INTRODUCTION

Pancreatic carcinoma is a highly aggressive malignancy characterized by very low 5-year survival rate (1). This pessimistic results of the treatment might be due to the poor responsiveness of pancreatic cancer cells to apoptotic stimuli and in consequence to tumor progression and resistance to commonly used oncologic therapies (2). Nearly 95% of pancreatic neoplasms are adenocarcinomas arising from the exocrine ductal system, in which high level of heat shock proteins expression has been described (3).

Heat shock proteins (HSPs, chaperones) are grouped into several families depending on their molecular weight (HSP100, HSP90, HSP70, HSP60 and the small HSPs). Numerous members of these groups are involved in the modulation of cell proliferation, cell cycle regulation and protection of cells against damage (4-6). HSP27 is an ATP-independent chaperone with antiapoptotic properties (7-8). Oligomers, the main form of HSP27 with chaperone activity are disrupted by phosphorylation to dimers and monomers (8). In human cells the upregulation of HSP27 expression and synthesis were found to be proportional to the exposure of the cells to multiple stress stimuli (9). HSP27 is able to block apoptosis at different steps but its capacity to interact with respective target seems to be determined by the oligomerization/phosphorylation status (10). HSP27 essentially diminish caspase-dependent apoptotic pathway, mainly by inhibiting caspase-3 activation (11). Overexpression of HSP27 is implicated in the resistance of pancreatic cancer to chemotherapy. High level of HSP27 expression in pancreatic tumors might be correlated with the resistance to gemcitabine treatment (12).

Kynuramines, metabolites of melatonin and L-tryptophan, are synthesized endogenously by oxygenases or in spontaneous reaction by an interaction with free radicals. We have reported previously that melatonin stimulates expression and phosphorylation of heat shock protein (HSP) 27, as well as production of HSP70 and HSP90αβ in pancreatic carcinoma cells (PANC-1). Based on those results, we hypothesized that above processes could have been involved in the interruption of intrinsic proapoptotic pathway. Herein, we report that incubation of PANC-1 cells with N'-acetyl-N'-formyl-5-methoxykynuramine (AFMK) or with L-kynurenine (L-KYN) lead to the overexpression of heat shock protein synthesis and these effects are partially reversed by 5-HT3 or MT1/MT2 receptor antagonists. PANC-1 cells in culture were treated with AFMK or L-KYN, with non selective MT1/MT2 receptor antagonist (luzindole), with 5-HT3 and 5-HT1 receptor antagonists (ketanserin and MDL72222), or combination of these substances. Both AFMK and L-KYN significantly decreased cytoplasmic HSP27 and this effect was presumably due to increased of its phosphorylation and consequent nuclear translocation, confirmed by immunoprecipitation of phosphorylated form of HSP27. These changes were accompanied by marked augmentation of HSP70 and HSP90αβ in the cytosolic fraction. Pretreatment of cell cultures with luzindole or MDL72222 followed by the addition of AFMK or L-KYN reversed the stimulatory effects of these substances on HSP expression in PANC-1 cells, whereas ketanserin failed to influence mentioned above phenomenon. We conclude that activation of HSPs in pancreatic carcinoma cells seems to be dependent on an interaction of AFMK or L-KYN with MT1/MT2 or/and 5-HT3 receptors.

Key words: N'-acetyl-N'-formyl-5-methoxykynuramine, L-kynurenine, heat shock protein 27, p-heat shock protein 27, heat shock protein 70, heat shock protein 90αβ, pancreatic carcinoma cells, melatonin receptors, 5-hydroxytryptamine receptors

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KYNURAMINES INDUCE OVEREXPRESSION OF HEAT SHOCK PROTEINS IN PANCREATIC CANCER CELLS VIA 5-HYDROXYTRYPTAMINE AND MT1/MT2 RECEPTORS

