Ph.D. THESES

THE EFFECT OF HISTAMINE ANTAGONISTS ON HUMAN MELANOMA MALIGNUM PROGRESSION IN SCID MICE

NÓRA SZINCSÁK

UNIVERSITY OF DEBRECEN

MEDICAL AND HEALTH SCIENCE CENTER

DEPARTMENT OF DERMATOLOGY

DEBRECEN, 2007.

THE EFFECT OF HISTAMINE ANTAGONISTS ON HUMAN MELANOMA MALIGNUM PROGRESSION IN SCID MICE

NÓRA SZINCSÁK

UNIVERSITY OF DEBRECEN
MEDICAL AND HEALTH SCIENCE CENTER
DEPARTMENT OF DERMATOLOGY
DEBRECEN, 2007.

I. INTRODUCTION

The frequency of malignant melanoma has increased

Malignant melanoma is a skin tumour with a very high mortality rate, and the incidence of this tumour is increasing rapidly throughout the world. The frequency of this disease has increased especially in the last two decades. According to statistics of European countries there are 4-15 melanoma cases in 100.000 persons. Consequently it stimulates a plethora of in vitro and in vivo research, modelling melanoma growth on the one hand and searching for effective drugs for possible human therapy on the other.

Mice suffering from severe combined immunodeficiency (SCID)

The technique of xenotransplantation of human tumours, among them melanoma, into immunodeficient mice is widely used. Human tumours, however, only show growth similar to their original growth if they are orthotopically implanted, that is if tumour tissue samples are inoculated into the same organs that they had originally been obtained from. In case of melanoma the organ involved is the epidermis and dermis of the skin. Due to the extremely thin epidermis and dermis in mice, however, the closest way to mimic the human situation is to use subcutaneous or subdermal implantation.

Mice suffering from severe combined immunodeficiency (SCID) are widely used for allo- and xenotransplantation of a variety of cells and tissues, since both T and B cell-mediated immunity have been damaged due to a congenital autosomal recessive mutation. The importance of the SCID mouse model system is that it is suitable for in vivo research which from an ethical point of view must not be allowed to be performed in humans. Substantial field of using SCID mice is xenotransplantation of several tumours. These mice have frequently been used to model tumours of the human haematopoietic system, human lung cancer, retinoblastoma, osteosarcoma and melanoma.

The role of histamine in cell proliferation

Melanoma cells and tissues like many cells produce large quantities of histamine and express L-histidine decarboxylase (HDC), the enzyme responsible for the synthesis of histamine. Histamine and HDC have been detected not only in mastocytes, basophilic cells and histaminergic neurons, but also in platelets, lymphocytes, macrophages and dendritic cells. HDC expression is also high in regenerating liver, in embryonic tissues and in experimental tumours. Consequently it has been evidenced in almost all proliferating cells, thus it may play an important roll both in benign cell division and malignant tumour growth.

Since melanoma cells and tissues but not melanocytes contain relatively large amounts of histamine, the functional significance of histamine can be examined by using specific antihistamines in vitro and in vivo in the human melanoma cell lines and severe combined immunodeficiency (SCID) mice.

Histamine antagonists, like cimetidine and ranitidine act by blocking binding to histamine receptors that are connected with inducible G protein-dependent intracellular signalling pathways and are located on cellular plasma membranes. In addition, it has been suggested that a small amount of locally produced histamine remains within the cell and binds to intracellular cytochrome P450 moieties found in the microsome and the nuclear fraction. This binding could be effectively blocked by the tamoxifen derivate N, N-diethyl-2-[4-(phenylmethyl)phenoxy]-ethanamine-HCl (DPPE).

The role of N, N-diethyl-2-[4-(phenylmethyl)phenoxy]-ethanamine-HCl (DPPE) in tumour proliferation

It was found that DPPE suppressed the in vitro proliferation capacity of lymphocytes from chronic lymphoid leukaemia patients with high HDC levels. According to in vitro research it reduces significantly the cell proliferation both in primary and metastatic melanoma cell lines. Others found in phase I, II trials that DPPE can increase the therapeutical efficiency of drugs for melanoma chemotherapy. In combination with chemotherapy, DPPE protected the body from cytotoxic effects in breast and prostate cancer. However DPPE alone stimulates in vivo tumour growth of breast tumours in rats and leukaemia in mice.

The role of cimetidine and ranitidine, histamine antagonists in tumour proliferation

Many research groups showed that cimetidine inhibits the growth of several types of tumours, both in vitro and in vivo. It has been shown to inhibit the growth of human gastric and colorectal cancer injected to nude mice. According to others it increased the survival of patients suffering from gastrointestinal tumours. Furthermore, cimetidine increased the volume doubling time of human bronchogenic carcinomas xenografted in immunosuppressed mice and inhibited the growth of human lung cancer as well as human neoplastic glia in vitro. Human ovarian carcinoma in nude mice has been shown to be favourably affected by cimetidine.

