Role of Nuclear Magnetic Resonance Spectroscopy (MRS) in Cancer Diagnosis and Treatment: $^{31}$P, $^{23}$Na, and $^1$H MRS Studies of Three Models of Pancreatic Cancer

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ABSTRACT

The role of nuclear magnetic resonance spectroscopy (MRS) in pancreatic cancer diagnosis and its treatment were assessed in three models of pancreatic neoplasms. Perfused MIA PaCa-2 human pancreatic cancer cells, s.c. implanted pancreatic tumors in hamsters, and pancreatic tumors induced in situ in rats by direct application of the carcinogen 7,12-dimethyl benzanthracene, were studied by phosphorous ($^{31}$P), sodium ($^{23}$Na), and proton ($^1$H) MRS. $^{31}$P spectra of pancreatic cancer were qualitatively similar to those of intact organs. There were, however, variations in peak intensities and ratios. Phosphomonoester signals were prominent in both normal pancreases and tumors, but their levels depended on the proliferation rate and on environmental conditions. Thus, the phosphomonoester:$\beta$-nucleoside triphosphate ratio was 1.15 ± 0.32 in 90% confluence and 1.31 ± 0.43 in 70% confluence, and this ratio increased upon lowering the perfusion rate. Total (intra- and extracellular) sodium concentrations, measured in the solid tumors, were 39–40 $\mu$mol/g wet weight in normal pancreases. Contrary to a previous hypothesis that malignant transformation is associated with increased sodium content, our $^{23}$Na MRS data showed that there were no significant differences between pancreatic tumors and intact organs. Proton spectra of perchloric acid extracts revealed several differences between tumors and control pancreases. The principal findings were elevated levels of the amino acid taurine, from 1.17 ± 0.39 $\mu$mol/g wet weight in healthy pancreases, to 2.79 ± 0.71 $\mu$mol/g wet weight in pancreatic carcinoma in rats, and lactate levels that increased from 0.92 ± 0.2 to 6.19 ± 1.93 $\mu$mol/g wet weight, respectively. On the other hand, creatine and glutamate were higher in the normal pancreases. Pancreatic cancer is usually resistant to chemotherapy, and we evaluated the effects of the metabolic inhibitors 2-deoxyglucose and lonidamine on the human pancreatic cancer cells by MRS and cytotoxicity studies. The IC$_{50}$ of Adriamycin and 2-deoxyglucose were 1.49 ± 0.18 $\times 10^{-6}$ and 136 ± 17 $\mu$g/ml, respectively. These results were similar to data obtained previously in multidrug-resistant human breast cancer cells, which were highly resistant (33-fold) to Adriamycin but were more susceptible (9-fold) to 2-deoxyglucose than their parental cells.

INTRODUCTION

The incidence of adenocarcinoma of the pancreas has steadily increased over the last several decades, and it is now the fourth most common cause of cancer deaths (1). Pancreatic cancer is a very aggressive and lethal neoplasm; more than 75% of the patients have widespread disease upon initial diagnosis (2). Furthermore, these tumors are usually resistant to conventional chemotherapy and irradiation, and the 5-year survival rate is only 3% (3). Accurate and reliable diagnostic means are, therefore, essential to improve the outcome for these patients. Imaging diagnostic modalities are based on spatial resolution of neoplastic tissues from their normal counterparts, and because a certain size of tumor is required for its detection, their usefulness for early cancer diagnosis is somehow limited. The genetic events that are associated with carcinogenesis are often accompanied by metabolic alterations. A technique that may discover biochemical features associated with the malignant changes may be an important tool for cancer research.

Nuclear MRS$^3$ is a powerful method that provides information on the biochemical status and physiological processes both in vitro and in vivo and is a qualitative as well as a quantitative method. The metabolism of intact cells and tissues can be studied in a continuous manner; thus, MRS is a unique, noninvasive biomedical research tool (4, 5). During the last two decades, there has been ongoing MRS research in malignant diseases. These studies provided valuable data on the biochemistry and metabolism of tumors and on the effects of nutrients, hormones, and growth factors (6, 7). The mechanisms of action of anticancer drugs and the acquired resistance to these agents were delineated (8, 9). MRS was used also for monitoring the response to therapy (10, 11).

Numerous MRS studies have focused on the value of MRS in cancer detection (12–15), but its efficacy as a diagnostic tool is still controversial. Size, location, and especially heterogeneity of tumors are major obstacles for clinical interpretations of experimental findings. Also, previous attempts to define characteristic and diagnostic MRS features in the plasma of cancer patients were found to be futile (16, 17).