Kynuramines, metabolites of melatonin and L-tryptophan, are synthesized endogenously by oxygenases or in spontaneous reaction by an interaction with free radicals. We have reported previously that melatonin stimulates expression and phosphorylation of heat shock protein (HSP) 27, as well as production of HSP70 and HSP90αβ in pancreatic carcinoma cells (PANC-1). Based on those results, we hypothesized that above processes could have been involved in the interruption of intrinsic proapoptotic pathway. Herein, we report that incubation of PANC-1 cells with N'-acetyl-N'-formyl-5-methoxykynuramine (AFMK) or with L-kynurenine (L-KYN) lead to the overexpression of heat shock protein synthesis and these effects are partially reversed by 5-HT3 or MT1/MT2 receptor antagonists. PANC-1 cells in culture were treated with AFMK or L-KYN, with non selective MT1/MT2 receptor antagonist (luzindole), with 5-HT3 and 5-HT1 receptor antagonists (ketanserin and MDL72222), or combination of these substances. Both AFMK and L-KYN significantly decreased cytoplasmic HSP27 and this effect was presumably due to increased of its phosphorylation and consequent nuclear translocation, confirmed by immunoprecipitation of phosphorylated form of HSP27. These changes were accompanied by marked augmentation of HSP70 and HSP90αβ in the cytosolic fraction. Pretreatment of cell cultures with luzindole or MDL72222 followed by the addition of AFMK or L-KYN reversed the stimulatory effects of these substances on HSP expression in PANC-1 cells, whereas ketanserin failed to influence mentioned above phenomenon. We conclude that activation of HSPs in pancreatic carcinoma cells seems to be dependent on an interaction of AFMK or L-KYN with MT1/MT2 or/and 5-HT3 receptors.
expression level was found to be significantly higher than described in non malignant ductal cells. Depletion of this protein leads to the activation of caspases cascade in the pancreatic tumor cell lines resulting in apoptosis recurrence (20, 21).

HSP90 occurs in two homologue isoforms, as an inducible HSP90α and a constitutive HSP90β. This chaperone inhibits apoptosis by direct binding to Apaf-1 and further prevention of recruitment of procaspase-9 to apoptosome (22, 23). HSP90 has been found to be overexpressed in many neoplasms and moreover it was frequently correlated with high invasiveness of carcinomas (22, 24, 25). Knock-down of HSP90β or its inhibition by geldanamycin or its analogues prevented HSP90β interaction with antiapoptotic protein Bcl-2 and resulted in cytochrome release, caspase activation and induction of apoptosis (26). This protein is localized throughout the pancreatic cancer tissue but its increased abundance has also been noticed in the cells adjacent to the tumor (27).

As we have shown in the previous papers melatonin effectively protected pancreatic tissue against the acute damage, and this beneficial effect could be related, at least in part, to the stimulation of HSP in pancreatic cells (28-31). However our subsequent studies have shown that overproduction of HSP27, HSP70 and HSP90αβ due to melatonin administration was in opposition to the ability of this indoleamine to stimulate the proapoptotic pathway on mitochondrial level and to the activation of procaspase-9 in pancreatic carcinoma cells. In spite of this, our results have evidenced that melatonin failed to activate executioner of apoptosis: caspase-3 and prevented DNA fragmentation in PANC-1 cells (32-33). This observation suggests that mentioned above HSPs could be involved in the inhibition of proapoptotic pathway at the level of caspases activation and thus HSPs could block the apoptotic cell death. In the present study we have assessed the changes in HSP27, HSP70 and HSP90αβ expression in human pancreatic carcinoma cells PANC-1 subjected to N-acetyl-N-formyl-5-methoxykynuramine or L-kynurenine and moreover we have investigated the involvement of MT1/MT2 and 5-HT receptors in this process.

MATERIALS AND METHODS

Reagents

L-kynurenine, luzindole (N-acetyl-benzyltryptamine), ketanserin and MDL72222 were purchased from Sigma-Aldrich Co. (St. Louis, MO), whereas AFMK (N-acetyl-N-formyl-5-methoxykynuramine) was from Cayman Europe OU (Tallinn, Estonia). Investigated substances were dissolved in different solvents according to the manufacturer’s procedures. The following materials were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA): protein A-Agarose, primary goat polyclonal HSP27, goat polyclonal p-HSP27 rabbit polyclonal HSP70, mouse monoclonal HSP70, rabbit polyclonal HSP90αβ antibodies and secondary rabbit anti-goat, goat anti-rabbit, mouse anti-rabbit antibodies linked to HRP (horseradish peroxidase-conjugated). SuperSignal West Pico Chemiluminescent Substrate was obtained from Pierce Biotechnology (Rockford, IL, USA). All of the other chemicals and reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Cell culture

Human pancreatic carcinoma cell line (PANC-1) was obtained from the American Type Culture Collection (Rockville, MD) and maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Sigma-Aldrich Co., St. Louis, MO) supplemented with 10% fetal bovine serum (HyClone, South Logan, UT), 15 μg/ml penicillin and 0.05 mg/ml streptomycin. PANC-1 cells were grown at 37°C in a humidified atmosphere of 5% CO₂. Twenty-four hours prior to the experiments, cells were supplemented with DMEM containing 0.2% BSA without antibiotics.

