UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

Doctoral School

PhD Thesis

(abstract)

Minimally Invasive Endoscopic Ultrasound as Palliative Therapy of Pancreatic Cancer

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Table of Contents

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STATE OF KNOWLEDGE

1. Pancreatic cancer

Pancreatic cancer is one of the most aggressive and lethal cancerous related disease with a survival rate of approximately 5% after 5 years. The American Cancer Society estimated that in 2015, around 48.960 new pancreatic cancer cases with an almost equal prevalence between women and men would be diagnosed. Regarding the mortality, it has increased considerably from 5 cases per 100.000 people in 1930 to over 10 cases per 100.000 people in 2003. In 2014 it was estimated that 39.590 people suffering from pancreatic cancer had died in USA. Romania is situated on the 15^{th} place globally when it comes to the standardised age of diagnosing this disease with an incidence of 7.9 cases per 100.000 people.

Surgical resection represents the elected treatment for patients with suspicious pancreatic tumours. Only 5-20% of the patients with pancreatic cancer present tumours that can be surgically removed at the moment of diagnosis. The resection percentage with the intention of curing is growing towards 33%, in the case of more aggressive interventions or of better preoperatory resectabilitty evaluation.

2. Endoscopic ultrasound pancreatic tumour assessment

The efficiency of the evaluation of the pancreas and the biliary ducts, through trans-abdominal ultrasound, is much lower than that of endoscopic ultrasound. The distance that separates the transductor from the targeted structures is reduced to a few millimetres, and the results are not affected by the fatty tissue surrounding the stomach or air. In the case of pancreatic affections, particularly pancreatic tumours (solid or cysts), there is no doubt regarding the strong rivalry between conventional EUS and computer tomography (CT) or magnetic resonance colangiopancreatography (MRCP). However, the development of EUS fine needle aspiration needle (EUS-FNA) towards the late 90', and the jump to therapeutic EUS in the 2000', have turned endoscopy into a key instrument in the diagnosis and therapeutic management of pancreatic diseases.

3. Radiofrequency ablation

The purpose of radiofrequency ablation (RFA) is to produce a thermic lesion in a tissue by using electromagnetic energy. The tissue alteration determined by heat, is dependant both temperature level and its exposure. Heating a tissue at a temperature between 50 and 55 degrees

Celsius from 4 to 6 minutes, will cause irreversible cellular lesions. The pancreas is a very thermo-sensitive tissue and the thermal ablation of a normal pancreas usually leads to an inflammatory response with subsequent oedema, fibrotic and sometimes cystic transformation. RFA can be performed laparoscopically, percutaneously or by using EUS-guidance.

4. Nanotechnology in Oncology

The progress in the field of nanotechnology has permitted the appearance of a wide range of possibilities in various fields, from data storage to biomedicine. Thanks to the attractive properties of magnetic nanoparticles (MNP), these have become the subject of numerous research studies, making possible the continuous development of this field.

By far, the most used MNPs are ferrites nanoparticles or iron oxide nanoparticles (IONs), which with their superparamagnetic properties, offer numerous possibilities in drug and gene delivery, diagnostics and therapeutics.. A large portion of their potential is oriented towards cancer diagnosis and targeted therapy of tumors. Mainly, three different aproaches can be used in the treatment of tumours with MNP: magnetic induced hiperthermia, targeted drug transport and selective supression of tumour growth.

5. Palliative therapy of pancreatic cancer

Because of the marked biological aggressiveness of pancreatic tumours, the curative results or long term remissions are very unlikely. In most of the patients, defining the therapeutic strategies implies adding new palliative treatment options, with the purpose of relieving the patients of unpleasant manifestations and to improve the life quality. Therefore, the large number of patients that can only benefit from palliative treatment represents an important area of interest for the study of mortality, morbidity and success rates for each palliative method. The relation between the possible results and the mortality of the procedure holds an important role in the establishment of the indications for implementing a palliative treatment.

The decision of going for a palliative treatment can be made in two different moments in the evolution of the disease. The first one is after the staging, making a selection of patients who can benefit from curative surgery, palliative surgery or palliative endoscopic methods. The second moment for selection appears during the surgical exploration when a decision can be made regarding curative resection, palliative resection or other surgical option. Therefore, maintaining a high accuracy in initial staging remains crucial, in the purpose of selecting the possibilities of curative or palliative therapies. In the case of pancreatic cancer, the most

important symptoms that can benefit from palliative treatment are jaundice, duodenal obstruction and pain.

Personal Contributions

The studies targeted the development of new therapeutic options for treating pancreatic cancer or its consequences, either local incision, metastasis or duodenal obstruction. The novelty is that all the procedures were performed under endoscopic ultrasound guidance as feasibility studies on experimental animals – pigs.