To assess the potential role of MRS in the diagnosis and treatment of pancreatic cancer and to address the problem of diversity and heterogeneity of tumors, we performed multinuclear MRS in three different models of pancreatic cancer. These models included perfused human pancreatic cells, s.c. implantation of human pancreatic cancer cells in hamsters, and in situ induction of pancreatic cancer in rats by the application of the carcinogen DMBA Data, which can be obtained from combined phosphorus ($^{31}$P), sodium ($^{23}$Na), and proton ($^1$H) MRS, cover a wide range of metabolic activities, and increase the potential of identifying useful biochemical properties. Previously, we described the effects of metabolic inhibitors on several cancer cell lines and found that these agents were much more toxic to MDR cells than to their parental drug-sensitive cells (8, 18). In the studies presented therein, we also evaluated the effects of the metabolic inhibitors, 2-DG and LND, on human pancreatic cancer cells.

MATERIALS AND METHODS

Cells. Human pancreatic carcinoma cells, MIA PaCa-2, were obtained from the American Type Culture Collection (Rockville MD) and grown in DMEM, supplemented with penicillin-streptomycin-nystatin, L-glutamine, and 10% FCS, under 5% CO$_2$ environment. The pancreatic cancer cell line of hamsters was established from nitrosamine-treated CB strain Syrian hamsters at the NIH (Bethesda, MD).

Materials. DMEM and supplements were purchased from Biological Industries (Beth-Haemek, Israel). 2-DG and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO), unless otherwise indicated, and were of the highest available purity. LND was kindly supplied by the F. Angelini Institute (Rome, Italy) and by the Upjohn Company.

$^1$ This work was supported by a research grant from the Israel Cancer Association.

$^2$ To whom requests for reprints should be addressed.
Animals. Male inbred golden Syrian hamsters (8–12 weeks of age; 100 ± 20 g) and male Sprague-Dawley rats (150 ± 30 g) were obtained from the Animal Laboratory, Tel-Aviv University School of Medicine. The animals were kept as outlined in the “Guides for the Care and Use of Laboratory Animals” (NIH publication no. 85–23, 1985) and were given food and water ad libitum. Prior to surgical procedures, they fasted for 8 h but had free access to water. All of the surgical procedures were performed under general anesthesia induced by ketamine hydrochloride (70 mg/kg, i.m.).

Perfused Human Pancreatic Cancer Cells. The essentials of cellular perfusion are that metabolic events are unhampered; thus, substrates and nutrients can be furnished continuously, and waste products removed, while stable pH levels and a temperature of 37°C are maintained. In the present studies, we used the method of cellular embedding in sodium alginate micro capsules (19, 20). MIA PaCa-2 cells were grown to either 70 ± 5% or 90 ± 5% confluency (six experiments), harvested with 0.25% trypsin-0.05% EDTA, centrifuged at 4°C at 1000 × g for 5 min, and washed twice with medium. Cells (1.5–2 × 10⁶) were used in each experiment, and the cellular pellet (1.0–1.2 ml) was mixed with an equal volume of 2.5% (w/w in PBS) sodium alginate. The mixture was manually extruded, under minimal pressure, through a 25-gauge needle, on the surface of a 0.1 M CaCl₂ solution. The small drops (~1-mm diameter) gelled and were immediately washed three times in the growth medium. The capsules were isolated by decantation and transferred to a 10-mm screw cap NMR tube, and perfusion was promptly initiated. The perfusion was performed through an insert with inlet and outlet tubes, and the volume of the perfusion chamber was 2 ml. A constant flow of 0.9 ml/min in a single-pass mode was maintained by a peristaltic pump (Cole Parmer), and the temperature was maintained at 37°C. In each experiment, control perfusion with ³¹P MRS recording was carried out for about 60 min, to ensure metabolic stability of the cells, before adding the metabolic inhibitor to the perfusion solution.

Toxicity Assays. Toxicity was measured by the MTT microculture assay (21), which is a colorimetric assay based on the formation of formazan by the viable cells. Plates were plated in 96-well plates in a 50-µl growth medium per plate. The drug was added following 1 day of incubation to allow the cells to settle and attach. Growth inhibition was measured 4 days later by the ELISA reader (Dinathex MR600) at a wavelength of 570 nm.

