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The Profile of Plasma Met-Enkephalin Concentration in Patients with Renal Cell Carcinoma*

Ocena zachowania się stężeń Met-enkefalin w osoczu krwi chorych na raka jasnokomórkowego nerki

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Abstract

Background. It is believed that Met-enkephalins are the modulators of carcinogenesis in the development of renal cell carcinoma (RCC). However, the Met-enkephalin plasma profile in patients with RCC has so far not been described.
Objectives. The aim of the study was to assess the changes in plasma Met-enkephalin concentration in patients with RCC who underwent radical nephrectomy. The influence of the RCC grade (according to the Fuhrman scale) on the Met-enkephalin profile was also investigated.
Material and Methods. The study included 39 patients, divided into two groups: Group I (n = 21) was comprised of patients with RCC, and Group II (n = 18) were healthy volunteers (the control group). All the patients with RCC underwent radical nephrectomy. The Met-enkephalin concentration was ascertained from blood samples using radio-immunological methods. In Group I the blood samples were taken three times: 24 hours before the radical nephrectomy, 24 hours after it, and on the 7th post-operative day. In the control group (II) the blood samples were taken at the same time intervals as in Group I.
Results. Plasma Met-enkephalin levels decreased 24 hours after nephrectomy, as compared to the value before the operation. Met-enkephalin was also lower on the 7th day after the procedure. However, no significant changes were observed in the post-operative period. In patients with more advanced RCC (Fuhrman G3), the plasma level of Met-enkephalin was significantly higher than in patients with RCC at G2 on the Fuhrman grading scale. Furthermore, the levels of both free and bound plasma Met-enkephalin were significantly higher in patients with RCC as compared to healthy volunteers.
Conclusions. Obtained observations showed a relationship between Met-enkephalins and RCC. However, further investigations are needed to reach a better understanding of the importance of Met-enkephalins in RCC pathogenesis, clinical and oncological outcomes (**Adv Clin Exp Med 2011, 20, 2, 149–156**).

Key words: renal cell carcinoma, Met-enkephalin, free fraction, bound fraction, cancerous marker.

Streszczenie

Wprowadzenie. Met-enkefaliny uczestniczą w karcynogenezie raka jasnokomórkowego nerki (RCC – *Renal Cell Carcinoma*). Jak dotąd profil Met-enkefalin w osoczu krwi u chorych z RCC nie został w pełni poznany.
Cel pracy. Ocena zachowania się stężeń Met-enkefalin w osoczu krwi chorych na RCC. Dodatkowo oceniono profil Met-enkefalin w zależności od stopnia zaawansowania raka w skali Fuhrman.
Materiał i metody. Badanie wykonano w grupie 39 pacjentów, których podzielono na dwie grupy: I (n = 21) – pacjenci z RCC oraz II (n = 18) – grupa kontrolna. Wszyscy pacjenci z rakiem nerki zostali poddani nefrektomii radykalnej. Stężenie Met-enkefaliny wolnej i związanej oceniano w osoczu krwi z użyciem technik radioimmunologicznych. Wykonano trzy pomiary – 24 godziny przed i po nefrektomii oraz w 7. dobie po zabiegu. W grupie kontrolnej oznaczono stężenia Met-enkefalin w identycznym odstępie czasowym jak w grupie I. Oceniając wyniki, porównywano stężenie Met-enkefalin wolnej i związanej w zależności od stopnia zaawansowania histopatologicznego G (skala Fuhrman).

*This study was carried out at the Department of Urology, Rydygier Memorial Hospital, Kraków, Poland.

