Systemic Cu/Zn-SOD activation in patients with pancreatic cancer

Aktywacja Cu/Zn-SOD u chorych na raka trzustki

Piotr Myśliwiec[1], Bogusław Kędra[1], Adam Kukliński[1], Krystyna Pawlak[2]

[1]2nd Department of General and Gastroenterological Surgery, Medical University, Bialystok
[2]Department of Monitored Pharmacotherapy, Medical University, Bialystok

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Słowa kluczowe: rak trzustki, dysmutaza nadtlenkowa Cu-Zn, nadtlenki.

Address for correspondence: Piotr Myśliwiec, MD, 2nd Department of General and Gastroenterological Surgery, Medical University, 68A Bacieczki St, 15-685 Białystok, Poland, phone +48 503 085 569, fax +48 85 746 86 22, e-mail: mpiotr@umwb.edu.pl

Abstract

Introduction: Reactive oxygen species (ROS) have been linked to pancreatic cancer for their potential to induce mitogenicity and to stimulate cell proliferation. Cu/Zn-SOD is emerging as an important element of the cellular antioxidant mechanisms.
Aim: The aims of this study were to assess Cu/Zn-SOD levels in plasma of patients with pancreatic cancer treated surgically and to analyze the association of Cu/Zn-SOD with total peroxide levels, along with clinical and pathological data.
Material and methods: We studied 31 patients with pancreatic cancer in comparison with 14 healthy subjects using ELISA techniques to determine Cu/Zn-SOD and total peroxides.
Results: We demonstrated a 3-fold increase in plasma levels of Cu/Zn-SOD in patients with pancreatic cancer ($p < 0.001$) in comparison with the control group. Also, total plasma peroxide levels were higher in the pancreatic cancer group ($p < 0.001$) than in the control group. As assessed at postoperative day 10, concentrations of Cu/Zn-SOD were enhanced in plasma of pancreatic cancer patients ($p < 0.05$) in contrast to total peroxides, which diminished postoperatively ($p < 0.05$). The levels of total peroxides were greater in patients with cancers of the pancreatic body when compared to those of the pancreatic head ($p < 0.05$).
Conclusions: We have shown for the first time that systemic Cu/Zn-SOD and total peroxides are increased in patients with pancreatic cancer. Surgical treatment resulted in greater plasma Cu/Zn-SOD and lower peroxide levels.

Streszczenie

Wprowadzenie: Reaktywne formy tlenu w przebiegu raka trzustki mogą działać mitogennie i stymulować proliferację. Cu/Zn-SOD coraz częściej opisuje się jako ważny element komórkowych mechanizmów antyoksydacyjnych.
Cel: Celem pracy była ocena stężenia Cu/Zn-SOD w osoczu chorych na raka trzustki, leczonych chirurgicznie, oraz analiza stosunku Cu/Zn-SOD do nadtlenków, a także danych klinicznych i histopatologicznych.
Materiał i metody: Stężenie osoczowe Cu/Zn-SOD oraz nadtlenków zbadano przy użyciu techniki ELISA u 31 chorych na raka trzustki oraz u 14 zdrowych ochotników. U pacjentów ponownej oceny dokonano w 10. dobie po operacji.
Wyniki: Wykazano 3-krotne zwiększenie stężenia osoczowego Cu/Zn-SOD u chorych na raka trzustki ($p < 0,001$) w porównaniu z grupą kontrolną. Także stężenie nadtlenków było większe u pacjentów z nowotworami ($p < 0,001$) niż w grupie kontrolnej. W porównaniu z badaniem przedoperacyjnym ocena w 10. dobie po operacji z powodu raka trzustki wykazała zwiększenie stężenia osoczowego Cu/Zn-SOD ($p < 0,05$) w przeciwieństwie do nadtlenków, których stężenie po operacji zmniejszyło się ($p < 0,05$). Stężenie nadtlenków było większe u chorych na raka trzonu trzustki niż u pacjentów z rakiem głowy tego narządu ($p < 0,05$).
Wnioski: Po raz pierwszy wykazano wzrost aktywności Cu/Zn-SOD oraz stężenia nadtlenków w krwiobiegu chorych na raka trzustki. Leczenie chirurgiczne prowadziło do zwiększenia aktywności Cu/Zn-SOD oraz zmniejszenia stężenia nadtlenków.

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States and its frequency is increasing [1]. Ductal adenocarcinoma is the most common tumour of the pancreas. At the time of diagnosis most pancreatic cancers are inoperable, frequently involve the lymph nodes and have distant metastases. Recently, growth factors have been shown to stimulate reactive oxygen species (ROS) generation by activation of membrane non-mitochondrial NAD(P)H oxidase. This, in turn, inhibits apoptosis and promotes cancer cell proliferation [2].

