Review

Other risk factors for pancreatic cancer: Hormonal aspects

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Summary

Exocrine pancreatic cancer is significantly more common in younger men than in younger women. The male-to-female sex ratio is, in most countries, between 1.25 and 1.75 to 1, but decreases with increasing age. Moreover, prior oophor-ectomy appeared in one study to be significantly more common in women with pancreatic cancer than in controls. This has raised interest in sex hormones in the development in pancreatic cancer. It has been questioned if there are estrogen receptors in ductal pancreatic cancer, but there are no doubt estrogen receptors and estrogen-binding protein in human healthy pancreas. It is also well proven that it is possible to influence experimental pancreatic cancer with estrogens. However, in clinical studies tamoxifen has repeatedly been shown to be without significant effects.

On the other hand, there are also androgen receptors in pancreatic cancer and testosterone has been shown to strongly promote growth in experimental pancreatic cancers. It is therefore of considerable interest that an androgen receptor was recently shown to significantly prolong life in patients with unresectable pancreatic carcinoma. However, in patients with advanced pancreatic carcinoma the serum testosterone is low, far lower than what could be expected due to weight-loss and malnourishment alone.

Pancreatic cancer has etiologically been connected to diet, for example the intake of fat. Cholecystokinin receptors have been found on human pancreatic cancer, possible to stimulate in vitro by cholecystokinin (CCK). Studies with CCK-receptor binding, hybridization with radiolabeled complementary DNA (cDNA) probes, or reverse-transcription polymerase chain reaction, have shown that CCK-A receptors also are present in rat pancreatic putative preneoplastic lesions and cancer tissue, rat pancreatic-cancer cell lines, pancreatic carcinomas in transgenic mice, hamster pancreatic cancer, and human pancreatic cancer cell lines and tumors. Also, CCK-B receptors have been found in some human pancreatic cancers. There are a vast number of experiments done on CCK-stimulation of pancreatic cancer. They indicate that CCK may have a promoting effect on exocrine pancreatic cancer, but it is not probable that hyperstimulation with CCK alone induce pancreatic cancer.

At present, however, despite a lot of evidence for a hormone-dependence of pancreatic cancer there are no data confirming a role for estrogens, androgens, CCK or their antagonists in clinical treatment of exocrine pancreatic cancer.

Key words: cholecystokinin, estrogen, hormones, testosterone, pancreatic cancer

Introduction

Substantial progress has been made towards a better understanding of pancreatic carcinoma due to not only clinical, but also basic, research. Molecular biology has made the carcinogenic process partly possible to understand. However, also the interaction between the cancer cells and their neighbours may be fruitful to study. It is long known that there is an exocrine-endocrine axis in pancreatic physiology, but its seems possible that also other hormones may be of importance and their action should be further explored.

The pancreas is not primarily regarded as a target organ for sex-hormones, but a number of observations have suggested a reassessment of this opinion. There are encouraging results from small series of patients with unresectable pancreatic carcinoma treated with low doses of octreotide and tamoxifen [1], but also for example combinations of aminoglutethimide and cytostatics [2]. Moreover a role for sex-hormones in pancreatic cancer development is suggested by epidemiological data. Exocrine pancreatic cancer is significantly more common in younger men than in younger women. The male to female sex ratio is in most countries between 1.25 and 1.75 to 1.00, but decreases with increasing age. In Sweden it is 1.75:1.00 below the age of 50 years but there is no difference beyond the age of 70 years [3-5].

The gut hormones cholecystokinin (CCK) and gastrin are phylogenetically and structurally related. Under normal conditions, postprandial CCK stimulates the secretion of the exocrine pancreas. Longterm endogenously induced hypercholecystokininemia in rats induces not only hypertrophy of the exocrine pancreas, but causes also pancreatic hyperplastic nodule formation [6]. Gastrin has been reported to stimulate growth of several human pancreatic cancer cell lines [7] and an increased incidence of pancreatic neoplasias has been found in patients with pernicious anemia, who are known to have raised levels of gastrin in serum [8]. However, these hormones will no further be discussed here.