Wyniki. Na podstawie przeprowadzonych badań zaobserwowano znamienny spadek stężeń Met-enkefalin w osoczu krwi u chorych 24 godziny po operacji w porównaniu do wartości ocenionymi 24 godziny przed zabiegiem. Kolejne obniżenie wartości stężeń Met-enkefalin stwierdzono w 7. dniu po operacji, niemniej różnica ta w porównaniu do stężeń tego parametru w 24 godziny po operacji nie była tak znamienna. W grupie chorych z RCC (Fuhrman G3) zaobserwowano znamiennie statystycznie wyższe stężenia Met-enkefalin w osoczu krwi w porównaniu ze stężeniami w grupie chorych z RCC (Fuhrman G2). Stężenie wolnej i związanej Met-enkefaliny w osoczu krwi było znamiennie statystycznie wyższe w grupie chorych z RCC w porównaniu z grupą kontrolną.
Wnioski. Wyniki badania wykazały istnienie zależności między stężeniem Met-enkefalin a RCC. Jakkolwiek dalsze badania są niezbędne w celu wyjaśnienia udziału Met-enkefalin w patogenezie, przebiegu klinicznym i onkologicznym RCC (**Adv Clin Exp Med 2011, 20, 2, 149–156**).

Słowa kluczowe: rak jasnokomórkowy nerki, Met-enkefaliny, frakcja wolna, frakcja związana, marker nowotworowy.

Renal cell carcinoma (RCC) of the kidney accounted for 3% of all malignancies. Recently, the number of diagnosed cases of RCC has risen by about 30%. There are many promising techniques for describing changes in the DNA, nuclear proliferation and blood serum antigens in patients with RCC [1–5]. Additionally, the typical carcinoembryonic antigen has been evaluated [6]. However, none of these diagnostic and molecular tools serve as a useful cancer marker in RCC staging and grading. In the literature, there are few data describing the carcinogenesis pathways and the immune system adjustment in these pathways [7, 8]. *In vivo* and *in vitro* experimental studies have proven the suppressing influence of Met-enkephalin on the development of neck and pancreatic cancer [9, 10]. Moreover, endorphins and enkephalins stimulate immunologic functions via the synthesis of antibodies and the proliferation of lymphocytes [11]. Previously published results have shown the presence of the Z-type receptor in fetal and cancerous tissues with an increased ability to bind Met-enkephalin [12]. In the literature, there is a lack of data on Met-enkephalin plasma concentration in patients with RCC. The authors of the current study repeatedly searched the Met-enkephalin studies carried out at various laboratories, but found no data about the basic blood plasma levels. Met-enkephalin evaluation is also difficult because of its rapid degradation by unique plasma enzymes.

The aim of the study was to assess the changes in plasma Met-enkephalin concentration in patients with RCC who underwent radical nephrectomy. The influence of the RCC grade (according to the Fuhrman scale) on the Met-enkephalin profile was also investigated.

Material and Methods

The Patients

The study involved 39 patients, divided into two groups: Group I (n = 21) was comprised of patients with RCC, and Group II (n = 18) were healthy volunteers (the control group). All the patients with RCC underwent radical nephrectomy in the Department of Urology at Rydygier Memorial Hospital (Krakow, Poland). The presence of kidney tumors had been confirmed on the basis of prior diagnostic tests (ultrasound, urography and computer tomography). All the qualified patients were stratified according to the degree of clinical advancement before the treatment, using the TNM classification.

Exclusion Criteria

Patients with RCC in their sole remaining kidney and those in whom bilateral cancer of the kidneys was suspected were excluded from the study.

Study Protocol

The study included patients in good general health, with no history of cancerous diseases of other organs, chronic inflammatory conditions, kidney or liver failure, rheumatoid disease or chronic allergic states. In Group I there were 11 men and 10 women of an average age of 63.8 years (range: 52–77 years). Healthy volunteers comprised the control group; their average age was 61.5 years (range: 30–80 years). All the patients in Group I were qualified for radical nephrectomy. Under general anesthesia, the kidney with the tumor was removed via retroperitoneal access. Two independent pathologists carried out pathological examinations of the specimens in the hospital's Department of Pathology.

