where national incidence data were unavailable, we averaged the incidence figures reported by regional registries. For Greece, we substituted incidence data for Italy. For the United States, we used data from the Surveillance, Epidemiology, and End Results[1] Program (*12*). The standardized incidence ratio (SIR)—the ratio of observed to expected pancreatic cancers—was used to estimate the relative risk. The 95% confidence interval (CI) for the SIR was calculated by assuming that the observed cases of cancer followed a Poisson distribution. We used the Kaplan—Meier procedure for calculating the cumulative incidence of pancreatic cancer. For this statistical routine and all other statistical tests, including chi-squared tests, Fisher's exact test, and calculation of means and standard deviations (SDs), we used SAS Institute (SAS Institute, Inc., Cary, NC) statistical software, release 7.07 (*13*). All reported *P* values are two-sided. For continuous data, such as age, we report the mean and the SD.

Testing for Pancreatitis Gene

Genetic testing has been performed for 30 (12%) members of this cohort. The methods used for identifying the locus of the hereditary pancreatitis gene on chromosome 7q35 and the subsequent characterization of the mutation in the cationic trypsinogen gene have been previously described (*3,4*).

Results

The final cohort consisted of 246 patients: 175 from the United States, 60 from Europe, and 11 from Japan. There were 125 males and 121 females. The mean age (± SD) at onset of symptoms of pancreatitis for all patients was 13.9 years ± 12.2 years. The mean duration (± SD) of follow-up for all patients was 14.6 ± 11.2 years. For patients who remained free of pancreatic cancer, the mean age (± SD) at onset of symptoms was 13.7 years ± 12.3 years; for the eight patients who eventually developed pancreatic cancer, the mean age (± SD) at diagnosis was 17.3 ± 6.9 years. The difference between age at onset of symptoms in the two groups was not significant ($P = .40$). Forty percent (n = 81) of adult subjects consumed alcohol in some amount, and 43% (n = 87) of adult patients were either current or former smokers. There was evidence of disease progression during the follow-up period: an additional 35 (14%) patients developed diabetes and another 32 (13%) patients developed pancreatic calcification during the course of their disease. One hundred forty-two (58%) patients required surgery on the pancreas or biliary tract.

Eight patients with pancreatic cancers (five males and three females) were diagnosed in six kindreds. The expected number of pancreatic cancers was 0.150,

yielding an SIR of 53 (95% CI = 23-105) (Table 1). The risk of developing pancreatic cancer was not significantly different for males and females or for patients diagnosed in the United States or in Europe. For the eight patients who developed pancreatic cancer, the mean age (± SD) at onset of pancreatic cancer was 56.9 ± 11.2 years, and the mean number of years from onset of symptoms of pancreatitis until diagnosis of pancreatic cancer was 39.6 ± 9.7 years.

The cumulative risk of pancreatic cancer in these patients to age 70 years was 40% (95% CI = 9%-71%) (Fig. 1, A). For the subgroup of 105 patients with a paternal parent of origin, the cumulative risk of cancer to age 70 years was approximately 75% (95% CI = 32%-100%) (Fig. 1, B). Pancreatic calcification and diabetes were found more often in patients with pancreatic cancer than in patients without pancreatic cancer. Information about these variables was known for six of the eight patients with pancreatic cancer. All six patients with pancreatic cancer had pancreatic calcification (P = .002; Fisher's exact test, compared with patients without pancreatic cancer). Diabetes was known to have occurred in four of six patients with pancreatic cancer (P = .04; Fisher's exact test, compared with patients without pancreatic cancer).

We observed an unusual parental inheritance pattern. A family history for the parental source of pancreatitis was available for 168 (71%) of the 238 patients in this cohort who remained free of cancer: 99 patients had a father or paternal relative with pancreatitis compared with 69 who had a mother or maternal relative with pancreatitis (P = .024). A family history of the parental-source pancreatitis was known for six of the eight patients with hereditary pancreatitis in the cohort who eventually developed pancreatic cancer: in all six patients, the paternal family had pancreatitis. Subsequent to the closure date for the cohort, we received information confirming a paternal source of pancreatitis for two new patients, a father and son, both of whom had hereditary pancreatitis and pancreatic cancer. Adding these two patients to the original group of six patients with pancreatitis and pancreatic cancer yields a total of eight patients with pancreatitis, pancreatic cancer, and a known parental inheritance pattern: in all eight patients, pancreatitis was inherited through the paternal side of the family (P = .025; two-tailed Fisher's exact test when compared with patients without pancreatic cancer).