Numerous antioxidants are present in human cells to prevent ROS-induced damage. Superoxide dismutases (SODs) dismutate superoxide anion O_2- into hydrogen peroxide H_2O_2, which is further converted into water by catalases and peroxidases [3]. Interestingly, antioxidant molecules mimicking SOD increase *in vitro* proliferation of normal cells but kill tumour cells [4].

Three isoforms of SOD have been described so far in eukaryotic cells according to their location and composition: manganese-containing SOD (Mn-SOD), copper- and zinc-containing SOD (Cu/Zn-SOD), as well as extracellular SOD (Ec-SOD). Cu/Zn-SOD represents about 90% of SOD activity. Besides its predominant cytosolic expression, it is also present in lysosomes, peroxisomes, in the nucleus and in the intermembrane space of mitochondria, the main site of superoxide generation [3]. Although Cu/Zn-SOD is located predominantly intracellularly, it can also be found in plasma, serum and other body fluids [5]. In the pancreas, the highest tissue expression was reported in duct cells, islet cells and centroacinar cells, whereas acinar cells showed almost no immunohistochemical staining for Cu/Zn-SOD. Cu/Zn-SOD was also found in pancreatic juice at a concentration similar to serum. Patients with pancreatic tumours had almost twice as high levels of Cu/Zn-SOD in pancreatic juice [6]. Experimental data show that overexpression of Cu/Zn-SOD in human pancreatic cancer cells resulted in decreased tumour cell growth whereas inhibition of endogenous SOD promoted tumour growth [3].

Aim

The aim of this study was to assess Cu/Zn-SOD levels in plasma of patients with pancreatic cancer treated surgically and analyze the association of Cu/Zn-SOD with total peroxide levels as well as clinical, pathological and laboratory data.

Material and methods

This study was performed on plasma samples from 31 patients with pancreatic cancers treated in the 2nd Department of General and Gastroenterological Surgery, Medical University of Bialystok, Poland. The control group consisted of 14 healthy subjects. The characteristics of the patients are given in Table I. The mean age was 66.1 (44 to 83). Twenty three patients with pancreatic cancer had a palliative procedure performed. Confirmation of cancer on pathological examination was performed in all patients.

The control group comprised 9 men and 5 women, aged 32 to 78 (mean 64.9). All these subjects were healthy. Patients and volunteers with clinical or laboratory signs of infection were excluded from the study. Exclusion criteria for control subjects also comprised malignancy, liver and kidney diseases and chronic inflammatory diseases. Ethics committee approval and informed consent from all patients were obtained.

In all patients, blood samples were collected before surgery and at post-operative day 10 (9-11). After an overnight fast, 4.5 ml of venous blood were taken from the antecubital vein and poured into a tube containing 0.5 ml EDTA. Immediately after sampling, specimens were centrifuged at 1000 rpm for 15 min at 4°C. The supernatant platelet-poor plasma was transferred to an Eppendorf tube and frozen at –70°C until analysis.

Cu/Zn-SOD and total peroxides were determined using ELISA kits purchased from Bender MedSystems, Vienna, Austria and Diagnostic Automation, Inc., USA., respectively. Biochemical and haematological parameters were determined by standard laboratory methods.

Data were analyzed using Statistica 5.1. computer software (StatSoft Inc, Tulsa). Normality of variable distribution was tested using Shapiro-Wilk W test. Normally distributed data are expressed as mean ± SEM. Non-Gaussian data are presented as median (full range). ANOVA was employed to test differences between groups. The Mann-Whitney test or Student's *t* test was used in statistical analysis to compare differences between individual groups. For differences between assessments performed on days 0 and 10 dependent *t*-test or Wilcoxon test was used. Correlations were determined using Spearman's analysis. A two-tailed p value < 0.05 was considered to be statistically significant.

Results

Cu/Zn-SOD was detectable in plasma of all the operated patients (100%) and in 57% of the normal controls. We found a significant 3-fold increase in plasma Cu/Zn-SOD in pancreatic ductal cancer patients when compared to the control group ($p < 0.001$) (Table II). Ten days after the operation, a significant

Table I. Clinical and pathological characteristics of patients with pancreatic cancer
Tabela I. *Kliniczna i histopatologiczna charakterystyka chorych na raka trzustki*