In Group I the blood samples were taken three times, as follows: 24 hours before the radical nephrectomy, 24 hours after surgery and on the 7th postoperative day. In the control group (Group II) the blood samples were taken at the same time intervals and prepared for examination the same way as in Group I. Radio-immunological methods were used to assess the peptide concentration on the basis of the competitive binding of marked-bond (exogenous) peptides and unmarked (endogenous)

peptides with unique antibodies. Separation of the free form from the bound form with the antibody complex was performed by applying polyethyleneglycol. The Met-enkephalin obtained from plasma as a free peptide was assessed by applying unique antibodies, and the peptide marked with J125 and the radioactivity of trace elements were measured in the Wizard counter. This procedure established the concentration of Met-enkephalin in the blood serum. The range of the standard curve covered concentrations from 3.1 pg/probe to 200 pg/probe. The sensitivity of the method was 1.2 pg/probe.

Study Group Outcomes

None of the patients required blood or plasma transfusions. In all the patients who underwent surgery the wound healed primarily. The hospitalization time was from 7 to 17 days (average: 9.9 days). In all the patients in Group I, the renal cell carcinoma was graded in accordance with the Fuhrman scale, and was found to be at the G2 (n = 10) and G3 (n = 11) stages of advancement.

Statistical Analysis

The results were expressed as mean and standard deviation (± SD). The results were statistically analyzed using the T-test and Mann-Whitney test. A p value of < 0.05 was considered significant. The data were analyzed with Statistica Software.

Results

Free and Bound Met-Enkephalin Plasma Concentration in the Control Group

In the control group (Group II) the concentration of Met-enkephalin (free and bound) was assessed. The concentration of the free fraction ranged between 73.0 pg/ml and 172.0 pg/ml. The average was 122.1 pg/ml. The level of the bound Met-enkephalin fraction ranged from 1900.0 pg/ml to 7100.0 pg/ml (average: 3802.7 pg/ml) (Figure 1).

Free Met-enkephalin Plasma Concentration in Patients with Renal Cell Carcinoma

In the Group I patients with both Fuhrman G2 and G3, the free Met-enkephalin plasma concentrations 24 hours before the treatment ranged from 327.0 pg/ml to 849.6 pg/ml, (average 438.9 pg/ml). In the samples taken from these patients 24 hours after the radical nephrectomy, the concentration of free Met-enkephalin was from 163.0 pg/ml to 292.5 pg/ml (average 191.0 pg/ml). In all the patients with RCC, the concentration of Met-enkephalin (both free and bound) was estimated on the 7th day after the surgery, and the concentra-