Experimental protocol

1. Determination of the effects of AFKM or L-kynurenine on HSP27, p-HSP27, HSP70 and HSP90αβ proteins expression in PANC-1

Cells cultured on 10 cm dishes were pretreated with various concentrations of AFMK or L-KYN (10⁻⁴, 10⁻⁸ or 10⁻¹² M). Cells were incubated with tested substance for 12, 24 or 48 hours. Time-course experiments pointed out that the most spectacular reaction of the cells was observed at 48 h of incubation and that time point was selected for further studies. Subsequently, the lowest used concentration of above substances (10⁻¹² M) was selected for further experiments. Control experiments were performed the vehicle only (0.1% DMSO; AFMK and 0.1 mM HCl; L-KYN).

2. Examination of the effects of melatonin receptor MT1/MT2 antagonist luzindole or 5-HT; receptor antagonist ketanserin, or/and 5-HT, receptor antagonist MDL72222 on HSP27, HSP70 and HSP90αβ proteins expression in PANC-1

Cells were incubated with luzindole or with ketanserin or with MDL72222 (MDL) at concentrations of 10⁻¹² M alone or in combination with AFMK (10⁻¹² M) or L-KYN (10⁻¹² M) for 48 hours. Control experiments were performed as described above.

Protien extraction

After 48 hours of incubation, cells were harvested by scraping with rubber ‘police man’ in ice cold PBS and collected by short spin centrifugation at 4°C. Then cell pellets were resuspended in 400 μl of extraction buffer containing 10 mmol/l Heps, 10 mmol/l KCl, 2 mmol/l MgCl₂, 1 mmol/l EDTA (ethylenediamine tetraacetic acid), 1 mmol/l DTT (dithiothreitol), 0.1 mmol/l PMSF (phenylmethylsulphone fluoride) pH 7.4 and kept on ice for 15 min. Subsequently, 25 μl 10% NP-40, was added and samples were mixed vigorously and centrifuged at 14,000 × g for 15 s at 4°C. The supernatant containing cytosolic fraction of proteins was removed and stored at −80°C until further analysis. Dry pellets were resuspended in 50 μl of buffer containing 10% glycerol, 50 mmol/l Hepes, 50 mmol/l KCl, 300 mmol/l NaCl, 0.1 mmol/l EDTA, 1 mmol/l DTT, 0.1 mmol/l PMSF pH 7.4 and incubated on ice with gentle agitation. After centrifugation at 14,000 × g for 5 min at 4°C supernatant (nuclear fraction) was aliquoted and stored at −80°C. The protein concentration was estimated using measurement of absorbancy at 280 nm.

Immunoprecipitation

Samples containing 5 – 10 μg of proteins were incubated for 3 h at 4°C on shaking platform with 5 μl of primary antibodies. 5 μl of A-agarose was added to each sample and samples were incubated overnight at 4°C. Immunoprecipitated complexes were washed in 1 ml of washing buffer (150 mmNaCl, 1% NP-40, 0.5% deoxycholate acid, 0.1% SDS, 50 mM TRIS pH 8.0) and centrifuged at 13,000 × g for 2 min at 4°C. This step was repeated 3 times. The pellets were proceeded according to immunoblotting protocol.
**Immunoblotting**

Proteins resuspended in loading buffer and denatured for 5 min at 95°C were separated using 12% SDS-polyacrylamide gel electrophoresis and then transblotted overnight at 4°C onto PVDF membranes (Bio-Rad Laboratories, Hercules, CA). Membranes were blocked in 5% non-fat milk solution in PBS and then incubated with primary antibodies at 1:1000 dilutions for 1hr at the room temperature on the rocking platform. Blots were washed three times for 10 min with TBS-Tween (TBST, 25 mM Tris-HCl, 0.2 mM NaCl, 0.1% Tween 20) and then incubated for 1 hour at room temperature with a secondary antibody, peroxidase conjugate. After washing with TBST, membranes were treated with enhanced chemiluminescence reagents according to the manufacturer’s protocol. The membranes were exposed to X-ray film for 1 to 15 min, time suitable for results the visualization. Protein expression was measured with the G-Box EF (Synoptics, GB). Protein loading between lanes was additionally controlled by GAPDH (glyceraldehyde-3-phosphate dehydrogenase) immunoblotting. The quantity of target protein was calculated relatively to the GAPDH expression and to the control.