Implanted Pancreatic Tumors. Cells (0.5 × 10⁶) of the established line of the hamsters’ pancreatic cancer cells were s.c. implanted in the interscapular area of 24 hamsters. Two hamsters died before MRS measurements were performed. At predetermined time intervals, a group of animals were operated upon, the tumors were excised without the skin and fat, and three of them were used for preparation of extracts for ³¹P MRS. The remaining tumors were placed in a chilled (1–2°C) isotonic (310 mosm), sodium-free mannitol solution in a 10-mm NMR tube, and spectra were promptly recorded at 4°C to minimize metabolic instability. Controls were pancreases of three animals in each group, which were excised and treated in an identical manner.

In Situ Induction of Pancreatic Tumors. Sixty Sprague-Dawley rats were operated under general anesthesia, the pancreases were exposed, and 1–2 mg (crystalline) of the carcinogen DMBA were directly applied to the pancreases parenchyma through a superficial cut in the anterior surface. The small incision was covered by the pancreatic capsule and omentum. Because we used a solid carcinogen, the operations were performed in a biological hood using strict no-touch techniques. The controls were eight animals in which physiological solutions instead of DMBA were used. The animals were kept for a period of 4–32 weeks, the size of the nodules was ~0.5 cm, and after 1 month, their diameter was ~1.5 cm. The tumoral mass seemed to be encapsulated by connective tissues, and most of the blood supply was from vessels that originated from the thoracic wall. In supernature tumors with no mortar and pestle. Four ml of precooled (~10°C) 0.5 M perchloric acid (22, 23) were added to every gram of tissue, and the mixture was stirred mechanically for 5 min at ~4°C. Samples were centrifuged at 2000 × g and ~4°C for 10 min, and the pH of the supernatant was adjusted to 6.5 with KOH in an ice-water bath. Potassium perchlorate was removed by a second centrifugation at 3500 × g and ~4°C for 15 min. The supernatant was lyophilized to dryness, dissolved in D₂O, adjusted to pH 7.0, and kept at ~20°C for less than 24 h before the MRS measurements were taken.

Magnetic Resonance Spectroscopy. These studies included ³¹P MRS of perfused cells and their extracts, ³¹P and ⁶²Na MRS of solid tumors, and ¹H measurements of extracts of solid tumors. For each nucleus, the acquisition and processing parameters were identical throughout all of the experiments. Spectra were recorded on a Bruker AM-360 WB spectrometer equipped with a variable frequency 10-mm probe and were analyzed on a SGI data station. ³¹P and ⁶²Na spectra of solid tumors were sequentially recorded, after which the tissues were weighed and processed for histological assessment. ³¹P spectra were measured at 145.78 MHz by applying 45° radiofrequency pulses; each spectrum of the whole organ was a collection of 400 scans and a relaxation delay of 2 s, whereas in the extracts, the parameters were 700 scans and 10 s. ³¹P chemical shifts were determined by standardizing GPC to 0.49 ppm (24). Comparisons between compounds were made by measuring the integrals of the peaks that had a 15-Hz line broadening. Sodium spectra were recorded at 95.26 MHz using 90° radiofrequency pulses and 200 scans with no relaxation delay. In experiments where tuning for ³¹P and ⁶²Na was alternately performed, a standard sample containing 30 mM NaCl was used as a reference. The signal/noise of the ⁶²Na signals was in the range of 40–45/1. The level of sodium in the pancreas or tumor was calculated by comparing the integrated area of the ⁶²Na peak to the integral of the reference signal. For ¹H MRS, 1.0 ml of D₂O dissolved extracts of normal pancreas or tumors were placed in a 5-mm tube. A 5-s repetition time and a 90° flip angle were used, and 1600 transients were accumulated for each spectrum. Chemical shifts of the signals were determined by standardizing to the reference compound TSP at 0 ppm, and concentrations of the metabolites were calculated by comparing the intensities of the signals with those of TSP methyl residues. Quantitative results are expressed as means ± SD. Statistical analyses were performed with the paired, double-tailed, Student’s t test (P < 0.05).

RESULTS

Histopathology. The carcinogenic effects of DMBA are shown in Table 1. Two rats died in the immediate postoperative period, and three rats died during the ensuing 15 months. In all pancreases, there were marked adhesions to neighboring organs, fibrosis, and foreign body granuloma. However, malignant changes were observed only after 10 months, and after 15 months, 70% of the animals had tumors. Macroscopically, most tumors were hard gray-white nodules of 2–4 cm in diameter, and in some animals, there was hemorrhagic ascites. The pancreatic tumors were papillary adenocarcinoma, some of them with cyst formation. Most tumors were of acinar rather than of ductal origin, and in almost all pancreases, areas of ductal hyperplasia were found after 12 months. Two of the tumors that were detected after 12 months and six of the tumors found after 15 months reached large sizes of 5–8 cm and invaded surrounding organs, the diaphragm and the abdominal wall, and had inner necrotic areas. In five of the adenocarcinoma detected after 15 months, there were distant metases; two in the liver, two in the lungs, and one in the right thigh. Four of the tumors were of mesenchymal origin; two of them were in the laparotomy incision, one was between the pancreas and the spleen, and one was in the lung. In the control group, neither tumors nor inflammatory or reactive changes were noted after 12 months.