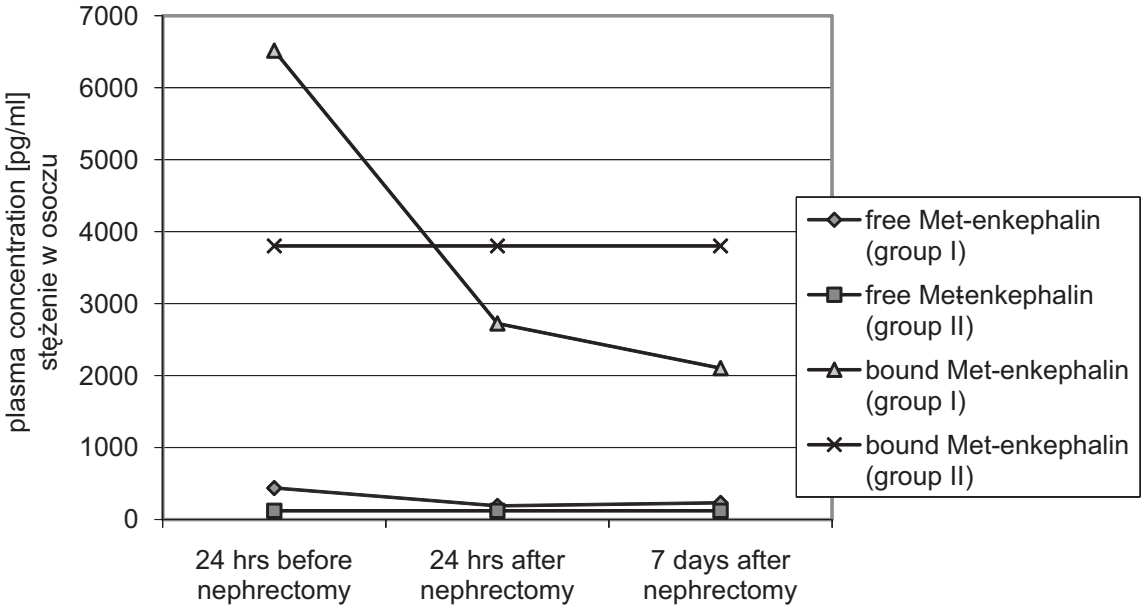

Fig. 1. The plasma profile of free and bound Met-enkephalin in patients with renal cell carcinoma (Group I) and healthy volunteers (Group II) at three times: 24 hours before, 24 hours after and on the 7th day after radical nephrectomy (Group I). In Group II the Met-enkephalin profile was evaluated at identical intervals

Ryc. 1. Profil stężeń Met-enkefaliny (wolnej i związanej) w osoczu krwi chorych z rakiem jasnokomórkowym nerki (grupa I) i w grupie kontrolnej (II) w trzech odstępach czasowych: 24 godziny przed i po nefrektomii radykalnej i w 7. dniu po zabiegu. W grupie II profil Met-enkefalin oznaczono w identycznym odstępie czasowym

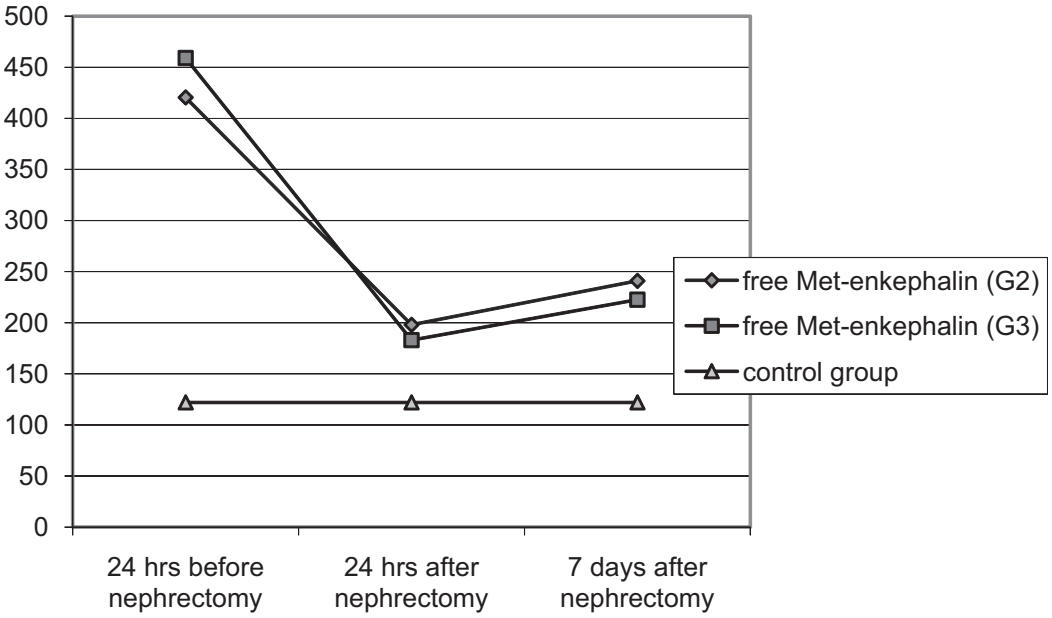

Fig. 2. Changes in the free Met-enkephalin plasma concentration in patients with renal cell carcinoma of Fuhrman grades G2 and G3, as well as in healthy volunteers (the control group – Group II) at three times: 24 hours before, 24 hours after and on the 7th day after radical nephrectomy (G2 and G3). In Group II the Met-enkephalin profile was evaluated at identical intervals