Patients with pancreatic adenocarcinoma		N	[%]
Gender: male		18	(58)
Gender: female		13	(42)
• head		25	80.6% of 31
• body		4	12.9% of 31
• generalized		2	6.45% of 31
TNM classification – grade:	I	2	6.45% of 31
	II	4	12.9% of 31
	III	14	45.2% of 31
	IV	11	35.5% of 31
TNM classification – tumour infiltration:	T1	3	9.7% of 31
	T2	4	12.9% of 31
	T3	9	29% of 31
	T4	15	48.4% of 31
TNM classification – lymph node status:	N0	3	9.7% of 31
	N1	28	90.3% of 31
TNM classification – metastases status:	M0	20	64.5% of 31
	M1	11	35.5% of 31
Procedure:			
• Whipple's operation		5	16.1% of 31
• left pancreatectomy		1	3.2% of 31
• Traverso-Longmire's procedure		2	6.45% of 31
• double bypass palliative operation		16	51.6% of 31
• laparotomy with biopsy		7	22.6% of 31

increase in plasma Cu/Zn-SOD was demonstrated in patients with pancreatic ductal cancer when compared to preoperative values (Table II).

Total peroxides were detectable in plasma of 85% of the cancer patients and in 100% of healthy subjects. As shown in Table II, total peroxides were significantly increased in plasma of pancreatic cancer patients (about 3,5-fold) ($p < 0.001$) in comparison to the control group. In contrast to Cu/Zn-SOD, total peroxides decreased after the operation for pancreatic cancer as assessed at postoperative day 10 ($p < 0.05$).

When analysing plasma Cu/Zn-SOD and total peroxides in relation to disease staging, we found a tendency to greater peroxide levels in advanced pancreatic cancer patients (with distant metastases compared to without metastases), although no significant difference was demonstrated ($p > 0.05$). Also, patients with irresectable tumours of the pancreas tended to have greater total peroxide levels but again without significant differences.

As far as location of pancreatic cancer is concerned, the patients with cancer of the body of the pancreas had greater concentrations of total peroxides in their plasma than those with cancer of the pancreatic head (Table II). No significant association was found however between either plasma Cu/Zn-SOD or total peroxides in pancreatic cancer patients and: sex, body mass, co-morbidities (arterial hypertension, diabetes), preoperative bile duct stenting and such laboratory parameters as: white blood count, haemoglobin, INR, albumin, bilirubin, CEA (carcino-embryonic antigen) or CA19-9.

Discussion

In the present study we assessed two recognized markers of oxidative stress, Cu/Zn-SOD and total peroxides, in patients with pancreatic cancer using ELISA tests. For the first time, we demonstrated significantly increased levels of plasma Cu/Zn-SOD in this group of patients. To our knowledge, there are no data on systemic Cu/Zn-SOD in pancreatic cancer patients.

Table II. Concentrations of Cu/Zn-SOD and total peroxides in plasma of healthy subjects (control group) and pancreatic cancer as a whole and divided into subgroups
Tabela II. *Stężenia Cu/Zn-SOD oraz nadtlenków w osoczu osób zdrowych (grupa kontrolna) oraz pacjentów z rakiem trzustki, z uwzględnieniem podziału na podgrupy*

Group/case	Cu/Zn-SOD [ng/ml]		Total peroxides [μM/l]	
	Day 0	Day 10	Day 0	Day 10
Control group	30 (4-48) (*N* = 8/14)		330 ±45 (*N* = 14/14)	
Pancreatic cancer	88 (28-284)*	126 (64-506)*#	1153 ±149*	737 ±106**#
Head of pancreas	*88 (28-284)**	*132 (72-506)*#*	*1044 ±171***	*709 ±135***
Body of pancreas	*86 (74-228)***	*76 (64-362)****	*1708 ±357*°*	*891 ±236***#
Resectable	*86 (74-144)***	*191 (82-434)***	*1044 ±316***	*1035 ±336****
Non-resectable	*88 (28-284)**	*114 (64-506)*#*	*1177 ±171***	*683 ±110***#
Metastases-negative	*81 (42-184)***	*126 (64-506)*#*	*1035 ±182***	*790 ±148***
Metastases-positive	*98 (28-284)**	*125 (72-476)***	*1359 ±257**	*619 ±94**#

*Values before the operation (day 0) and after the operation (day 10) are shown. Results are given as mean ± SEM. Non-Gaussian data are presented as median (range) depending on their distribution. For the control group, we show in brackets the ratio of the patients with detectable levels of Cu/Zn-SOD (8 out of 14) and total peroxides (14 out of 14). Significant differences as compared to the control group are marked with: *p < 0.001, **p < 0.01 or ***p < 0.05. Significant difference between day 0 and day 10 is marked with #p < 0.05. A difference between patients with cancer of the body and head of the pancreas is marked with °p < 0.05*
*Zamieszczono wartości przedoperacyjne (dzień 0.) i pooperacyjne (dzień 10.). Wyniki podano jako średnia ± błąd standardowy. Wyniki o rozkładzie niezgodnym z krzywą Gaussa przedstawiono jako mediana (zakres). W odniesieniu do grupy kontrolnej w nawiasach przedstawiono udział liczbowy osób z wartościami oznaczalnymi (powyżej progu detekcji) Cu/Zn-SOD (8 z 14); nadtlenki (14 z 14). Znamienne różnice w porównaniu z grupą kontrolną oznaczono jako *p < 0,001, **p < 0,01 czy ***p < 0,05. Znamienne różnice między dniem 0. oraz 10. oznaczono #p < 0,05. Różnicę między chorymi na raka trzonu i głowy trzustki oznaczono °p < 0,05*