All of the tumors that developed in the hamsters after s.c. cancer cell implantation were papillary ductal adenocarcinoma. After 2 weeks, the size of the nodules was ~0.5 cm, and after 1 month, their diameter was ~1.5 cm. The tumoral mass seemed to be encapsulated by connective tissues, and most of the blood supply was from vessels that originated from the thoracic wall. In supernature tumors with no...
<table>
<thead>
<tr>
<th>Time (mos)</th>
<th>No. of animals</th>
<th>Histopathological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6</td>
<td>Adhesions, fibrosis, foreign body granuloma</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>Adhesions, fibrosis, foreign body granuloma</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>Pancreatic adenocarcinoma (1 rat)</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>Pleomorphic spindle cell sarcoma in the laparotomy incision (1 rat)</td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>Pancreatic adenocarcinoma (12 rats)</td>
</tr>
</tbody>
</table>

We observed changes in peak intensities and in the ratios between the phospholipid and energy metabolites, which were associated with cellular confluency and with the perfusion rate. The PDE:βNTP ratios were 0.53 ± 0.19 and 0.69 ± 0.23, respectively. However, the differences between 70 and 90% confluency were of no statistical significance, probably due to the large SDs. As expected, reduction of the perfusion rate was followed by a gradual depletion of the high-energy compounds. At a perfusion rate of 0.3 ml/min, PCr depleted below detectable levels after two spectra were recorded (~28 min), and βNTP depleted after six to eight spectra were recorded (~98 min). At this perfusion rate, we noted a 39 ± 17% elevation of GPC compared to its levels at a perfusion rate of 0.9 ml/min, but no changes in the PME signals were found.

Unlike intact pancreases, there were no PDE signals in 31P MRS of pancreatic tumor implanted s.c. in hamsters (Fig. 3). It should be mentioned, though, that in spectra of intact perfused rat pancreas (27), as well as in those of perfused pancreatic cancer cells and pancreatic cancer induced in situ by DMBA (see below), there were large signals of PDE. PME compounds dominated these spectra, and because the resolution of spectra of tissues is inferior to that of extracts and other compounds (mainly sugars) may appear in the PME region, it was essential to achieve unambiguous assignment of these signals. Therefore, we also performed a two-dimensional 1H-31P correlation map (Fig. 4), which showed that PE and PC were indeed the two main compounds in the PME region. However, contrary to the spectra of MIA PaCa-2 cells, the PE was the dominant signal of the PME region.

Macroscopic dimension increase, skin and thoracic wall invasion, together with central degeneration, were observed, and the amount of necrosis correlated with the size of the tumors.

31P MRS. 31P MRS spectra of perfused MIA PaCa-2 human pancreatic cancer cells are shown in Fig. 1a. Peak assignments were based on previously reported 31P MRS data (25, 26), and to validate the assignments of the signals, perchloric acid extractions of the cells were prepared (Fig. 2), and the genuine compounds were added to the extracts. 31P MRS spectra of perfused human pancreatic cancer cells were qualitatively similar to those of intact hamsters pancreases (Fig. 3a), which were used for comparison because we had no immortal normal human pancreatic cell line. The only difference was that whereas PE was the dominant PME signal in the normal pancreas, PC was the dominant signal in the PME region in human pancreatic cancer cells. For acquisition parameters, see "Materials and Methods." Pi, inorganic phosphate; NAD, nicotinamide dinucleotide; DPDE, diphosphodiester. b, 31P MRS of the same cells 175 min after the addition of 5 mM 2-DG to the perfusion solution. The dominant signal is the phosphorylation product of 2-DG.