Ryc. 2. Zmiany stężenia wolnej Met-enkefaliny w osoczu krwi chorych z rakiem jasnokomórkowym nerki w zależności od stopnia w skali Fuhrman (G2 i G3) oraz w grupie kontrolnej (II) w trzech odstępach czasowych: 24 godziny przed i po nefrektomii radykalnej i w 7. dniu po zabiegu. W grupie II profil Met-enkefaliny oznaczono w identycznym odstępie czasowym

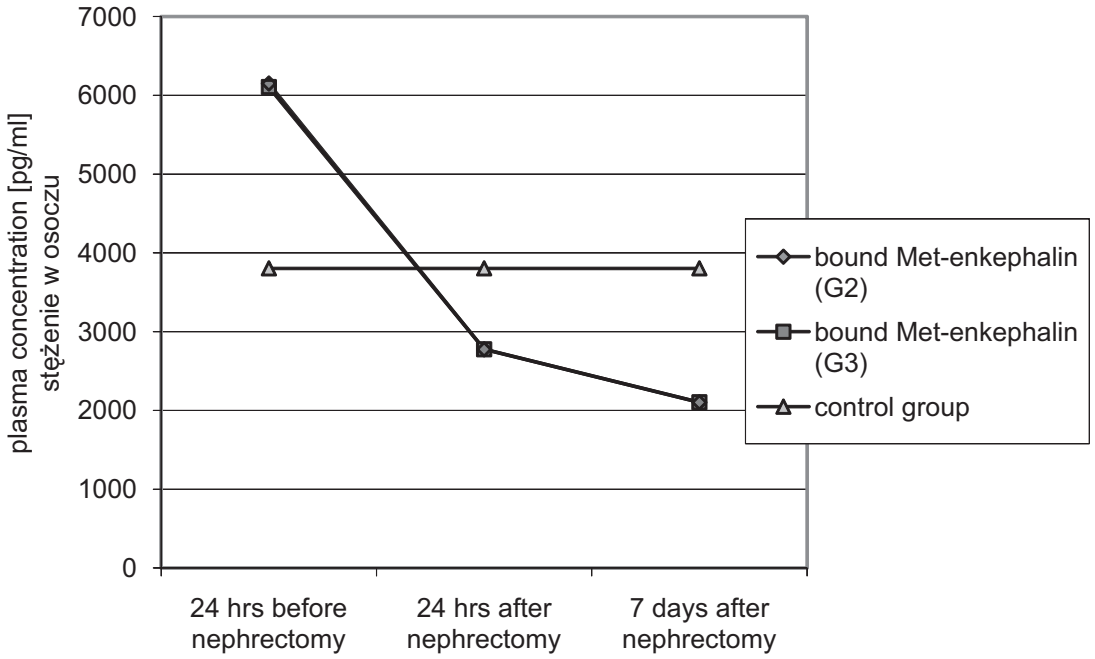

Fig. 3. Changes in the bound Met-enkephalin plasma concentration in patients with renal cell carcinoma of Fuhrman grades G2 and G3, as well as in healthy volunteers (the control group – Group II) at three times: 24 hours before, 24 hours after and on the 7th day after radical nephrectomy (G2 and G3). In Group II the Met-enkephalin profile was evaluated at identical intervals

Ryc. 3. Zmiany stężenia związanej Met-enkefaliny w osoczu krwi chorych z rakiem jasnokomórkowym nerki w zależności od stopnia w skali Fuhrman (G2 i G3) oraz w grupie kontrolnej (II) w trzech odstępach czasowych: 24 godziny przed i po nefrektomii radykalnej i w 7. dniu po zabiegu. W grupie II profil Met-enkefaliny oznaczono w identycznym odstępie czasowym

tion ranged from 156.0 pg/ml to 363.0 pg/ml (average 232.5 pg/ml) (Figure 2).

Bound Met-enkephalin Plasma Concentration in Patients with Renal Cell Carcinoma

The same procedures were used to assess the concentration of bound Met-enkephalin in the entire study group. The average concentration of bound Met-enkephalin 24 hours before treatment was 6516.6 pg/ml, with a range between 4400.0 pg/ml and 12480.0 pg/ml. In the samples taken 24 hours after the surgery, the concentration of bound Met-enkephalin ranged from 2010.0 pg/ml to 3610.0 pg/ml, with an average concentration of 2725.2 pg/ml. On the 7th day after the treatment the average concentration of bound Met-enkephalin was 2104 pg/ml, with a range from 1660.0 pg/ml to3000.0 pg/ml (Figure 3).