**Statistical analysis**

The results of representative experiment were shown and each point in Figs. 1-5 represents the means ± S.D. (standard deviation) of triplicate determination. Multiple group comparisons were performed by one-way ANOVA with a post hoc Dunnett’s test for comparison each of a number of treatments with a single control.

**RESULTS**

Expression of HSP27, p-HSP27, HSP70 and HSP90αβ in PANC-1 was detected in the control cells as well as in cells stimulated with AFMK or L-KYN. HSPs expression have also been found in the PANC-1 cells incubated with AFMK or L-KYN combined with luzindole, ketanserin or MDL72222 (Figs. 1 - 5).

The effects of AFMK or L-kynurenine on HSP27, p-HSP27, HSP70 and HSP90αβ proteins expression in PANC-1

Incubation of PANC-1 cells in medium supplemented with AFMK (10^{-12} M) leads to the significant reduction of cytoplasmic abundance of HSP27 protein when compared to the values obtained in cell cultures subjected to vehicle only. To the contrary, administration of AFMK, melatonin degradation product, significantly (almost 3 fold) increased nuclear accumulation of HSP27 protein and stimulated its phosphorylation. The changes in p-HSP27 were even more evident in nuclear fraction than in cytosol and averaged 312% of control (Fig. 1).

Application of L-KYN to PANC-1 also resulted in marked decrease of cytoplasmic HSP27 protein after 48 hours of incubation (Fig. 1). The most prominent inhibitory effect on the expression of HSP27 was observed after application of L-KYN at the dose of 10^{-12} M as compared to the control cultures subjected to vehicle alone. These changes were accompanied by the rise of HSP27 abundance in the nucleus that reached 275% control values (Fig. 1). Moreover, L-KYN added to medium significantly increased abundance of p-HSP27 in both cytoplasmic (165%) and nuclear (233%) fractions of PANC-1 cells (Fig. 1).

As shown in Fig. 2, addition of AFMK or L-KYN to the PANC-1 cell culture also influenced HSP90αβ protein expression. The highest level of this protein was observed after stimulation with L-KYN at concentration of 10^{-12} M and averaged 412% of control values. Similarly, the almost 3 folds elevation of cytoplasmic HSP90αβ protein level was detected following treatment with AFMK compared to the control. The changes of HSP90αβ protein were accompanied by marked increase of HSP70 protein expression in cytoplasm of PANC-1 incubated with both AFMK and L-KYN (Fig. 2).

The effects of various concentration of melatonin receptor MT1/MT2 antagonist luzindole or 5-HT1α receptor antagonist ketanserin, or 5-HT3 receptor antagonist MDL72222 on HSPs expression in PANC-1

Luzindole, melatonin receptor antagonist (10^{-12} M), used together with AFMK (10^{-12} M) or L-KYN (10^{-12} M) reversed the
inhibitory effect of these substances on HSP27 (Fig. 3). The most spectacular effect on cytoplasmic level of HSP27 was observed after administration of luzindole and averaged about 816.6% (for AFMK) and 392.4% (for L-KYN) of control values. Similarly, to the previously described effect, incubation of PANC-1 with 5-HT$_3$ receptor antagonist MDL72222 (10$^{-12}$ M) followed by the addition of AFMK or L-KYN resulted in the rise of cytoplasmic HSP27 protein level by 6 or 5 folds respectively. Administration of ketanserin 5-HT$_2$ receptor antagonist (10$^{-12}$ M) followed by L-KYN or AFMK failed to affect HSP27 protein expression level (Fig. 3).

As shown on Fig. 4, application of AFMK together with luzindole or MDL72222 has reversed stimulatory effect of this melatonin metabolite on cytoplasmic HSP70 protein abundance (Fig. 4). The most prominent inhibitory effect was observed after administration of MT1/MT2 or 5-HT$_3$ receptor’s antagonists used at concentration of 10$^{-12}$ M (29.6% or 40.4% respectively). Administration of ketanserin 5-HT$_2$ receptor antagonist (10$^{-12}$ M) followed by L-KYN or AFMK failed to affect HSP27 protein expression level (Fig. 4).