Nine other histologically confirmed tumors were diagnosed during the study period: one at each of the following sites—the ampulla of Vater, stomach, lung, cervix, breast, bone (metastatic or primary site unknown), and three skin cancers. The expected number of nonpancreatic, noncutaneous cancers was 8.1 (SIR = 0.7; 95% CI = 0.3-1.6). Twenty patients died during the follow-up period: all eight with pancreatic cancer; four with cancers of the ampulla of Vater, lung, bone, and stomach; four from diseases related to pancreatitis (three with diabetes and one

Table 1. Numbers of patients, person-years, and observed and expected pancreatic cancer cases in patients with hereditary pancreatitis (HP)

Group	No. of HP patients	Person-years*	Pancreatic cancer		SIR‡ (95% CI)
			Observed	Expected†	
United States	175	5677	5	0.0736	68 (22-159)
Europe§	60	2496	3‖	0.0690	44 (9-127)
Japan	11	358	0	0.0071	—
Males	125	4351	5	0.1083	46 (15-108)
Females	121	4180	3	0.0414	73 (15-212)
Total	246	8531	8	0.1497	53 (23-105)

*Person-years are calculated from birth to time of last follow-up or time of death.

†Expected number of pancreatic cancer cases using national age- and sex-stratified incidence data for the period 1983 through 1987 (*7*).

‡SIR = standardized incidence ratio; CI = confidence interval.

§Includes: Italy (19 cases), Federal Republic of Germany (11 cases), Sweden (eight cases), France (seven cases), Spain (six cases), Switzerland (four cases), U.K. (four cases), and Greece (one case).

‖Spain (two cases) and U.K. (one case).

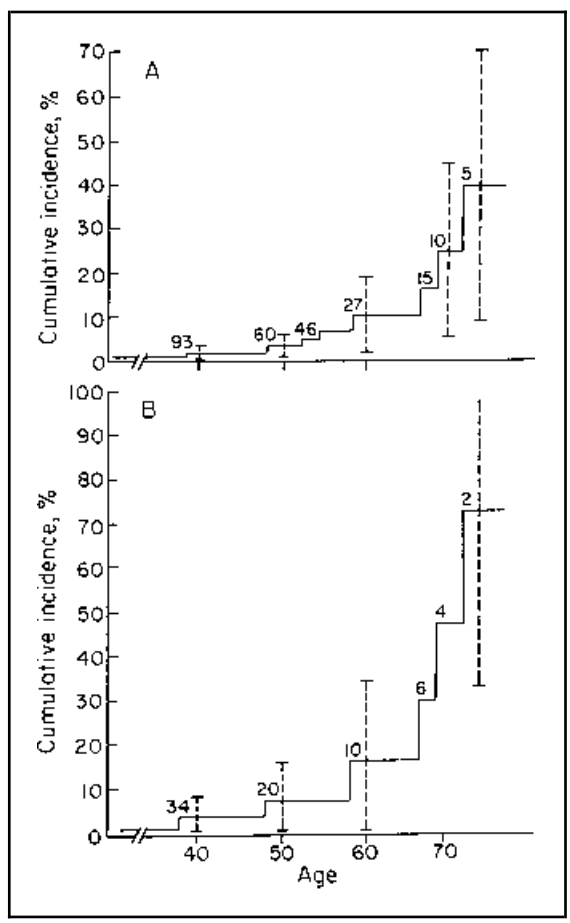

Fig. 1. A) Cumulative incidence (%) of pancreatic cancer since birth in entire cohort of 246 patients with hereditary pancreatitis. **B)** Cumulative incidence (%) of pancreatic cancer in 105 patients with a paternal inheritance pattern. Vertical dotted lines = 95% confidence intervals, and numbers within graph indicate patients at risk.

with acute pancreatitis); and four from other causes—two from heart failure, one from a motor vehicle accident, and one from liver failure.

Discussion

The main finding in this cohort study of a large group of patients with hereditary pancreatitis was an exceptionally high risk of pancreatic cancer. This excess risk was observed in both sexes and in patients from different geographic regions. The absence of any excess of other tumor types implies that the genetic abnormality responsible for this disease does not increase overall susceptibility to cancer. The risk of pancreatic cancer was considerably higher than has been previously observed in several studies (*5-8*) of patients with other forms of chronic pancreatitis. The high risk in patients with hereditary pancreatitis may be related to the early onset of pancreatitis, so that over a prolonged time period, there is progression of disease, tissue destruction, and, eventually, development of defects in cellular repair. This sequence of events has been described in other organs (*14*).