In contrast, ranitidine, another potent histamine H2 antagonist, did not inhibit the proliferation or in vivo tumour growth of a human colon cancer cell line. Others claim that ranitidine as well as cimetidine decreased the in vitro growth of human gastric carcinoma, but the effect was not significant.

Cimetidine and ranitidine have been shown by many authors to inhibit the growth of melanoma stimulated by histamine, both in vitro and in vivo in mice. Promising data from a phase II trial also prove the strength of cimetidine in the therapy of metastatic melanoma. Ranitidine, similarly to cimetidine can be an active immunotherapeutical agent in the treatment of human melanoma.

II. OBJECTIVES

In our work we planned to examine the following :

1. The examine of the in vitro proliferation of HT168 and M1 human melanoma cell lines under treatment with several histamine antagonists, like cimetidine, ranitidine and a tamoxifen derivate N-diethyl-2-[4-(phenylmethyl)phenoxy]-ethanamine-HCl (DPPE).
2. To detect the presence of H2 receptor mRNA (by reverse transcription-polymerase chain reaction) and its activity in HT168 melanoma cells.
3. To follow up of the in vivo growth of HT168 and M1 melanoma cell lines in SCID mice.
4. To the effect of several antihistamine agents (cimetidine, ranitidine, DPPE) and their combinations on the growth characteristics in SCID mice inoculated with human HT168 melanoma cells in vivo, and to compare the mean tumour volumes after treatments.
5. To examine the effect of antihistamines on the tumor development potential of melanoma cells.
6. To follow the survival characteristics of SCID mice after treatment and in controls.
7. To compare the HDC expression in HT168 cells in vitro and in vivo by Western blot analysis.
8. To study the histology, immunoreactivity and types of cells infiltrating into the growing tumours.

III. MATERIALS AND METHODS

Drugs

Cimetidine and ranitidine were purchased from Tocris Cookson Ltd (London, UK), DPPE was synthesized by Department of Organic Chemistry, Eotvos University (Budapest).

Melanoma cell lines

We used a primary melanoma HT168 cell line with a low liver metastatic capacity. Initially we also used the M1 cell line with a high liver metastatic capacity, isolated from the liver metastases of the HT168 cell line.

Melanoma cell culture

Human melanoma cell lines were cultivated in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) and 0.16 g/l gentamicin. Cultures were incubated at 37^{o}C in a water-saturated atmosphere of 5% CO_2/95% air. Cells were passaged every 3 days. Before treatment, cells were incubated for 24h after the last passaging at 37^{o}C in RPMI 1640 medium containing

10% FCS in a 5% CO_2 humidified incubator, at a density of 4,5 x 10^4 cells/ml medium. The medium was then replaced and the HT168 cells were treated with DPPE (10^{-5} to 10^{-6} M), ranitidine (10^{-5} M), cimetidine (10^{-5} M) or combinations of these. Cultures were incubated at 37°C in a 5% CO_2 for 48h. After incubation the cells were washed with phosphate buffered saline (PBS) and then lysed for further analysis.

Protein extraction and Western blot analysis

Cultivated cells or snap frozen (liquid nitrogen) tissue specimens were lysed in lysis buffer for protein extraction. The nuclear fraction was separated by centrifugation and the supernatant was used to determine the expression of HDC by Western immunoblotting.

Antibodies

The primary polyclonal antibody was produced in chicken against the epitopes containing the 318–325 (VKDKYKLQ) amino acid sequence of human HDC (Promega, Madison, Wisconsin, USA).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

For this 15 µg protein per sample was loaded onto the denaturing polyacrylamide gel. The blotting was done using a Hoeffer instrument on Amersham nitrocellulose Hybond-C Extra membrane in transfer buffer at 100V and 200mA for 80min. The primary antibody (affinity-purified anti-human $HDC_{318–325}$ IgY (chicken); Promega, USA) was used at a concentration of 40 ng/ml for 60 min. The secondary antibody (horseradish peroxidase-conjugated anti-chicken IgY (rabbit); Promega, USA) was used at a concentration of 0.5 µg/ml for 30 min. Immunoreactive bands were visualized using the ECL (Amersham, England) Western blotting protocol.

MTT assay

For studying cell proliferation, the colorimetric MTT method was used, in which a water-soluble tetrazolium salt (MTT) is converted into an insoluble purple formazan by dehydrogenase enzymes. The supernatants were discarded and the precipitates were dissolved in DMSO. Absorption was read at 540 and 620 nm. In previous experiments we have confirmed that results with the MTT assay correspond to those of [^{3}H]thymidine incorporation.