Tendencies shown in Figs. 3 and 4 were accompanied with decrease of HSP90$\alpha\beta$ protein abundance in cytoplasm of PANC-1 after application of each kynuramine (AFMK or L-KYN) together with luzindole. MT1/MT2 receptor antagonist reduced the ratio of HSP90$\alpha\beta$/GAPDH/control to 28.8% (AFMK + luzindole) and to 67% (L-KYN + luzindole) respectively (Fig. 5). Similarly, incubation of PANC-1 cells with 5-HT$_3$ receptor antagonist MDL72222 followed by AFMK or L-KYN resulted in the decrease in cytoplasmic (48.9% or 61.5%) level of HSP90$\alpha\beta$. Administration of kynuramines with the combination of ketanserin did not affect significantly the level of HSP90$\alpha\beta$ (Fig. 5).

**DISCUSSION**

The results of present study provide the evidence that AFMK or L-KYN stimulate production of antiapoptotic heat shock proteins; p-HSP27, HSP70 and HSP90$\alpha\beta$ in PANC-1. As a reference to the previous study, we have shown that melatonin; induced synthesis, phosphorylation and nuclear translocation of HSP27 and caused overexpression of HSP70 and caused overexpression of HSPs resulted in the interruption of the intrinsic proapoptotic pathway and inhibition of caspases activation, leading to the blockade of apoptosis (33).

AFMK (N$^1$-acetyl-N$^2$-formyl-5-methoxykynuramine) is one of melatonin metabolites (34). It can be formed endogenously by oxygenases such as IDO-1, IDO-2 (indoleamine-pyrrole 2,3-dioxygenase 1 or 2) or myeloperoxidase. Despite this enzymatic
process, AFMK could be also generated by numerous free radicals (34-35). It has been reported that AFMK interacts with various reactive species of radicals, mainly with hydroxyl radical (OH) and through its antioxidant properties could reduce lipid peroxidation and DNA damage (36-37).

Rozov et al. (35) have found a detectable level of AFMK in serum, retina and lateral ventricle of the brain in the rats injected with melatonin but not in the serum from untreated animals. In contrast to these observations, Niu et al. (38) have not identified AFMK and its deformylated product AMK (N-acetyl-5-methoxykynuramine) neither in human urine nor in the urine, feces, liver, brain and eyes of mice treated with melatonin. Unlike, some authors described the presence of very low concentration of AFMK in mouse urine (0.15 mM) following the application of large dose of exogenous melatonin (39). The expression of IDO, the enzyme involved in the metabolism of L-tryptophan or melatonin to kynuramines, could be detected in most normal and cancer human cells (40, 41). Some groups have presented data confirming expression of IDO2 gene as well as its enzymatic activity in pancreatic ductal adenocarcinomas cell lines (42-44). Furthermore, many studies have shown correlation between increased IDO expression and significant shortening of survival expectation of cancer patients. Koblish et al. (45) reported that IDO-1 inhibitors suppressed L-tryptophan catabolism in colon and pancreatic carcinoma cells in culture and in vivo in tumors and their draining lymph nodes (45, 46). Moreover, it is well known that excessive production of free radicals is characteristic for the solid tumor tissue e.g. pancreatic adenocarcinomas (47-48). Based on this information we have decided to employ PANC-1 cell line as a model of pancreatic neoplasm in our study concerning involvement of kynuramines on heat shock system activation. As we have been using the end product of the melatonin and L-tryptophan metabolic pathway - AFMK and L-KYN, the activity of IDO2 seems not to be the most essential for the result of our observations. Additionally, we suggest that kynuramines could promote proliferation and survival of PANC-1 through activation of antiapoptotic heat shock proteins.

Kynurenines derived from L-tryptophan: L-KYN and 5-hydroxykynurenine were detected in human plasma, reaching the values of 1.14 – 3.02 µM/l (L-KYN) and less than 0.13 µM/l in case of 5-hydroxykynurenine (49). Studies of Paluszkiewicz et al. (50) have shown the presence of measurable level of kynurenic acid in bile and in pancreatic juice of pigs. This observation indicates the important role of liver and pancreas as a plausible source of kynuramine in gastrointestinal tract due to their involvement in L-tryptophan metabolic pathway (50-51).