Two studies previously assessed tissue expression of Cu/Zn-SOD in pancreatic cancer patients using gel electrophoresis [7] or immunohistochemical methods [8]. In both cases decreases in Cu/Zn-SOD tissue expression were observed in pancreatic cancer when compared to normal pancreas.

Since no studies on systemic Cu/Zn-SOD in pancreatic cancer are available, we examined reports on other malignancies for comparisons. Lin *et al.* [9] reported that serum levels of Cu/Zn-SOD were significantly elevated in gastric cancer patients compared with healthy controls and concluded that higher Cu/Zn-SOD levels may be associated with an increased risk of gastric cancer. Meanwhile a decrease in the tissue expression of Cu/Zn-SOD has been reported in gastric cancer [10]. Also in patients with other digestive cancers, serum Cu/Zn-SOD levels were significantly elevated when compared to healthy subjects [11]. As tissue expression of Cu/Zn-SOD is diminished in pancreatic cancer, augmented plasma levels of the enzyme are unlikely to be of pancreatic origin, even though Cu/Zn-SOD levels are increased in pancreatic juice in the course of pancreatic cancer [6]. The most probable source of plasma Cu/Zn-SOD is peripheral blood mononuclear cells (PBMC). Activation of PBMC has been shown in blood of patients with advanced pancreatic, colon and breast cancers [12]. Surgical treatment induces polymorphonuclear blood cells. In our study, we have observed that after the operation Cu/Zn-SOD increased. At the same time postoperative H_2O_2 levels decreased, as compared to preoperative values. We speculate that surgical stress stimulates not only Cu/Zn-SOD but also the H_2O_2 scavengers catalase and/or peroxidase, and this out of proportion to Cu/Zn-SOD.

A question arises, what is the significance of elevated Cu/Zn-SOD in plasma of patients with pancreatic cancer? The answer does not appear clear-cut.

Reactive oxygen species have been shown to enhance pancreatic cancer cell proliferation and have antiapoptotic activity [2]. These pro-survival effects can be modulated by antioxidant enzymes. Overexpression of Cu/Zn-SOD in human pancreatic cancer cells using adenoviral vector transfection resulted in decreased tumour cell growth. This was accompanied by decreased superoxide and increased H_2O_2 levels. The authors also demonstrated that inhibition of endogenous SOD with small interfering RNA promoted tumour growth [3]. Thus, Cu/Zn-SOD seems to exert an anti-tumour effect on pancreatic cancer cells.

In this study we have demonstrated significant increases in total peroxides in plasma of patients with pancreatic cancer, which is in line with enhanced Cu/Zn-SOD levels. An anti-tumour role of Cu/Zn-SOD seems to be due to decreased superoxide levels, since generation of ROS is at least in part responsible for resistance of pancreatic cancer cells to apoptosis [2, 13]. However, there is also a potential impact of H_2O_2 on tumour cell growth. As the anti-tumour effect of SOD overexpression could be reverted by glutathione peroxidase or catalase, H_2O_2 seems to be important for cancer cell damage [14]. H_2O_2 has been suggested to be a mediator of pro-apoptotic action of some therapeutic agents, which could be due to down-regulation of anti-apoptotic Bcl-2 [15]. In a model of chemically-induced pancreatic adenocarcinoma in the Syrian hamster, treatment of animals with vitamins A and C decreased the incidence of pancreatic cancer with concomitant increase in the activity of SOD. The authors speculated that decreased incidence of pancreatic cancer was due to increased intracellular hydrogen peroxide levels as a result of its selective toxicity to tumour cells [16]. On the other hand, Moskovitz *et al.* [17] have demonstrated that chronic exposure to hydrogen peroxide caused chromosomal abnormalities in cultured pancreatic ductal epithelium, possibly an early event in the development of pancreatic cancer. The role of systemic H_2O_2 increase in pancreatic cancer patients needs clarifying.

Conclusions

We have shown for the first time that systemic copper/zinc superoxide dismutase and total peroxides are increased in patients with pancreatic cancer. Surgical treatment resulted in greater plasma Cu/Zn-SOD and lower peroxide levels, which supports the role of PBMC in systemic increase of Cu/Zn-SOD.

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