Detection of H2 histamine receptors on HT168 melanoma cells

H2 receptor binding capacity was measured in the presence of 3 nM of [^{3}H]tiotidine. Non-specific binding was defined with 5 mM histamine HCl. The reaction was stopped by rapid filtration under vacuum over Whatman GF/B glass fibre filters. Radioactivity retained on the filters was measured by liquid scintillation counting in 3 ml of OptiPhase HiSafe cocktail.

Detection of H2 histamine receptor mRNA by reverse transcription-polymerase chain reaction

Reverse transcription (RT) was carried out using Perkin Elmer reverse

transcriptase on a Pharmacia Ataq Gene Controller. RNA samples (1.5 µg) were reverse transcribed at 42°C for 30 min, then at 99°C for 5 min. For polymerase chain reaction (PCR) amplification, the reaction mixture consisted of 1x buffer, 1mM $MgCl_2$, 0.1 mM dNTPs, 2.25 U Taq polymerase, 25 pmol sense and antisense primers, templates (10 µl) and destilled water in a final volume of 50 µl. The oligonucleotide primers used for the H2 receptor were 5'-TCG TGT CCT TGG CTA TCA C for the sense primer, and 5'-CCT TGC TGG TCT CGT TCC T for the antisense primer. The resulting PCR products were separated on 2% agarose gels containing 0.01% ethidium bromide. Samples were run under 100 V for 45 min. The size of the PCR product corresponding to H2 receptor mRNA was 330 bp.

Animals

The immunodeficient CB-17 scid/scid mouse population originated from the laboratory of the Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA. Typical SCID features were proven by measuring serum IgM in the mice. In the initial experiments animals were injected with 5×10^6 M1 or HT168 melanoma cells in 200 µl PBS, subdermally into the abdominal skin. In further experiments we injected the mice with HT168 melanoma cells and divided them into groups (7–12 mice), which were given the following agents in their drinking water, keeping the doses constant, from the day after the injection of cells: DPPE alone (4 mg/kg per day per mouse); DPPE (4 mg/kg per day per mouse) in combination with cimetidine (50 mg/kg per day per mouse); DPPE (4 mg/kg per day per mouse) in combination with ranitidine (50 mg/kg per day per mouse); cimetidine alone (50 mg/kg per day per mouse); ranitidine alone (50 mg/kg per day per mouse). The individual test groups had their own control groups who were given only plain drinking water from the day after the injection of cells.

Measurement of tumour growth

The size of individual subdermal growing tumours was measured weekly by calliper for 6–9 weeks after injection of the cells, and tumour volume was calculated using the formula tumour volume = length × width × height. The mean tumour volume was then calculated for each group.

Histopathological assessment

The mice were killed 6–9 weeks after the injection of cells and the tumours together with a thin stripe of the mouse skin were resected. Tissue samples were embedded in paraffin and 5 µm sections were stained with haematoxylin and eosin. At the same time frozen sections were also prepared. Tumours were examined with respect to grade of differentiation, proportion of nucleus and cytoplasm, pleiomorphism of cell nucleus, and presence of melanin. In addition, deparaffinated tissue sections were incubated in 1% bovine serum (BS) and 1% saponin/PBS, stained with either fluorescein isothiocyanate-labelled rat anti-mouse interferon-γ IgG1 antibodies or phycoerythrin-labelled rat anti-mouse CD11b IgG2b antibodies, which detect infiltrating murine macrophages, washed and then examined using a confocal microscope. Similarly labelled isotypic antibodies were applied as controls.

Statistical analysis

The Student's t-test, analysis of variance (ANOVA), Holm-Sidak, Fisher and Logrank tests were used to measure differences resulting from the different treatment regimens in all experiments. Results were expressed as the mean ± SEM. Differences between results were considered to be significant if $P < 0.05$.

IV. RESULTS

In vitro studies

Study of the in vitro proliferation of HT168 and M1 human melanoma cell lines with several histamine antagonists, like cimetidine, ranitidine and DPPE

The effect of histamine antagonists on the in vitro proliferation of human melanoma cell lines HT168 and M1 has been examined. In HT168 cells, a combination of cimetidine and DPPE significantly reduced (37% inhibition) the proliferation of the cells ($P<0.01$), and ranitidine and DPPE produced a moderate (16%) but significant reduction ($P<0.05$). Ranitidine alone and cimetidine alone produced a moderate and non significant reduction.