It has been put forward that hormonal treatment may be of value also in pancreatic cancer [9-11]. Even if the benefit were small such an approach would be of significance given the overall prevalence of the disease and the relatively poor results achieved with current treatment. The sex-hormones and peptides of the cholecystokinin- and the gastrin family are hitherto the most studied hormones in pancreatic cancer research.
Influence of estrogens on the exocrine pancreas

More than 30 years ago Ullberg and Bengtsson [12] reported increased accumulation of radiolabeled estrogen in the exocrine pancreas. Labeled estradiol or estriol has also been found to be selectively retained in the pancreas of male dogs, rats and baboons in addition to other organs including the kidney, prostate, testis, muscle and thyroid [13, 14]. Such selective concentration in the pancreas was unaffected by testosterone or cortisol. Using autoradiography Sandberg and Rosenthal [15] confirmed that the estrogen was predominantly localized in the cytoplasm of the acinar cells. Moreover, normal human pancreatic tissue has been shown to be capable of converting both estrone and estrone sulphate into the biologically active estrogen 17-beta-estradiol at a rate comparable with that of established estrogen target tissue, such as breast cancer [16].

Boctor and coworkers reported [17] that the accumulation of zymogen granules, which occurred after adrenalectomy in male rats, was reversed by giving 17-beta-estradiol. A putative receptor for 17-beta-estradiol, distinct from the uterin receptor, was also identified. Evidence that this receptor was restricted to the exocrine pancreas was based on experiments using streptozotocine which selectively destroys islet cells but does not affect the estrogen receptors [16].

The binding of steroid hormone to pancreatic acinar tissue requires the presence of a co-ligand [18], one possible candidate being somatostatin [19]. This hormone, however, also inhibits the translocation of proteins into the endoplasmatic reticulum which might explain how somatostatin reduces exocrine secretion.

Estrogen appears to be necessary for the synthesis of pancreatic digestive enzymes. Following adrenalectomy in male rats and adrenalectomy and oophorectomy in female rats, there is a marked depletion of zymogen granules in the acinar cells. Treatment with either triamcinolone or estradiol reverses this effect, whilst there is no influence by testosterone [20]. Moreover, estrogen administration results in decreased secretion of zinc and bicarbonate in pancreatic juice, whereas amylase and lipase are increased. In pancreatic tissue triglycerides and total lipids are increased by estrogens [21, 22].

In contrast to the effects on pancreatic enzyme synthesis, estrogens appear to have an inhibitory effect on pancreatic growth. Lhoste et al [23] showed that the pancreatic weight of normal male rats was 35 per cent higher than that of normal females, even when corrected for body weight. Guo and Singh [24] demonstrated that chronic treatment of guinea-pigs with estradiol resulted in a significant reduction in pancreatic weight, and an amylase content comparable with that in castrated control animals, without an associated change in body weight. The pancreatic weight loss with estradiol treatment was primarily due to reduced pancreatic growth in terms of cell numbers, as the DNA content per unit pancreatic weight was identical between treated and control groups.

In contradistinction to estrogens, some other male and female sex-hormones appear to have a trophic effect on the pancreas. Orchidecotomy in male rats was found to decrease pancreatic weight, which was reversed by testosterone administration [23]. Beaudoin and co-workers [25] reported that dexamethasone replacement therapy in castrated and adrenalectomized rats partially restored the level of pancreatic amylase, whilst estradiol had no effect on the amylase content and was associated with the presence of unusual "halo" granules. While some have shown that glucocorticoids produce a significant increase in pancreatic weight, total DNA, RNA and protein content in the rat [26, 27], others have shown that corticosteroids produce a decrease in pancreatic weight, protein, DNA content [20, 28] and cell proliferation [28, 29], in the same species.

Of certain interest is that Guo and Singh [24] have shown increased release of amylase by cholecystokinin (CCK) in oophorectomized guinea-pigs treated with estradiol. Although the total amount of amylase released was less in the latter due to a reduction in the amount of acinar tissue, the proportional release of amylase was significantly greater. This appeared to be due to specific up-regulation of the pancreatic CCK receptors as the receptor density for vasoactive intestinal polypeptide (VIP) was unaltered. That the estrogenic effect on the CCK receptor was organ specific was suggested by the finding of normal receptor density for both CCK and VIP in the gallbladder.

The mechanism by which estradiol mediates its multiple effects on pancreatic growth, enzyme production and release remains uncertain. At least some of these effects, however, may be due to a direct interaction of estradiol with estradiol-binding protein observed in the rat pancreas [15, 17, 36].

Steroid receptors

Steroid receptors, including the receptor for estradiol, are located in the nucleus. Estrogen receptors (ER) belong to a large family of hormone-dependent DNA-binding proteins with a "zinc-finger" motif, a family including receptors for other steroids, thyroid hormone, retinoic acid, vitamin D3, and some "orphan receptors" the ligands of which have yet to be identified [31]. The hormones, at least estradiol, cross the cell membrane probably by diffusion, are bound to the receptor and the complex can then bind to specific sites on the chromosome.

