Clinics in Oncology

Research Article
Published: 09 Sep, 2016

Pancreatic Alteration Induced by Incretins is Consistent with the Changes at the Early Stages of Pancreatic Carcinogenesis in the Hamster Model

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Abstract

Background: GLP-1 analogs and DDP4 inhibitors, known as incretins, are used for the treatment of type 2 diabetes. Although several published clinical and experimental studies point to the beneficial effects of these drugs with negligible mild side effects, except for acute pancreatitis, the detailed long-term effects of these drugs on the pancreas was missing. A recent report by the investigators at UCLA showing a profound expansion of pancreatic endocrine cells, hyperplasia of ductal epithelium and induction of endocrine lesions initiated serious concern about the safety of these drugs.

Methods: These alterations were almost identical to those found at the early stages of pancreatic carcinogenesis in the hamster model. Therefore, in the present study, we compared the alterations published by the UCLA investigators with the alterations of the pancreas occurring during pancreatic carcinogenesis in the hamster model. Results and Discussion: The results heighten the safety concern of these drugs and suggest that in cretins should be considered promoters of silent malignant pancreatic lesions. The use of these drugs should be restricted to genuine long-standing diabetics and be withheld from individuals with new-onset diabetes (Type 3 diabetes) who exhibit asymptomatic pancreatic cancer.

Keywords: Glp-1; Pancreatic cancer; Islet

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Received Date: *01 Aug 2016*
Accepted Date: *17 Aug 2016*
Published Date: *09 Sep 2016*

Citation:
Pour PM, Talmon G. Pancreatic Alteration Induced by Incretins is Consistent with the Changes at the Early Stages of Pancreatic Carcinogenesis in the Hamster Model. Clin Oncol. 2016; 1: 1085.

Introduction

Glucagon-like peptide 1 based therapies [GLP-1 receptor agonists (exenatide, liraglutide and lixisenatide) and DPP-4 inhibitors (sitagliptin, vildagliptin, saxagliptin and linagliptin)] have been available since 2005 for the treatment of Type 2 diabetes.

The efficacy of GLP-1 receptor agonists and DPP-4 inhibitors has been demonstrated. In terms of safety, the most common adverse events seen in clinical trials with GLP-1 receptor agonists are of gastrointestinal character, mainly nausea, vomiting and diarrhea. However, the incidence diminishes over time. Other identified risks include pancreatitis, immunogenicity, acute renal failure and rapid weight loss. Identified and potential risks with DPP-4 inhibitors include hypoglycemia, hypersensitivity, gastrointestinal disorders, pancreatitis, skin disorders, transaminase elevation and infections.

Although the manufacturers of the drugs have recently incorporated acute pancreatitis as a serious side effect of the GLP-1 drugs, two fundamental concerns have been disregarded, including:

1. The long-term effects of the drugs on the structure of the pancreas and its consequences; and
2. The physiological and pathological effects of the drug and their duration after their cessation.

The first concern was highlighted by a study of Butler et al. [1].Using donor pancreases of Type 2 diabetics treated with in cretins, they demonstrated massive and extended proliferation of pancreatic islet cells. Remarkably, the patterns of the morphological changes in their publication were almost identical to the early lesions induced in the hamster pancreatic cancer model. The present report compares the pancreatic lesions published by Butler et al. [1] with those found during pancreatic carcinogenesis in the Syrian hamster model [2].

Material and Methods

Histological and immunohistochemical material from published article are presented [1].

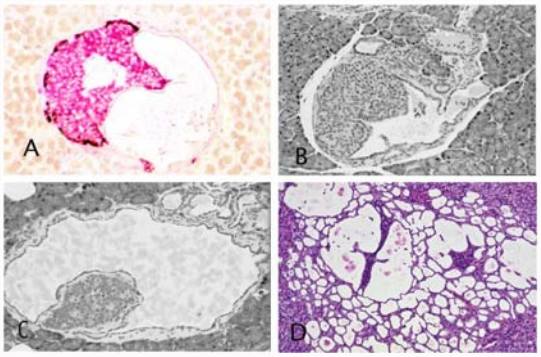

Figure 1: Intrainsular ductular patterns. A and B: Is lets of Syrian hamster treated once with pancreatic carcinogen, N-nitrosobis (2-oxopropyl) amine show cystic ducts (d)occupying almost half the islet(I) and are filled with floccus material. Insulin, red and glucagon, brown. ABC method x 40. C and D: Atrophic islets (I) attached to the wall of the cystic ducts directly (C) or by a thin connective tissue (D). There are also numerous peri-insular ductular structures. H&E x 40 (C), x20 (D). A,B and D are almost identical to Figure 2A published by Butler et al. [1].