1. Pancreatic Radiofrequency Ablation on an experimental pig model

Objective - to assess the feasibility and safety of EUS-guided RFA of the pancreas using an experimental survival porcine model. The secondary aim included the pathological assessment (size, shape, location, etc.) of the EUS-guided RFA ablation zone, based on standardized settings of the RFA procedure.

Materials and Methods. The RFA procedure was performed on ten pigs using a radiofrequency generator and the transduodenal approach was performed using a linear endoscopic ultrasound scope with a large interventional channel, coupled with the corresponding Evis Exera system and an AlokaProSound5500 US system. An experimental RFA probe, with a working length of 200 cm was inserted through a 19G EUS-fine-needle aspiration. The head of the pancreas underwent four sessions of consecutive ablations with 4-6 mm of the catheter exposed at 5 W, 10 W, 15 W, and 20 W for 120 s each. Recovery from anesthesia was carried out either spontaneously or by antagonizing the analgesic, the neuromuscular blocker, and/or the benzodiazepines. Biological samples were harvested before the procedure as well as 3 days and 5 days after intervention. One week later, the pigs were euthanized and necropsy was immediately performed.

Results. All the pigs showed no significant changes regarding their behavior and no signs of complications were encountered. There were no problems in identifying the pancreas of the pigs using EUS even though its position when compared to humans was a bit higher on the posterior wall of the stomach. A hyperechogenic, elliptic lesion appeared surrounding the inserted RFA probe, with a median diameter of 2.65 cm and an interquartile range (IQR) of 0.5 cm. No immediate complications were noted. Blood analysis revealed increased values of amylase, AP, and GGT on the 3rd day. However, their levels started to decrease within the 5th day after the procedure. No variations were found in liver transaminases or bilirubin. When slicing the pancreas, on the part located near the gastric wall, a lesion (2 × 3 cm in size), with a whitish peripheral rim of 15 to 20 mm, of pseudocystic structure with liquid inside was revealed.

Conclusions. EUS-RFA might represent a promising therapy for the future treatment of pancreatic cancer, because of its feasibility and low level of complications during and post procedures.

2. Endoscopic ultrasound magnetic nanoparticle injection - a new approach for treating pancreatic and hepatic tumors

Objective. EUS-guided MNP injection through various methods as a potential vector for angiogenic therapy in pancreatic cancer and hepatic metastases. The novelty was the EUS-fine needle injection (EUS-FNI) through the portal vein on experimental pig models.

Materials and Methods. The study was performed on 8 pigs maintained in special conditions. The animals were divided in 4 groups: two pigs were injected through a peripheral vein, two were injected in the portal vein, and other four were subjected to local exposure of IONs in the liver and pancreas, two each. A 19-gauge EUS needle (Boston Scientific, Cook Medical) was inserted and after withdrawing the stylet 2 ml of MNP solution was directly injected either in the portal vein or directly in the liver and pancreas. For the liver, MNP were injected in the left lobe, as for the pancreas the MNP were directed to the head of the pancreas. Portal vein EUS-FNI consisted of puncturing the vascular wall and inserting the MNP solution into the bloodstream. Regarding the peripheral access, the catheter placed on the ear lobe was the point of distribution.

The pigs were followed for the next 7 days, with close monitoring regarding any change in their behavior, food intake and body temperature. Animals were euthanized with a pentobarbital overdose and necropsy was performed with their liver and pancreas and other organs being harvested. Gross examination was performed and organs were stored in formalin and sent for a 3T MRI (Philips Ingenia 3T, Netherlands) scanning with a special research coil.

Results. Macroscopic changes were observed only after local injection and vascular EUS-FNI. Portal vein injection showed no sign of thrombosis, even though large deposits of MNP were visualized up the distal hepatic branches . Regarding the pancreas, MRI images were only relevant after local EUS-FNI procedures.

Pathological assessment was done with Prussian blue staining which showed various deposits within the selected organs. Local injection in the liver showed a large volume of MNPs in the targeted area and several deposits in small quantities further away. In contrast, EUS-FNI in the portal vein revealed a large amount of IONs scattered in the hepatic lobules as well as between the lobules and in the hepatic periportal space.

In both cases the deposits were encapsulated in the macrophages, in the Ito cells and

hepatocytes and stored within the capillary sinusoids. Peripheral vascular injection also showed deposits in other macrophages-like tissues. Small deposits were seen in the white and red pulp of the spleen, within the medullar interstitial space and collector tubes of the kidneys and even in the myocardium space. However, systemic injection showed smaller quantities of MNPs within the liver as compared to local and portal vein EUS-FNI, with considerable larger deposits within the other harvested organs. Local pancreas EUS-FNI showed a large area of MNP deposits with a localized inflammatory tissue reaction at about 2 cm away from the injected area. A mild inflammation of the pancreatic parenchyma with fat necrosis and atrophy characterized the surrounding tissues.