There are several biases that might lead to a spuriously high risk of pancreatic cancer. An excess risk would arise if centers with cases of pancreatic cancer were more likely to join the study than centers without this cancer. The aim of the project as outlined in the original letter to all centers was to study the natural history of a rare form of pancreatitis; cancer was not mentioned. Furthermore, the 3% prevalence of pancreatic cancer in our cohort is the same as the prevalence of pancreatic cancer reported in follow-up data of patients with hereditary pancreatitis from the Mayo Clinic and lower than the 6% prevalence of pancreatic cancer in literature reports collected prior to 1985 (*15*). Underestimation of pancreatic cancer in the background population would yield inflated risk ratios. However, even if half of the pancreatic cancers in the general population were unrecognized, our findings would still be highly statistically significant. If patients who eventually developed pancreatic cancer were followed more closely than patients who did not develop pancreatic cancer, our results would be spuriously elevated. To estimate the possible extent of this bias, we have performed an analysis assuming that all patients without pancreatic cancer with incomplete follow-up lived until the end of the study period but remained free of pancreatic cancer. This assumption adds 592 patient-years and lowers the SIR to 39.2 (95% CI = 17-77), but the results are still statistically significant. None of these biases would account for the observed paternal inheritance pattern observed in patients with pancreatic cancer.

The results of the actuarial analysis must be interpreted cautiously because follow-up intervals for most patients were shorter than follow-up times for patients who developed pancreatic cancer. More complete follow-up is needed before we can conclude that the high actuarial rates are real.

Because there were so few pancreatic cancers, we were unable to evaluate the importance of smoking—a strong and consistent risk factor for this neoplasm. However, since hereditary pancreatitis appears to be associated with pancreatic cancer, it would be unwise for these patients to smoke, since there could well be an interaction between these two causative factors. Also, since heavy alcohol consumption is the most common cause of pancreatitis, these patients should be advised to drink only small quantities of alcohol, if they drink at all.

A paternal inheritance pattern appears to be a strong predictor for the development of pancreatic cancer. This finding was entirely unexpected and could represent a chance finding. If this finding is real, one explanation could be genetic imprinting—a term used to describe differential expression of maternally or paternally inherited copies of a gene. Imprinting is known to be necessary for normal development (*16*) and has been linked to altered DNA methylation in cancer (*17*). Imprinting has not been described in pancreatic cancer but has been reported in various other neoplasms, including Wilms' tumor, retinoblastoma, osteosarcoma, paraganglioma, and other tumors (*18,19*). As in this report, a paternal inheritance pattern is usually observed. We plan additional studies to evaluate the evidence for genomic imprinting observed in this cohort.

This cohort study suggests that an autosomal-dominant genetic disorder, hereditary pancreatitis, may markedly increase the risk of pancreatic cancer. The gene for this disease has been located on chromosome 7q35, and the specific mutation appears to be an Arg-His substitution at residue 117 of the cationic trypsinogen gene (*3,4*). To date, 30 patients included in our cohort have been tested for this gene; all have tested positive. After appropriate genetic counseling, other members of the cohort and their relatives may wish to be tested. Also, it may be prudent to screen young patients who have recurrent attacks of pancreatitis or unexplained bouts of abdominal pain accompanied by hyperamylasemia to determine if they carry the mutant gene. Eventually, it may be worthwhile to search for a similar genetic defect in more common forms of pancreatitis or in sporadic cases of pancreatic cancer.

References

(*1*) Sibert JR. Hereditary pancreatitis in England and Wales. J Med Genet 1978;15:189-201.

(*2*) Le Bodic L, Bignon JD, Raguenes O, Mercier B, Georgelin T, Schnee M, et al. The hereditary pancreatitis gene maps to long arm of chromosome 7. Hum Mol Genet 1996;5:549-54.

(*3*) Whitcomb DC, Preston RA, Aston CE, Sossenheimer MJ, Barua PS, Zhang Y, et al. A gene for hereditary pancreatitis maps to chromosome 7q35. Gastroenterology 1996;110: 1975-80.

(*4*) Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 1996; 14:141-5.