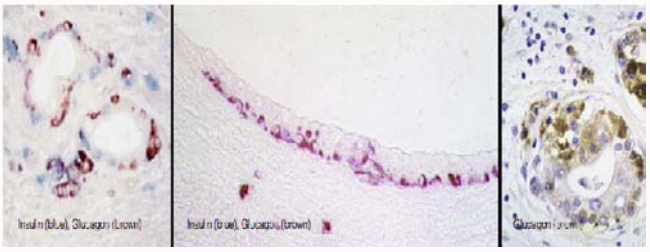

Figure 2: Large population of endocrine cells in malignant pancreatic adenocarcinoma in humans (A and C) and hamsters (B). A: Insulin (*blue*), glucagon (*brown*), somatostatin (*black*), ABC method, x40; B: insulin (*red*), glucagon (*brown*), ABC, x40; C: insulin (brown), ABC, x 40. These figures are strikingly similar to the figures C,D and E, respectively, published by Butler et al. [1].

In cretin Mimetics Products Liability Litigation is still on-going, therefore, we were unable to obtain permission for the reproduction of the photomicrograph published by Butler et al. The readers can have access to the referenced figures by using the online version of that publication with the following URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3712065/

The presented figures from the hamster study (1974-2010) were obtained from the Tumor Archive at the UNMC eppley Cancer Center.

Results

As described earlier in detail in the hamster model [2, Chapter 12], the earliest alteration in carcinogen-treated animals is the development of ductular structure within (intrainsular ducts) and around the islets (peninsular duct). The initially tiny intrainsular ductules gradually enlarge. Due to serous and mucinous secretion by ductular cells, the ductules undergo cystic distention and the increasing pressure of liquid within the intrainsular ductules causes atrophy of the islet cells that appear as a small ortiny cell aggregate attached to the cystic wall (Figure 1 and 2).In animals exposed to a high dose of carcinogen the intra-and peri-insular ductules undergo gradual hyperplasia, dysplasia and culminate in the malignant gland that finally destroys the islets and invades the surrounding tissue [2, pp 92-94]. In hamsters exposed to a single injection or repeated low doses of the carcinogen, the earliest and the solitary alteration is

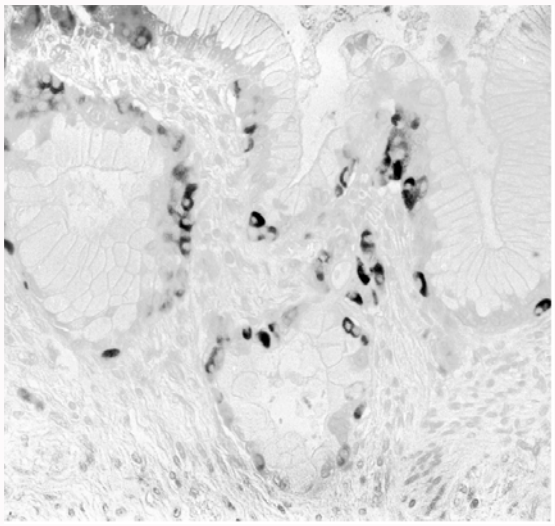

Figure 3: Human pancreatic mucinous adenocarcinoma with numerous endocrine cells (black) in the basal epithelial layer. Identical lesions were illustrated as figure 7A and 7C by Butler et al. [1] in diabetic patients treated with incretin. ABC method, x 40.

the formation of intrainsular ductules that either remain stationary during the animal's lifespan or slowly and gradually progress to neoplastic lesions. Figure 2A in the publication of Butler et al.