Fig. 1. a, 31P MRS of perfused MIA PaCa-2 human pancreatic cancer cells at 37°C. For acquisition parameters, see "Materials and Methods." b, 31P MRS spectra of extracts of MIA PaCa-2 human pancreatic cancer cells. Cells were grown to 80 ± 5% confluency, harvested with 0.25% trypsin-0.05% EDTA, centrifuged at 4°C at 750 × g for 10 min, and washed three times in PBS. The pellet (10³ cells) was treated with 5 volumes of 0.5 M cold perchloric acid, followed by sonication on ice for 5 min. The mixture was then centrifuged at 3000 × g and −4°C for 10 min, and the pH of the supernatant was adjusted to 6.5 with KOH in an ice-water bath. Potassium perchlorate was removed by a second centrifugation at 3500 × g and −4°C for 15 min. The supernatant was lyophilized to dryness, dissolved in 1 ml of D2O, and adjusted to pH 7.0. For acquisition parameters, see "Materials and Methods." Assignments of 31P signals were based on data reported previously and verified by adding the genuine compounds to the extract solution.
MRS STUDIES OF PANCREATIC CANCER

ments of the signals were according to reported data (25, 30) and were validated by adding the specific compounds to the extracts' solution. These spectra demonstrated consistent MRS differences between intact pancreases and pancreatic cancer, which appeared in both models of solid tumors. The principal features were elevated signals of the amino acid taurine and of the lactate in the tumors and the high creatine, as well as glutamine/glutamate, signals in the healthy pancreases. The concentrations of the metabolites are shown in Table 3. Taurine, lactate, and creatine/PCr levels in the two models of tumors were all significantly different from those of normal pancreases (P < 0.05). These differences were noted as early as 19 days after tumor implantation in hamsters and were persistent, i.e., the proton spectra after 19 and 108 days were similar. There were intermediates of the glycolytic pathway, in which lactate is the end product, but at low concentrations. They were represented by pyruvic acid at 2.48 ppm. The most abundant amino acids in the spectra, except for taurine, were alanine, glutamic acid, and glycine. Valine, leucine, proline, and aspartic acid appeared at low concentrations. The dominant peaks centered at 3.20 ppm represented the N-methyl and N-methylene terminals of choline, PC, and PE, and the differences between healthy pancreases and tumors were not significant. The GPC, GPE, and also glycerol signals appeared at 3.29 ppm, partially overlapping the high field triplet of taurine (S-CH2). In agreement with 31P spectra (Fig. 3), these peaks were prominent in healthy pancreases but were very low in implanted pancreatic tumors. The spectra of the necrotic inner parts of the overgrowing tumors revealed only very high signals of lactate (data not shown).

Effects of Metabolic Inhibitors We assessed the toxicity of the two metabolic inhibitors, 2-DG and LND, on MIA PaCa-2 cells (Table 4) and compared the results with their effects on drug-sensitive compounds. After 3.5 months, the inner parts of the tumors were necrotic, and as the size of the tumors increased, there was a decrease of PCr and NTP signals (Fig. 5). The PME:NTP ratios 19 and 108 days after implantation of pancreatic cancer cells were 2.6 ± 0.5 and 3.5 ± 0.6, respectively (P > 0.05). 31P spectra of the necrotic fluid showed only a large broad peak of inorganic phosphate at 2.05 ppm, corresponding to a pH of 6.7, and a group of signals in the PME region, representing species from disorganized membranes and nucleic acids (28).

31P spectra of pancreatic tumors, which were induced in situ by DMBA, were characterized by high signals from the precursors of phospholipids synthesis, the PME compounds, as well as from their degradative products, the PDE compounds (Fig. 6). Similar to s.c. implanted tumors, but unlike the human pancreatic cancer cell line, the PE, and not the PC, was the dominant signal in the PME region. These spectra were qualitatively similar to those of the controls.

23Na MRS. Most of the sodium in tissues is extracellular, and the 23Na spectra are characterized by a dominant extracellular peak and a very small intracellular signal, both of which were integrated to determine sodium concentrations (Table 2). Contrary to a previous hypothesis that assumed that neoplastic changes are associated with increased sodium content (29), 23Na MRS of both models of solid tumors showed that it was similar to normal pancreases, and total sodium content did not change during tumor growth until necrosis occurred.

1H MRS. Proton spectra of perchloric acid extracts of an intact pancreas and of a pancreatic tumor are shown in Fig. 7. The assign-
MRS STUDIES OF PANCREATIC CANCER

Tumors originating from s.c. implantation of human pancreatic cancer cells in hamsters were of ductal origin and morphologically resembled human pancreatic adenocarcinoma (32, 33). However, their unnatural location may be associated with remarkable changes in

and -resistant human breast cancer cells (31). The human pancreatic cancer cells were very resistant to Adriamycin. On the other hand, these cells were relatively sensitive to both LND and 2-DG. Incubations with low concentrations of 2-DG (0.1 mM) were followed by stimulating effect to 124 ± 13% above initial MTT readings; this observation requires further investigation. The effects of these agents on perfused cells were also monitored by 31P MRS. Following the addition of 5 mM 2-DG to the perfusion solution, there was a gradual accumulation of its phosphorylation product 2-deoxyglucose-6-phosphate, concomitant with a decrease in the levels of the high-energy compounds, PCr and NTP (Fig. 1b). When LND was added to the perfusion solution, the pH decreased to 6.76 ± 0.12. This finding was in accordance with our previous data that the mechanism of action of LND is through intracellular acidification due to inhibition of extracellular lactate transport (9).