(*5*) Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, et al. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. N Engl J Med 1993;328:1433-7.

(*6*) Ekbom A, McLaughlin JK, Karlsson BM, Nyren O, Gridley G, Adami HO, et al. Pancreatitis and pancreatic cancer: a population-based study. J Natl Cancer Inst 1994;86:625-7.

(*7*) Fernandez E, La Vecchia C, Porta M, Negri E, d'Avanzo B, Boyle P. Pancreatitis and the risk of the pancreatic cancer. Pancreas 1995;11: 185-9.

(*8*) Bansal P, Sonnenberg A. Pancreatitis is a risk factor for pancreatic cancer. Gastroenterology 1995;109:247-51.

(*9*) Kattwinkel J, Lapey A, Di Sant'Agnese PA, Edwards WA. Hereditary pancreatitis: three new kindreds and a critical review of the literature. Pediatrics 1973;51:55-69.

(*10*) Miller AR, Nagorney DM, Sarr MG. The surgical spectrum of hereditary pancreatitis in adults. Ann Surg 1992;215:39-43.

(*11*) Parkin DM, Muir CS, Whelan SL, Gao YT, Ferlay J, Powell J, editors. Cancer incidence in five continents. Vol. VI. IARC Sci Publ No. 120. Lyon: International Agency for Research on Cancer, 1992.

(*12*) Cancer statistics review 1973-1988. NIH Publ No. 91-2789. Washington (DC): National Cancer Institute, 1991.
(*13*) SAS system, release 7.07. Cary (NC): SAS Institute, 1992.
(*14*) Preston-Martin S, Pike MC, Ross RK, Jones PA, Henderson BE. Increased cell division as a cause of human cancer. Cancer Res 1990;50: 7415-21.
(*15*) Gross JB. Hereditary pancreatitis. Chapter 56. In: Go VL, editor. The exocrine pancreas: biology, pathobiology, and diseases. New York: Raven Press, 1986:829-39.
(*16*) Barlow DP. Gametic imprinting in mammals. Science 1995;270:1610-3.
(*17*) Jones PA. DNA methylation errors and cancer. Cancer Res 1996;56:2463-7.
(*18*) Feinberg AP, Rainier S, DeBaum MR. Genomic imprinting, DNA methylation, and cancer. J Natl Cancer Inst Monogr 1995;17:21-6.
(*19*) Peterson K, Sapienza C. Imprinting the genome: imprinted genes, imprinting genes, and a hypothesis for their interaction. Annu Rev Genet 1993;27:7-31.

Notes

[1]*Editor's note:* SEER is a set of geographically defined, population-based central tumor registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Each registry annually submits its cases to the NCI on a computer tape. These computer tapes are then edited by the NCI and made available for analysis.

The International Hereditary Pancreatitis Study Group—United States: G. Aranha, Maywood, IL; P. Banks, Boston, MA; F. R. Burton, St. Louis, MO; D. Carr-Locke, Boston; W. P. Dyck, Temple, TX; R. G. Gish, San Francisco, CA; R. L. Goodale, Jr., Minneapolis, MN; G. Lehman, Indianapolis, IN; S. P. Martin, Cincinnati, OH; J. Potts III, Houston, TX; S. Sherman, Indianapolis; C. D. Ulrich II, Cincinnati; P. Yakshe, Minneapolis; P. Yeaton, Charlottesville, VA. Japan: Y. Hamanaka, Yamaguchi; M. Koizumi, Sendai; T. Tomioka, Nagasaki City; T. Tsunoda, Kurashiki City; K. Yamadera, Asahikawa. Europe: J. P. Delmont, Nice, France; H. G. Beger, Ulm, Federal Republic of Germany (F.R.G.); A. Holstege, Regensburg, F.R.G.; V. Keim, Leipzig, F.R.G.; P. Layer, Essen, F.R.G.; J. Triantafillidis, Nicea, Greece; P. Boyle, Milan, Italy; G. Cavallini, Verona, Italy; L. Gullo, Bologna, Italy; S. Pedrazzoli, Padua, Italy; G. Uomo, Naples, Italy; D. Gonzalo de las Heras Castano, Santander, Spain; I. Ihse, Lund, Sweden; M. Büchler, Bern, Switzerland; and E. Elias, Birmingham, U.K.

Supported in part by grants from the C. D. Smithers Foundation.