The estrogen receptor gene consists of more than 140 kilobases of genomic DNA divided into eight translated exons. The interaction of estradiol with the steroid-binding domain of the receptor leads to a strong binding of the receptor to the estrogen-response elements of target genes. This binding is brought about by the zinc fingers of the receptor resulting in a range of transcription events to produce several types of mRNA. The resulting translations induce the production of progesterone receptor and a variety of other proteins and peptides [32]. These estrogen-regulated agents include c-fos, classical growth factors such as transforming growth factor (TGF) alpha, insulin-like growth factors (IGF) I and II, acid and basic fibroblast growth factors (FGF) and epidermal growth factor-like protein, which when secreted can activate membrane receptors on the same cells. Also influenced by the estrogen level are proteases such as plasminogen activator and precursors of...
cathepsins, which stimulate cell proliferation as well as enhance invasiveness by breaking down the extracellular matrix. The extent to which these growth factors are involved in pancreatic cancer is currently debated [33].

Currently much research worldwide focuses on the steroid receptor homologies similar to those of the viral oncogene v-erb A and its cellular equivalent c-erb A. This has inevitably raised questions as to the oncogenic potential of steroid receptors, particular as c-erb A has been found to be a thyroid hormone receptor [34]. Since the DNA-binding domains of this receptor family are similar there is the possibility of interaction (either competitive or synergistic) between their respective ligands at the DNA level [31]. It might be added that two different types of ERs have been defined, and that it has been proposed that ER type II is of special interest for estrogen induced proliferation [35, 36].

There is increasing attention on the effect of steroid hormones on the structure and function of the exocrine pancreas [20, 25, 37]. There are sex-steroid biosynthetic enzymes in pancreatic tissue [13, 25] and the demonstration of testosterone biotransformation by the isolated perfused pancreas [38] strongly suggests the participation of androgens in the pancreatic function. Specific sex-steroid binding sites have been demonstrated in the pancreas of dogs [14, 39], rats [15, 17, 40, 41], hamsters [30], and humans [42, 43]. Various effects of other steroid hormones on the exocrine pancreatic functions have also been reported [14, 24, 26, 44-47].

**Influence of estrogens on experimental pancreatic cancer**

Strong suggestions for a role for estrogen in promoting pancreatic cancer come from studies both in vivo and in vitro. Lacaine et al [48] reported that the in vivo growth of a grafted hamster ductular pancreatic cancer cell line which binds 17-beta-estradiol and dihydrotestosterone was significantly reduced in estrogen-treated animals. It has also been shown that estradiol treatment is highly effective in a dose dependent manner in inhibiting the development and growth of preneoplastic pancreatic lesions in rats treated with azaserine, which induces acinar, but not ductular, pancreatic cancer [49].

The possible hormone sensitivity of the pancreas is further indicated by the presence of several sex-steroid biosynthetic enzymes in normal and malignant tissue. Estrone sulphate, which is quantitatively the most important estrogen in non-pregnant individuals, is converted into the biologically active 17-beta-estradiol by human pancreatic homogenates in vitro. Also, the enzymes 5-alpha-reductase (which converts testosterone to the more potent androgen 5-dihydrotestosterone) and aromatase (which converts delta-4-androstenedione to estrone and testosterone to estradiol) have been found in human pancreatic cancer tissue [50].

In a series of in vitro experiments, Benz and co-workers [51] compared the steroid responsiveness of four human and one rodent pancreatic tumor cell line with an estrogen receptor-positive breast cancer cell line. They found that all four human pancreatic tumor cell lines contained measurable levels of specific estradiol binding sites with a $K_d$ that ranged from 1 to 9 nM, which contrasted with the higher affinity found in the breast cancer cell lines, with a $K_d < 1$ nM. Growth of one of the pancreatic tumor cell lines was stimulated by about 40 per cent following exposure to nanomolar concentrations of estradiol. Moreover, both glucocorticoids and androgen also stimulated the proliferation of the pancreatic tumor cell lines by as much as 30 per cent. The growth of the other pancreatic tumor lines was inhibited by varying degrees following exposure to micromolar concentrations of estrogen, anti-estrogen, anti-androgen, progesterone and inhibitors of steroid-metabolizing enzymes but with little relationship to the estrogen receptor content of the tumors. In general, progesterone was slightly more growth inhibiting to these pancreatic tumor lines than the other endocrine agents, including tamoxifen. An inhibitor of 5-alpha-reductase with minimal affinity for androgen receptors inhibited the growth of the pancreatic tumor cell lines to less than 40 per cent of controls, whereas a potent androgen receptor antagonist with no direct influence on 5-alpha-reductase inhibited growth of two cell lines but not the others. This suggests that the steroid-dependent growth-inhibitory mechanisms of some pancreatic cancers might involve both receptor antagonism and direct inhibition of steroidal oxidoreductases.