DISCUSSION

The role of MRS in cancer diagnosis will ultimately be determined only by human studies. However, to define unambiguous MRS characteristics of malignant processes, laboratory and animal studies are essential. Furthermore, delineation of the mechanisms of action of anticancer modalities is necessary for optimal treatment, and animal models are very useful for this purpose. To minimize experimental artifacts and simulate human pancreatic neoplasms, we performed MRS studies of three different models of pancreatic cancer and used human pancreatic cancer cells. Perfusion of intact cells is a very valuable approach for the noninvasive study of metabolism. In contrast to the in vivo situation, these cells are homogeneous, and there are no artifact data from connective tissues and blood vessels. The cells are metabolically stable for prolonged periods during perfusion under physiological conditions, and the effects of drugs can be continuously assessed. The combination of perfusion and in vivo studies is necessary to determine the relevancy of experimental models to the human clinical setting.

Table 2 23Na MRS of experimental pancreatic cancer: sodium content

<table>
<thead>
<tr>
<th>Time</th>
<th>No. of animals</th>
<th>Sodium µmol/g wet weight*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implanted tumors in hamsters</td>
<td>5</td>
<td>38.8 ± 3.6</td>
</tr>
<tr>
<td>19 days</td>
<td>5</td>
<td>41.4 ± 3.3</td>
</tr>
<tr>
<td>38 days</td>
<td>5</td>
<td>40.5 ± 6.1</td>
</tr>
<tr>
<td>108 days</td>
<td>3</td>
<td>39.1 ± 3.1</td>
</tr>
<tr>
<td>Intact pancreas</td>
<td>9</td>
<td>40.2 ± 5.9</td>
</tr>
<tr>
<td>Tumors induced in situ in rats by DMBA</td>
<td>6</td>
<td>41.3 ± 4.3</td>
</tr>
<tr>
<td>12 mo</td>
<td>3</td>
<td>39.7 ± 4.1</td>
</tr>
<tr>
<td>15 mo</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* Results are expressed as means ± SD. Statistical analyses were performed with the paired, double-tailed, Student’s t test (P < 0.05), and the differences between the results were not significant.
issue about which the reported data were controversial (34, 35). It should be pointed out that in our studies, most tumors developed more than a year after DMBA applications, whereas other investigators found a tumor incidence of about 50% after 6 months (34, 35). This prolonged induction period must be taken into consideration when planning studies with this tumor model.

Phosphorous has been the most widely used nucleus in MRS studies of metabolism. $^{31}$P spectrum is easy to interpret, but the number of compounds that are detectable is small, and this limits its potential as a diagnostic means. Our $^{31}$P MRS studies showed that there were no consistent spectral features of pancreatic neoplasms, and there were no characteristic $^{31}$P MRS signals of the malignant processes. Only in the model of s.c. implantation of human pancreatic cancer cells in hamsters was there a qualitative difference between normal pancreases and tumors, i.e., the absence of PDE signals in spectra of the latter. It was reported previously that PDE species were elevated in tumors (36—38), reflecting perhaps enhanced membrane biosynthesis. The spectra of pancreatic cancer were dominated by the PDE signals; however, they were also notably high in normal pancreases. Environmental conditions can modify signal intensities, and most experimental solid tumors cannot be studied under physiological conditions, i.e., perfusion at 37°C. We suggest, therefore, that comparisons between signal ratios should be used only for spectra measured under identical experimental conditions. Clinical $^{31}$P MRS studies demonstrated reduced levels of PDE and decreased PDE:NTP ratio, following therapy (10, 11).

These studies also demonstrated a conspicuous amount of PCr in the pancreas. Although this is the dominant $^{31}$P signal in MRS of striated and smooth muscles (39), it is often absent from spectra of parenchymatous and glandular tissues. This finding may point to the high metabolic rate and extensive energy requirements of the pancreas. Another finding was the elevation of the GPC signal when environmental conditions of the cells were compromised, i.e., decreased perfusion and supply of nutrients. We have previously observed this phenomenon in a model of perfused rat pancreas (27), and GPC appears to be an indicator of a reversible ischemic insult.