We thank D. Lowenfels for computer assistance and database management and G. P. Jarvik for reviewing the manuscript.

Manuscript received August 2, 1996; revised November 25, 1996; accepted January 6, 1997.

Apoptosis of N-Type Neuroblastoma Cells After Differentiation With 9-*cis*-Retinoic Acid and Subsequent Washout

*Penny E. Lovat, Helen Irving, Margherita Annicchiarico-Petruzzelli, Francesca Bernassola, Archie J. Malcolm, Andrew D. J. Pearson, Gerry Melino, Christopher P. F. Redfern**

Background: **The overall survival rate for patients with neuroblastoma has improved over the past two decades, but long-term survival for the subgroup of patients with high-risk disease remains low. In recent years, there has been interest in the potential clinical use of drugs able to induce differentiation of neuroblastoma cells. Since 9-*cis*-retinoic acid induces better and more sustained differentiation of neuroblastoma in vitro than other retinoic acid isomers, this may be a more appropriate retinoid for use in neuroblastoma therapy.** ***Purpose:*** **The purpose of this work was to compare the long-term effects of all-*trans*- and 9-*cis*-retinoic acid on neuroblastoma differentiation using an N-type (neuroblastic) cell line, SH SY 5Y, as an in vitro model. In addition, we wanted to find out whether 9-*cis*-retinoic acid would induce programmed cell death (apoptosis) in these N-type neuroblastoma cells and to determine whether the effects of either 9-*cis*- or all-*trans*-retinoic acid are dependent on their continued presence in the culture medium.** ***Methods:*** **SH SY 5Y cells were incubated in either the continued presence of all-*trans*- or 9-*cis*-retinoic acid or for 5 days with retinoic acid followed by culture in the absence of retinoid for up to 13 days. Morphologic changes were observed using phase-contrast and scanning electron microscopy. Apoptosis was determined by flow cytometry of propidium iodide-stained cells and by using terminal deoxynucleotidyl transferase to end-label DNA fragments in situ in apoptotic cells.** ***Results:*** **Culture of SH SY 5Y cells with all-*trans*- or 9-*cis* retinoic acid for 5 days induced morphologic differentiation and inhibited cell growth. These effects were maintained in the continuous presence of each retinoic acid isomer but were more profound in cells treated with 9-*cis*-retinoic acid. The differentiation of cells treated with all-*trans*-retinoic acid was reversible once retinoic acid was removed from the medium. Conversely, apoptosis was induced in cells treated with 9-*cis*-retinoic acid for 5 days and cultured for 9 days (4 days after washout) but not in cells cultured in the continuous presence of 9-*cis*-retinoic acid. This effect was specific to 9-*cis*-retinoic acid.** ***Conclusions:*** **Previous studies have demonstrated differential responses to all-*trans*-retinoic acid in N- and S-type (substrate-adherent or Schwann-like) neuroblastoma cells: Apoptosis is induced in S-type cells, whereas differentiation occurs in N-type cells. The present results show that, unlike all-*trans*-retinoic acid, 9-*cis*-retinoic acid induces both differentiation and apoptosis in N-type SH SY 5Y neuroblastoma cells. However, apoptosis was dependent on removal of 9-*cis*-retinoic acid from the culture medium.** ***Implications:*** **Since both differentiation and apoptosis are involved in tumor regression, 9-*cis*-retinoic acid may be a more appropriate retinoid for clinical trials in neuroblastoma. The dependence of apoptosis on treatment and subsequent removal of 9-*cis*-retinoic acid implies that drug scheduling may be an important parameter affecting therapeutic efficacy. [J Natl Cancer Inst 1997;89:446-52]**

**Affiliations of authors:* P. E. Lovat (Departments of Medicine and Child Health), C. P. F. Redfern (Department of Medicine), H. Irving, A. D. J. Pearson (Department of Child Health), A. J. Malcolm (Department of Pathology), University of Newcastle Upon Tyne, U.K.; M. Annicchiarico-Petruzzelli, F. Bernassola, G. Melino, Istituto Dermopatico dell'Immacolata-Istituto di Ricovero & Cura a Carattere Scientifico Laboratorio, Università di Tor Vergata, Rome, Italy, and Università di L'Aquila, Italy.

Correspondence to: Christopher P. F. Redfern. M.M.B.G., The Medical School, Framlington Pl., Newcastle Upon Tyne, NE2 4HH, U.K.

See ''Notes'' following ''References.''