[http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3712065/] presents an identical pattern. In addition to the scattered islets of various sizes in the figure, there is one large islet in the lower middle field that contains a cystic duct and, in the upper middle field, there is another cystic duct with an atrophic islet inside. The remaining three cystic ducts appear to have lost the insular mass but still contain islet cells within their epithelium.

Another striking feature in the induced lesions in the hamster model, as well as in human pancreatic cancer, is the presence of endocrine cells within the hyperplastic ducts, the number of which may seem to exceed the number of the ductal cells (Figure 3). Identical patterns have been observed in the study of Butler et al. [1] shown in Figure 2C,D, and E. Mucinous ductal cell hyperplasia bearing endocrine cells at the base of the epithelium, a characteristic finding in human pancreatic cancer and in the hamster model has also been demonstrated in Figure 7A and C of the publication(www.ncbi.nlm.nih.gov/pmc/articles/PMC3712065/).

Discussion

The present comparative study provides evidence that GLP-1 treatment in type 2 diabetics causes profound alterations of the pancreas, consistent with early lesions occurring during pancreatic carcinogenesis in hamsters.

The origin of pancreatic cancer has remained controversial. Genetic studies have suggested that under diverse experimental conditions different pancreatic epithelial cells, including pre-existing acinar cells, pre-existing β-cells, pancreatic ductal cells, and cells expressing the mesenchymal marker nestin, may undergo malignant transformation. [3-8]. The development of the hamster pancreatic cancer model, that in clinical, morphological, biological and genetic aspects mimics the human disease [9], to the surprise of many investigators, showed that islet cells play a major role in pancreatic cancer development [2]. The initial lesion in hamsters is

Table 1: In 9 out of 15 cases pancreatic cancer has been occurred to occur within three months. In Saxagliptin group the time was 4-18 months. In others no definite time lapse was recorded.

Drug	Number of cases
Exenatide	2
Liraglutide	1
Lixisenatide	3
Sitagliptin	2
Saxagliptin	8
Vildagliptin	3
Linagliptin	A few?

the appearance of ductular structures within the islets in the form of tiny conduits that gradually increase in size and undergo cystic or malignant changes that totally destroy the islets. The alterations of large ducts occur later [2, pp92-94]. The occurrence of intrainsular ductules was thought to be related to the transdifferentiation of islet cells to ductular cells, a process that was confirmed by several studies [10-15]. The purified human and hamster pancreatic islets in culture were shown to form ductal, acinar and intermediary cells (cells with both exocrine and endocrine phenotypes) [10-15]. Hamster islet cells treated in vitro with pancreatic carcinogen, N-nitrosobis (2-oxopropyl) amine formed ductal adenocarcinoma *in vivo* [11]. Based on the silent clinical course of pancreatic cancer and difficulties in dissecting the entire pancreas in serial sectioning, the possibility that human pancreatic cancer also initiates from the islets remained obscure.

The fundamental role of islet cells in pancreatic carcinogenesis was evident by the observation that any procedure that stimulated islet cell replication, such as induction of nesidioblastosis, feeding a diet high in fat, which enlarges the islets, enhanced the cancer formation [2, Chapter 17].Although the increased incidences of pancreatic cancer in obese people, who have enlarged pancreatic cells [16-19], appear to be in line with the findings in hamsters, the link was bleak. The study by Butler et al. offered the missing link [1].

Some academic and industrial researchers [20,21] criticized the paper of Butler et al. [1]. Although some of their points from toxicological standards were reasonable, considering the parameters that they have recommended, thousands or even more brain-dead organ donors would have been required to obtain statistically significant data. These authors did not propose how to obtain the adequate number of treated and untreated sex-, age- and BMI-matched donor pancreases receiving the same agent (of more than 20 that are currently in use) and disregarded the simple fact that the described unique alterations have never been reported in the pancreas of any normal or diabetic persons treated or untreated with various agents. Yet, the lesions were found only in the eight treated patients but in none of the 14 controls [1].

The Committee for Medicinal Products for Human Use (CHMP) reviewed the publication by an ad-hoc expert meeting held on July 10, 2013 and concluded that the results of the study by Butler et al. [1] are not considered to constitute a new safety signal for the GLP-1 based therapies with respect to pancreatic safety. It was argued that, due to the mechanism of action, there are still some uncertainties with respect to the long-term pancreatic safety associated with these products and updates to the risk management plans (including planned and ongoing studies) and that harmonization of warnings in the product information should be taken forward.