Increased sodium concentrations are associated with several pathological processes, especially when there is remarkable tissue edema. Previously, it was hypothesized that lowered transmembrane potential, leading to reduced activity of the sodium pump and elevation of intracellular sodium, would initiate and sustain the mitogenic process. Using X-ray microanalysis, Cameron et al. (29) found that in four lines of transformed cells, sodium levels were up to 5-fold higher than in their normal counterparts (29). In previous NMR studies, reported by Goldsmith and Damadian (41), sodium contents of four

**Table 3** $^1$H MRS of perchloric extracts of experimental pancreatic cancer: metabolites concentrationsa (µmol/g wet weight)

<table>
<thead>
<tr>
<th></th>
<th>Taurine</th>
<th>Lactate</th>
<th>Creatine and PCr</th>
<th>Choline-containing compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pancreases</td>
<td>1.17 ± 0.39</td>
<td>0.92 ± 0.28</td>
<td>4.15 ± 0.63</td>
<td>6.52 ± 1.88</td>
</tr>
<tr>
<td>Implanted pancreatic tumors in hamsters</td>
<td>2.56 ± 0.63</td>
<td>5.71 ± 1.67</td>
<td>1.38 ± 0.36</td>
<td>5.80 ± 1.63</td>
</tr>
<tr>
<td>In situ induction of pancreatic cancer in rats by DMBA</td>
<td>2.79 ± 0.71</td>
<td>6.19 ± 1.93</td>
<td>1.54 ± 0.46</td>
<td>5.97 ± 1.59</td>
</tr>
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</table>

a Results are expressed as means ± SD. Statistical analyses were performed with the paired, double-tailed, Student's t test. Taurine, lactate, and creatine/PCr levels in the two models of tumors were all significantly different from those of normal pancreases (P < 0.05). The differences between the two models, and between the levels of the choline-containing compounds, were not significant.

**Table 4** Toxicity studies: MTT tests

<table>
<thead>
<tr>
<th></th>
<th>MIA PaCa-2 cells</th>
<th>MCF-7 drug-sensitive cells</th>
<th>MCF-7 drug-resistant cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>LND</td>
<td>184 ± 22</td>
<td>85 ± 15</td>
<td>135 ± 12</td>
</tr>
<tr>
<td>2-DG</td>
<td>136 ± 17</td>
<td>780 ± 52</td>
<td>91 ± 8</td>
</tr>
<tr>
<td>LND + 2-DG (5 ms)</td>
<td>94 ± 13</td>
<td>38 ± 8</td>
<td>70 ± 14</td>
</tr>
<tr>
<td>Adriamycin (1.49 ± 0.18) × 10⁻⁶</td>
<td>(5.5 ± 0.12) × 10⁻⁸</td>
<td>(1.86 ± 0.10) × 10⁻⁶</td>
<td></td>
</tr>
</tbody>
</table>

a Data are presented as IC₅₀ ± SD (µg/ml).
types of experimental tumors were found to be elevated compared to normal tissues. Recently, Stubbs et al. (42) found by atomic absorption spectroscopy of extracts that sodium levels in Morris hepatoma were twice those of normal livers. Liebling and Gupta (43) found increased intracellular sodium concentrations in neoplastic tissues compared to their normal counterparts. However, this elevation has only minimal contribution to the total sodium NMR signal. Traditional methods like atomic absorption and ion-selective electrodes are destructive, and $^{23}\text{Na}$ MRS offers the advantage of noninvasive measurements of the sodium content in tissues and organs. Magnetic resonance imaging studies of sodium demonstrated greater sodium signals in experimental neoplasms (44), as well as in human brain tumors (45, 46), than in their surrounding tissues. Sodium bound to macromolecules may be MRS invisible, and by combined atomic absorption and MRS measurements, we found previously that $^{23}\text{Na}$ visibility in the intact pancreas was 65 ± 20% (47). To simulate the clinical setting and to assess the applicability of $^{23}\text{Na}$ MRS for cancer diagnosis, we measured total visible (intra- and extracellular) sodium levels of tumors without artifacts from surrounding tissues. We found that sodium levels of the tumors were similar to those of normal pancreases. The elevation of bound intracellular sodium in malignant cells may increase its invisibility, and this may account for some of the discrepancy between our MRS results and data obtained from other techniques. It is noteworthy, though, that most sodium in tissues is in the extracellular compartment, and major ion shifts are required for the above-mentioned extreme changes. Also, there are often areas of edema in and around tumors that may be responsible for some of the elevations in sodium levels that are detected by localized images.