In 1982 Benz et al [52] using the estrogen receptor expressing human pancreatic cancer cell line COLO-357 found that the binding of estradiol to the receptor could be inhibited with diethylstilbestrol. When cytotoxic concentrations of 5-fluorouracil were combined with estradiol, progesterone or tamoxifen, there was a five-fold increase in cytotoxicity. On the other hand randomized addition of aminogluthethimide, an inhibitor of the aromatase enzyme, did not add to the survival of patients with advanced pancreatic carcinoma.

**Tamoxifen and pancreatic cancer**

In 1982 Wilking et al [53] described the distribution of radioactively labeled tamoxifen - considered to be a competitive inhibitor of estrogens on the ERs - given intravenously to oophorectomized mice. Rather low amounts of uptake were found in normal estrogen target tissues, such as breast and uterus, but high concentrations of tamoxifen were detected in the lungs, adrenals and pancreas. This observation coupled with the in vitro effects of tamoxifen in pancreatic cancer cells might suggest a benefit for tamoxifen in vivo. However, tamoxifen had no effect on the growth of human pancreatic cancer xenographs in nude mice [54]. In that study there was neither any effect of the synthetic non-steroidal estrogen stilboestrol. An analogous situation to breast cancer may pertain in which hormone manipulation with anti-estrogens is more likely to be effective if the pancreatic cancer expresses estrogen receptor. Thus, in a recent study of xenografted human pancreatic cancer cell lines in nude mice, growth was significantly reduced by somatostatin given alone or as a combined regimen with tamoxifen [55]. Also the DNA, RNA and protein content in the tumors was reduced. In one of the cell lines tamoxifen was effective. These data were supported by a non-
randomized human series of twelve patients treated with a low dose of a synthetic somatostatin, octreotide, combined with tamoxifen which showed a longer survival than expected, 12 months [1].

A few phase II clinical trials of tamoxifen treatment in patients with advanced pancreatic cancer have been published. Theve et al [56] found in a small study a median survival of nine months in patients with unresectable and histologically proven adenocarcinoma given tamoxifen 20 mg a day. In three of the men the survival extended to 22 months. The overall survival was significantly better as compared to a historical control group surviving in median only three months. Tønnesen and Kamp-Jensen [57] similarly reported a median survival of seven months in patients treated with tamoxifen 10 mg daily. However, Crowson et al [58] failed to demonstrate any effect by tamoxifen. Based on serial computerized tomography studies the mean interval for disease progression was three months (range 1-8 months) in this study. The loading dose of 160 mg tamoxifen was given followed by 40 mg once daily. Only one of the patients survived more than six months.

Wong et al [59] treated 24 pancreatic cancer patients with tamoxifen 20 mg daily with a median survival of seven months. In men the median survival was four months and in women (all of whom were postmenopausal) ten months. Six of these women survived for more than one year and three survived for more than two years. There are also some phase III studies of tamoxifen treatment in pancreatic cancer. In the prospective controlled trial reported by Keating et al [60], 108 patients with pancreatic adenocarcinoma were randomly allocated to receive daily tamoxifen 20 mg, cyproterone acetate 100 mg or no active treatment. The median survival of those receiving tamoxifen was five months compared with four months for the cyproterone acetate group. There was no survival benefit for either of these groups compared to the control group which had a median survival of three months. A regression analysis of twelve clinical and biochemical features showed, not surprisingly, that for the entire group of patients survival was significantly longer in younger patients, those undergoing surgical bypass and those with better performance status. Adjustment for the distribution of these prognostic variables confirmed the lack of benefit of different treatments.

In a Norwegian multicenter study of 173 patients with pancreatic cancer and cancer of the papilla of Vater a randomization was done between 30 mg tamoxifen daily or placebo [61]. Treatment was continued until death or ten months after accrual into the trial. The median survival in the tamoxifen group was 115 days compared with 122 days in the placebo group. Although there was no difference in survival between men and women, a retrospective analysis of women with stage III cancer showed that the median survival was 195 days in the treatment group compared to 45 days in the control group, which is a significant difference. Three women (7 per cent) were still alive after 2.5 years in the treatment group and none in the control group; no men survived after this time. The findings in the Norwegian study in women with stage III cancer might be conceived as supporting the view of Wong et al [59] that tamoxifen is beneficial in postmenopausal women. The final answer to the question of whether tamoxifen is of benefit in postmenopausal women with pancreatic cancer require further phase III trials.

References

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