Changes in Free Met-enkephalin Plasma Concentration in Healthy Volunteers and Patients with Renal Cell Carcinoma

In the control group the average concentration of free Met-enkephalin was 122.0 ± 8.0 pg/ml. This was similar to the values obtained by Olson et al. [13]. However, in the blood serum of patients with RCC with a G2 Fuhrman grade, the concentration of Met-enkephalin before the operation was about 244% higher than in the control group (420.0 ± 19.0 pg/ml). This difference was statistically significant ($p < 0.001$) (Figures 1 and 2). After the operation (24 hours) in spite of the sudden decrease in the concentration of free Met-enkephalin to 198.0 ± 12.0 pg/ml, this value was still significantly higher – by about 62% – than the opiate concentration in the blood serum of the control group ($p < 0.05$). Additionally, it is interesting that after 7 days, the concentration of free Met-enkephalin didn't reach a value similar to what was observed in the control group, but increased to 241.0 ± 20.0 pg/ml ($p < 0.001$).

Moreover, the concentration of free Met-enkephalin in the blood serum of the patients with Fuhrman G3 RCC was higher before the operation than in those with G2 RCC ($p < 0.1$). Similarly to patients with Fuhrman G2 RCC, the opiate concentration in patients with G3 RCC was about 290% higher than in the control group: 476.0 ± 14.0 pg/ml ($p < 0.001$) (Figure 2). A considerably larger decrease in the concentration was observed 24 hours after the operation in the Fuhrman G3 group than in the Fuhrman G2 group: 62% vs. 51% ($p < 0.05$), but the value in the G3 group – 183.0 ± 6.0 pg/ml – was still about 50% higher than the opiate concentration in the control group ($p < 0.01$). This tendency was still observed on the 7th day after the operation, when the concentration of encephalin was 222.0 ± 15.0 pg/ml. This was about 82% higher than in the control group ($p < 0.01$) and about 22% higher than on the first day after the operation ($p < 0.05$).

Comparing the changes in the free Met-enkephalin concentration in the blood serum of patients in the Fuhrman G2 and G3 groups emphasizes that in spite of a similar course, the dynamics of the changes in the opiate concentration were clearly higher in the Fuhrman G3 group, but with no statistical significance in the treatment period.

Changes in Bound Met-enkephalin Plasma Concentration in Healthy Volunteers and Patients with Renal Cell Carcinoma

The concentration of bound Met-enkephalin in the control group blood serum was 3802.0 ± 296.0 pg/ml, which was comparable to the values observed by other authors. The concentration of bound Met-enkephalin in the blood serum of Fuhrman G2 RCC patients before the operation was significantly higher – by about 82% – than in the control group, measuring 6891.0 ± 633.0 pg/ml ($p < 0.001$). The concentration of the opiate 24 hours after the operation was 2679.0 ± 173.0 pg/ml, which represents a decrease of about 61% from the value in the first blood sample ($p < 0.05$) and was about 29% lower than in the control group ($p < 0.05$) (Figures 1 and 3). In the samples taken 24 hours after the surgery the concentration of Met-enkephalin decreased to 2063.0 ± 121.0 pg/ml, which was about 70% lower than in the first blood sample ($p < 0.01$), about 45% lower than in the control group ($p < 0.05$) and about 23% lower than on the 7th post-operative day ($p < 0.05$). Additionally, the concentration of Met-enkephalin in the Fuhrman G3 group was 6105.0 ± 257.0 pg/ml before the operation, which was about 61% higher than the level in the control group ($p < 0.01$). In the Fuhrman G3 group, as in the G2 group, the concentration of bound Met-enkephalin 24 hours after the operation decreased to 2776.0 ± 149.0 pg/ml, which was about 54% of the level in the first blood sample ($p < 0.01$). This value was also lower than in the control group (about 27%; $p < 0.05$). It is also important to note that after six post-oper-

ative days (on day 7), the concentration of bound Met-enkephalin underwent a further decrease, to to 2103.0 ± 108.0 pg/ml, which was about 46% of the level in the control group and about 24% of the level in the second blood sample ($p < 0.01$).