The current results indicate that the strong immunoblotting signal of nuclear p-HSP27, cytoplasmic HSP90αβ and HSP70 was observed when all studied concentrations of AFMK were used. It is well known that AFMK could act as an antioxidant, but its effect is weaker than antioxidant properties of melatonin and requires the use of higher doses of AFMK (1 – 500 µM) (37, 52). As we have shown in the previous paper, precursor of AFMK, melatonin could exert its stimulatory effect on HSPs production and proapoptotic pathway at a lower (perhaps

![Fig. 4. Immunoblotting analysis of HSP70 in cytoplasm of PANC-1 treated with AFMK or L-KYN at 10^{-12}M concentration and in combination of these substances with luzindole (10^{-12} M), MDL72222 (10^{-12} M) or ketanserin (10^{-12} M) after 48 h of incubation. Changes in HSP70 protein abundance presented in graph shows the effects of AFMK or L-KYN (10^{-12} M) given alone (control) or in combination with LUZ (luzindole), MDL (MDL72222) or KET (ketanserin) as compared to the control values. *P = 0.05 versus AFMK or L-KYN (10^{-12} M) administration.](image-url)
physiological) concentration. Variable and contradictory action of different concentration of this hormone on the production of arginine vasopressin has also been reported by Juszczak et al. (53). It might suggest that these processes perhaps involve not only MT1/MT2 high affinity surface receptors but also ROR/RZR nuclear receptors. Our result seems to confirm this hypothesis.

Our research has also revealed that the application of MT1/MT2 receptor antagonist (luzindole) significantly reversed AFMK induced stimulation of anti-apoptotic HSPs production in PANC-1 cells. Similarly, the use of an inhibitor of 5-HT, receptor resulted in the reduction of this melatonin metabolite activity, but this effect was not as spectacular as that observed in case of luzindole administration. Many researchers have confirmed the binding of AFMK to the melatonin membrane receptor, while pointing out that AFMK has a weaker affinity to this receptor than that observed with melatonin itself (35, 36, 52, 54). Furthermore, Budu et al. (55) reported that cell cycle synchronization of malaria parasites caused by AFMK is abrogated with MT1/MT2 receptor antagonist luzindole. Moreover, studies of Dragicevic et al. (56) also confirmed reversible action of luzindole on melatonin metabolite effects as in case of its precursor. In their study, application of MT1/MT2 receptor blocker strongly inhibited the restorative effect of AFMK on mitochondrial function in Alzheimer’s mice.

In our studies we observed that application of 5-HT1 receptor antagonist; MDL72222 resulted in the most pronounced inversion of L-KYN action on HSPs. There was also a partial blockage of its effects after luzindole application but this action was distinctly weaker than that of serotonin antagonist. The earlier studies have demonstrated that 5-hydroxykynuramine, derived from L-KYN, could possible bind to the serotonin (5-HT1,2,3) receptors and moreover acts either as serotoninergic agonist or as an antagonist mainly depending on its concentration (37, 56). Similar reaction in regard to the 5-HT1, and 5-HT2 receptors and serotonin action was reported by Chojnacki et al. (57). Despite the affinity of this kynuramine to some serotonin receptors subtypes, its binding and effect is less pronounced than that of its parent indoloamines (34). To our knowledge L-KYN could employ several different receptors e.g. arylhydrocarbon receptor (AhR) to evoke the response in pancreatic ductal adenocarcinomas. According to the previous publications AhR seems to be one of them, but not the most important. However, there are reports showing that AhR is not expressed in PANC-1 as well as in MIA-PaCa-2 and T3M4 cell lines, so we did not find it necessary to evaluate its status in our study (58). This is why we have focused on the signal transduction pathways that involves L-tryptophan, serotonin and melatonin.

In summary, the results presented here indicate that kynuramines stimulate production of antiapoptotic heat shock proteins; p-HSP27, HSP70 and HSP90α/β in pancreatic carcinoma cells. We also suggest that these effects seems to be dependent on interaction of AFMK or L-KYN with MT1/MT2 or/and 5-HT3 receptors.

Abbreviations: AFMK, N1-acetyl-N2-formyl-5-methoxykynuramine; DTT, dithiothreitol; EDTA, ethylenediamine tetracetic acid; GAPDH, phosphate dehydrogenase; HSP, heat
shock protein; KET, ketanserin; L-KYN, L-kynurenine; LUZ, luzindole; PANC-1, human pancreatic carcinoma cell line 1; PMSF, phenylmethylsulphone fluoride.

Acknowledgements: This study was supported by statutory grant from Jagiellonian University Medical College.

Conflict of interests: None declared.

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Received: April 9, 2015
Accepted: July 27, 2015

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