Conclusions. Our study focused on showing the feasibility of EUS-FNI of MNPs in the liver and pancreas by local or vascular access and their organ distribution.

3. Endoscopic ultrasound guided gastrojejunoanastomosis – a new approach for palliative treatment in gastric outlet obstruction

Objective - to develop and to evaluate an efficient method of palliative treatment in gastric or duodenal obstructions caused by pancreatic cancer. The gastrojejunalanastomosis was created by apposing the gastric wall to the first jejunal loop, entirely through an endoscopic approach. The GJJ implied the creation of a communicating canal using fully covered expandable metal stent.

Materials and Methods. The procedure was performed on 8 pigs, with weight between 25 and 30 kg as follows:

1. Endoscope insertion until the second portion of the duodenum was reached;
2. An enteroscopic balloon was placed approximately 30 cm away from the duodenojejunal junction, and filled with NaCl 0,9%;
3. The endoscope was retracted after placing the enteroscopic balloon;
4. An echoendoscope was inserted;
5. The balloon was identified on EUS-imaging;
6. The fully covered expendable metal stent was inserted through the woking channel of the echoendoscope;
7. The stent was released under EUS-guidance;
8. The anastomosis canal was dilated using an esophageal dilation balloon;
9. Visual endoscopic assessment of the anastomosis was performed.

Results. The procedures were performed in a proper environment, with no major immediate complications. There were no signs of bleeding, perforation or injury of the surrounding structures. The introduction and filling of the enteroscopic balloon was accomplished without any impediments. In all cases, the balloon was localised on EUS-imaging on the anterior part of the stomach. Applying the expandable stent did not create any technical problems, and it was successfully placed in all of the eight cases between the gastric wall and the jejunal loop. This aspect was confirmed by additional imaging using the endoscope. The necropsy allowed the identification of the complete adhesion between the gastric and the jejunal wall. The GJJ was identified at a medium distance of 27 cm away from the pylorus, and after sectioning the anastomosis, the stent was extracted without encountering any problems. The medium time for the stent placement was 36 minutes, with an obvious increase in procedure efficiency after the first intervention, leading to a decreased total time of almost half in the last procedure.

The pathological examination revealed a fusion of the mucosa and the muscular layer, with minor inflammatory changes. The anastomosis implied the presence of multiform granulomatous tissue with lymphocytes, macrophages and neutrophils. The submucosal layer revealed the presence of collagen fibres, fibroblasts and angiogenesis. There were no adverse reactions determined by the presence of a foreign body.

Conclusions. As a concept in palliative treatment of malignant gastric outlet obstruction, EUS-guided GJJ may represent a valid option which may require more attention in the future. This technique may be able to offer more advantages than current therapies, which have a high complication rate. Also, it has to be taken into consideration the possible use of this technique in benign stenosis as well as for the treatment of obesity and type II diabetes.

4. General Conclusions

- Pancreatic cancer represents one of the most agressive forms of malignancy, with a poor prognosis. Most of the times, the disease is diagnosed in advanced stages, when curative opstions are not available, which resulted in an increase in the need for potential new therapies that cand prolong the patient's life and improve their condition.
- Palliative treatment of pancreatic cancer focuses on controlling symptoms and preventing secondary effects;

- Endoscopic ultrasound is the investigation of choice for pancreatic masses because it offers the opportunity to examine the entire pancreas, in almost all patients regardless of morphological variants;
- RFA may be considered as valid option for nonmetastatic local extended pancreatic cancer;
- The RFA study demonstrated that EUS-guided RFA on a pig's pancreas is a well tolerated and feasable technique, with no morbidity and mortality a week from the procedure as well as no major complications.
- Recent developments on oncology recomment EUS-guided fine needle injection as potentially promising new technique for local transport of drugs;
- EUS-FNI in the portal vein revealed a larger amount of MNP within the hepatic lobulesas well as in the periportal space than other tested methods.
- EUS-FNI in a tumor amy be an important option for local treatment of pancreatic cancer;
- EUS-GJJ was successfully performed in all experimental models;
- The use of a fully covered metal stent is a feasible option, laborious with lower complications when comparing to other NOTES techniques;
- GJJ on animal models is technically feasible and may proposed in patients with gastric or duodenal obstruction as a result of pancreatic cancer invasion.

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