The proton has the highest magnetic resonance sensitivity and is the most abundant nucleus in biological molecules. Therefore, $^1\text{H}$ MRS bears the greatest potential for the detection of biochemical properties of cancer, and oncological proton MRS has attracted a lot of interest in recent years (48–51). High resolution MRS of extracts, with unambiguously assigned signals, provides the means for pursuing such biochemical markers. Indeed, our $^1\text{H}$ MRS studies demonstrated that pancreatic malignant transformation is characterized by high levels of the amino acid taurine. Taurine is the end product of methionine and cystine and is unique among other amino acids in that it is a free amino acid in the cytosol and is not a constituent of a protein. It plays a role in the absorption of fats, in membrane protection, possibly through detoxification, antioxidation, and osmotic regulation mechanisms, and also as a neurotransmitter in the central nervous system (52, 53). The association between taurine and neoplastic diseases has been described in the past (54), and increased taurine levels in other tumors were found recently by MRS studies (55–58). Alterations in taurine levels were found following therapeutical manipulations (59) and in relation to MDR (60). Learning the mechanisms of these changes may provide important data for basic cancer research. In case these high-resolution features will be likewise detected by localized $^1\text{H}$ MRS, taurine may as well serve as a clinical diagnostic marker.

The elevated lactate and decreased glutamate levels in pancreatic tumors, compared to normal pancreases, illustrate the differences in energy production; i.e., preference of glycolysis in tumors, and of oxidative phosphorylation in normal tissues. Proton spectra showed that creatine and phosphocreatine were higher in normal pancreases than in pancreatic cancer, but in $^{31}\text{P}$ spectra, $\text{PCr}$ was higher in tumors than in intact pancreases. It should be concluded, therefore, that creatine was much higher in normal pancreases, perhaps because of changes in creatine kinase activity induced by the malignant transformation. However, these results should be considered prudently, because $\text{PCr}$ is a very labile molecule and could undergo hydrolysis, even in the short interval between tissue excision and extract prepartion.

Pancreatic neoplasms seldom respond to conventional chemotherapy. This inherent resistance may be partly due to their excretory capabilities, because the MDR phenomenon is often associated with an energy-dependent efflux mechanism (61). Toxicity assays and MRS were used to study the effects of the anti-metabolism drugs, 2-DO and LND, on experimental pancreatic neoplasms. The mechanism of action of 2-DO was found to be through depletion of high energy compounds; therefore, it may be effective in drug-resistant tumors that have increased energy requirements (8, 18). MRS studies of LND delineated a novel anticancer mechanism, i.e., markedly intracellular acidification caused by inhibition of lactate transport (9). Because we had no drug-sensitive and drug-resistant MIA PaCa-2 cell lines, we compared our results with data concerning breast cancer cells. 2-DO had similar effects on pancreatic cancer cells as on MDR MCF-7 cells, and its cytotoxicity was much higher in these cells than in the drug-sensitive cells. Although these comparisons should be considered with caution, they point to the usefulness of metabolic inhibitors in the treatment of pancreatic cancer. Additional studies are needed to elucidate whether the stimulation of pancreatic cancer by cerulein or secretin, which induce synthetic and metabolic activities, followed by treatment with 2-DO and/or LND, augments their therapeutic effects.

The role of MRS in the diagnosis and treatment of pancreatic cancer would be ultimately determined by human studies. These multicellular MRS studies of three experimental models of pancreatic carcinoma demonstrated some of the potential of this technique. The data that can be obtained from MRS of each element should be defined. Thus, it seems that although $^{31}\text{P}$ MRS may have only a limited role in cancer diagnosis, it can be a useful means to monitor the effects of the treatment and can be used in the development of new therapeutical modalities. Similarly, we found no $^{23}\text{Na}$ MRS characteristics of pancreatic cancer, and its role in the treatment should be evaluated in additional studies. $^1\text{H}$ MRS showed that the amino acid taurine and lactate were elevated in pancreatic tumors compared to normal pancreases, and creatine and glutamate were higher in the intact organs. We believe that the proton is the most promising nucleus for cancer detection by MRS.

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Role of Nuclear Magnetic Resonance Spectroscopy (MRS) in Cancer Diagnosis and Treatment: $^{31}$P, $^{23}$Na, and $^1$H MRS Studies of Three Models of Pancreatic Cancer

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