Comparing the changes in the serum concentration of bound Met-enkephalin in patients with Fuhrman G2 and G3 RCC shows that the changes were considerably less pronounced in the G3 group, but without statistically significant differences between the two groups in second and third blood sample. The only statistically significant differences ($p < 0.05$) observed between these two groups of patients were in the Met-enkephalin concentrations in the first blood samples, taken before the operation.

Discussion

Cancer of the kidney is recognized as immunological cancer, which means that the clinical course depends on the relationship between the tumor and the patient. Various authors have compared the immunological effects of opiate and somatostatin activity [14]. These immunological effects are present in both healthy and cancerous cells and can adjust their levels through unique receptors. Experimental studies using ligands (substances which uniquely bind to receptors) provide a better evaluation of some types of opioids receptors.

Among these are the so-called Zeta receptors, which have a higher ability to bind Met-enkephalin. Met-enkephalin activity is transferred through Zeta receptors as well as Delta receptors in different tissues. The presence of these receptors has been established in all examined fetal and cancerous cells [15, 16]. Tests have demonstrated a reduction in the proliferation of cancer cells of the breast, ovary, pancreas, kidneys, large intestine, lungs and brain within 2–12 hours of adding Met-enkephalin to the cell culture in 48–70% of all cultured cells. This suppressing opiate effect is maintained for several days, leading to long-term impairment of the growth of cancerous cells [17, 18].

The suppressing effect of Met-enkephalin is reversible and nontoxic. The opiates act on the cell membrane directly through the receptors and initiate the activation of G proteins, which participate in conducting information from the receptor to the effectors, regardless of the primary signal. (Gilman and Rodbell received a Nobel Prize in 1994 for discovering G proteins and their role in transmitting information to cells.)

It is hard to ascertain the origin of enkephalins in blood, because it isn't certain that they are synthesized in tumors. The majority of experiments are being conducted on cell lines or on human cancer grafts (mouse cancer models), so the test results must be treated with caution. It is also difficult to establish the baseline concentration of Met-enkephalin in blood plasma due to the rapid degradation of Met-enkephalin through certain specific enzymes.

Some researchers assume that opiates can stimulate apoptosis [19, 20]. Maneckjee et al. and Zagon et al. demonstrated the role of Met-enkephalin in cell development. Opiates can be considered growth factors. These factors suppress the replication of cancerous cells during carcinogenesis, and this process is conducted through Zeta receptors, which aren't expressed in healthy cells of an adult organism. The opiate concentration in a tumor changes with the intensity of the carcinogenesis [10]. The results of the present study show that the changes in the concentrations of free and bound Met-enkephalin are dependent on the degree of pathological advancement (Fuhrman grading).

It is difficult to measure the opiate concentration in blood, since this concentration is a result of many processes: Firstly, the opiate level depends on the degree of its synthesis in the central nervous system, as well as in the peripheral nerves, endocrine glands (the pancreas, adrenal glands) and other tissues (intestines, the heart). Secondly, opiates are secreted either directly from the place of synthesis or from various opiate-storing tissues. Thirdly, changes in the activity of the enzymes (aminopeptidases and carboxypeptidases) that cause the hydrolysis of the precursor of bound and free Met-encephalin may lead to changes in opiate concentration. Fourthly, the opiate receptors' binding abilities can alter the opiate concentration. Fifthly, the opiate system's interactions with the nervous, immunologic and endocrine systems seem to affect its profile.