According to the assessment report for GLP-1 based therapies 25July 2013 EMA/474117/2013, in clinical trials, a few cases have been reported for some products (Table 1). Although the data currently available from clinical trials do not indicate an increased risk for pancreatic cancer with these medicines, cases of pancreatic cancer have been reported in the post-marketing setting. A cumulative review of the cases has been undertaken and the majority (19 out of 29) had a time to onset of less than six months, a period considered too short to suggest a causal relationship.

The role of GLP-1 analogs in pancreatic cancer varies widely between the studies and based on the findings, multi-district litigation (MDL) were established. Some trials, including SAVOR-TIMI (of saxagliptin) and EXAMINE (of alogliptin), found no difference between dipeptidyl peptidase 4 (DPP-4) inhibitor treatment and placebo with regard to pancreatitis or pancreatic cancer [22]. In a recent study, the risk was actually elevated for insulin. The pancreatic cancer rate was 9.39% vs. 2.61% for population controls, with an adjusted odds ratio of 3:6, suggesting that these drugs are strongly associated with pancreatic cancer [22].

Although certain classes of nitrosamines act as pancreatic carcinogenin hamsters [2, Chapter 7], the over production of insulin seems to be the underlying factor in humans. Insulin is known as a strong mitogenic factor and accelerates cell growth [23,24]. In diabetics, where the insulin-producing beta cells have lost the ability to produce insulin, GLP-1-enforced beta cell neogenesis and insulin synthesis could well lead to the formation of immature beta cells or to transdifferentiation of emerging beta cells to ductular cells associated with errors in DNA synthesis and genetic mutations. The ability of human islet cells to readily transdifferentiate into ductal (and acinar) cells has been covered previously.

The fate of the lesions described [1] in the patients cannot be predicted. Will it remain stationary, regress or progress to frank malignancy? In hamsters, the described alterations occur following low doses of pancreatic carcinogens and remain mainly stationary. In some instances, the alterations advance to hyperplastic change, indicating that the lesions virtually present the early stages of cancer in these patients. It is possible that the advanced lesions have escaped their detection. Although the observed morphological alterations in the patients do not provide definite signs of malignancy and, regretfully, the genetic alteration of the lesions was not performed, the findings remain a controversial issue. In diabetics the amount of locally secreted insulin and IGF-1 may not be sufficient to stimulate the growth of the altered cells, but the GLP-1-induced insulin certainly can affect the latent premalignant and small cancers, which are reported to occur in up to 36% of individuals over 50years old [25-37], and in a 9.5% incidence of “silent” pancreatic cancer” in smokers [31]. These “silent” cancers are vulnerable to rapid growth in any condition leading to increased insulin and IGF-1 production. Support for this view is the increased pancreatic weight and ductal/ductular hyperplasia in incretin-treated animals and patients [1]. The reported and registered short latency of pancreatic cancer in clinical trials, mentioned previously is in-line with this likelihood.

The problem with this assessment is the lack of data from the pancreas of incretin-treated diabetics, the same as Butler and associates. The ideal study to answer the question definitively would involve large-scale pathohistological examination, databases from

multiple countries, providing enough data on new users as well as prevalent users in order to eliminate bias from the duration of use. Ideally, such studies would also last longer than any performed thus far. Most cancer epidemiologists would like to see a drug exposure of at least 8 to 10 years before they consider it usable.

Hence, the presented data suggest that incretins could act either as promoters or the initiator of pancreatic cancer and their use should be restricted to genuine long-standing diabetics and be withheld from individuals with new-onset diabetes (Type 3 diabetes) who present with asymptomatic pancreatic cancer [38-43].

Given the >20 million known patients with type 2 diabetes in the United States alone, and the numerous GLP-1–based drugs either available now or in the final stages of development, the potential impact of the adverse effects of this class of drugs is considerable.

In summary, pushing the organism to perform a desirable function (to produce insulin) that the body, for its own justified reasons, does not want to do or is unable to do, can lead to unexpected, unwanted and sometimes disastrous results.

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