Multicenter studies conducted on animal models (mice, rats, sheep) as well as clinical trials have demonstrated that serum opiate levels undergo major changes during stressful situations such as carcinogenesis or surgical intervention [13]. The present study analyzed changes in the level of Met-enkephalin in the pre- and post-operative periods: first in the presence of cancerous cells in the kidney (the first blood sample); next shortly after surgery, which is a strong stress factor (the second blood sample); and third after six days of hospitalization, when the regeneration processes are taking place in the organism (the last blood sample). The results observed in RCC patients showed an increased concentration of both free and bound Met-enkephalin in comparison with healthy volunteers.

Met-enkephalin acts as an anticarcinogenic factor. Perhaps that is the reason for the observed increase in the concentration of the opiate in the plasma of RCC patients. Similar higher concentrations of free and bound Met-enkephalin in patients with pancreatic cancer and with neck cancer have been described [9, 10].

In the current study patients with Fuhrman G2 or G3 RCC had opiate concentrations about 244% and 290% (respectively) higher than the levels in the control group. This indicates increased activity of the opiate system in RCC patients. It may also suggest that as carcinogenesis increases, so does the release of opiate free forms. Simultaneously the concentration of bound encephalin in the Fuhrman G2 groups decreased, which may confirm this hypothesis. The results and conclusions of this study correlate with the results and observations in a study by Gustin et al. on serum enkephalin concentration and clinical stage in brain tumor patients [21].

Another observation was that free Met-enkephalin concentration in the Fuhrman G3 group was significantly higher than the concentration in the G2 group. It is obvious that the organism is responding more strongly to the greater malignancy of the cancer in group G3, which may explain the increased level of circulating Met-enkephalin.

In vivo, Met-enkephalin suppresses the growth of cancer. Also, the dynamics of the changes in free Met-enkephalin concentrations after the operation provide information about the organism's diversified reaction to the kidney tumor. The decrease in free Met-enkephalin levels 24 hours after the operation in the Fuhrman G3 group was bigger than in patients in the Fuhrman G2 group. This fact also confirms the statement that more malignant tumors evoke a stronger reaction from the organism. In the next six days after the operation, it is not the absence of the tumor that stimulates the increase of the level of Met-enkephalin – the operation itself and the healing processes are responsible for the elevated Met-enkephalin concentration.

Another observation was that the bound Met-enkephalin levels in RCC patients were about 60–80% higher than in the control group. It seems that the stressful situation activated other opiate resources, like the core of the adrenal glands, bowel etc. It appears to be an increased demand for enkephalins, which have a strong analgesic action. On the other hand, it may be an attempt to suppress the proliferation of cancerous cells. It is also possible that Zeta receptor activity is changing in the cells and that this acts as a signal for increased free enkephalin synthesis. This observation correlated with the sudden decrease in the concentration of free and bound Met-enkephalin 24 hours after the operation in the RCC patients. Because the cancer had been removed at that time, the receptors did not need to maintain a high opiate level for analgesic reasons or for apoptosis. It is difficult to explain the decreased level of bound Met-enkephalin, which at 24 hours after the surgery was even lower in the RCC patients than in the control group. The authors do not know exactly when the increase in bound Met-enkephalin starts in the organism. The regeneration of the organism, the lack of negative factors, local recurrence or metastasis may be responsible for the release of Met-enkephalin. Other authors have observed very high levels of Met-enkephalin in the blood of patients with cancer of adrenal glands, which decrease after the operation [22]. Yoshida et al. suggested that the tumor was the source of opiates in this case. It is also possible to speculate that Met-enkephalin was released from the core of the adrenal glands and sympathetic nerve endings. The high level of enkephalin could also be an effect of impaired degradation and increased hydrolysis of free Met-enkephalin from plasma proteins. They also demonstrated that patients with cancer of the kidneys who were receiving interleukin II had a reduced level of Met-enkephalin in plasma, which probably resulted from the stimulating effect of interleukin and from the tumor necrosis factor (TNF), which suppressed free Met-enkephalin release.

Further investigation of these interactions is needed for a better understanding of the importance of Met-enkephalins in RCC pathogenesis, clinical and oncological outcomes.

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