In M1 cells, H2 histamine receptor antagonists in the presence of DPPE significantly decreased proliferation (cimetidine + DPPE, 27%; ranitidine + DPPE, 23%) ($P<0.05$). Interestingly, in M1 but not in HT168 the inhibition produced by DPPE alone was significant (21% and 26% at concentrations of 1 and 10 µM, respectively). Ranitidine alone and cimetidine alone reduced the proliferation of the melanoma cells, in non significant manner.

Study of the presence of H2 receptor mRNA and its activity in HT168 melanoma cells

The presence of H2 receptor mRNA and its activity in HT168 cells has been proved. H2 receptor binding capacity was measured in the presence of [^{3}H]tiotidine, non-specific binding was defined with excess of histamine. The maximum specific binding (B_{max}) and equilibrium dissociation constant (K_d) values ($0.98 \pm 0.2 \times 10^6$ sites/cell and 18.08 ± 6.77 nM, respectively) from the Scatchard plot analysis clearly indicate the presence of active H2 receptors on HT168 cells. Similar results were obtained with M1 cells.

According to the data in the Materials and methods section we demonstrated the presence of active H2 histamine receptor mRNA in HT168 cells, by reverse transcription-polymerase chain reaction. The size of the PCR product corresponding to H2 receptor mRNA was 330 bp.

In vivo studies

Follow up of the in vivo growth of HT168 and M1 melanoma cell lines in SCID mice

In vivo tumour growth of HT168 and M1 cells has been examined. Tumours appeared in 75% of the mice injected with the HT168 cell line and in 91% of the mice injected with the M1 cell line. The mean tumour volume and in vivo tumour growth in mice injected with the M1 line was higher than that in mice injected with the HT168 line. Except at week 7 ($P = 0.015$), the difference was not statistically significant ($P > 0.05$). For all further experiments the HT168 melanoma line, which showed lower tumour growth potential, was used.

The effect of several antihistamine agents (cimetidine, ranitidine, DPPE) and their combinations on the growth characteristics in SCID mice inoculated with human HT168 melanoma cells

DPPE in combination with cimetidine effectively reduce the in vivo growth of human melanoma in SCID mice

Growth after treatment with ranitidine, cimetidine and DPPE, alone and in various combinations, was compared with tumour growth in controls.

While cimetidine alone enhanced the tumour volume after 3 weeks, when combined with DPPE a very large reduction in tumour mass was found.

The mean tumour volumes in SCID mice after various treatments are given according to the data in the Materials and methods section. Growth after treatment with ranitidine, cimetidine and DPPE alone, and in various combinations, was compared with tumour growth in controls.

Cimetidine combined with DPPE reduced the tumour growth after 3 weeks and it was statistically significant after 4 weeks (t test, $P < 0.05$).

Ranitidine with DPPE or DPPE alone had no inhibitory effect on the growth of HT168 melanoma in SCID mice.

Interestingly ranitidine alone decreased the growth of melanoma in SCID mice as compared to the control group, although it evoked a weaker and more transient inhibition in tumour growth (weeks 3-4 only), while the simultaneous application of DPPE with ranitidine had no inhibitory effect on the growth of HT168 cells in SCID mice.

The effect of H2 receptor antagonists (cimetidine, ranitidine) and DPPE on the tumor development potential of melanoma cells in SCID mice

83% of the mice developed tumours in the control group, but only 33% in the group of mice receiving DPPE in combination with cimetidine. The differences being statistically significant (Fisher test). 55% of the mice developed tumours in the group receiving ranitidine alone. In all mouse treated with DPPE alone, cimetidine alone or DPPE and ranitidine grew tumour.

We examined the effect of antihistamines on the tumor development potential of melanoma cells. The rate of mice with tumours and all living mice in the same treated or non treated groups has related.

Tumours have appeared in control group at the first week. In the group treated with cimetidine and DPPE tumours grew from third week. In addition, both the mean tumour volume and the tumour growth potential in mice receiving DPPE and cimetidine together were lower than in control mice, the differences being statistically significant (Fisher test).

Detection of the survival characteristics of SCID mice after treatment and in controls

Very similar conclusions can be drawn if survival of mice is examined. The survival of mice treated with cimetidine and DPPE significantly exceeds that of controls and other treatment groups.

The HDC expression in HT168 cells in vitro and in vivo by Western blot analysis

The HDC expression of melanoma cells in vitro and in vivo was compared by Western blot analysis. Regardless of the presence or absence of antihistamines, HDC expression in HT168 cells increases under in vivo conditions compared with that of in vitro cultivated cells; however, the various in vivo treatments did not influence the amount of HDC protein in the tissue extracts. There is a tiny shift in the position of the HDC band in ranitidine-treated mice, but the reason for this is not known.

The histology, immunoreactivity and infiltrating cells of tumours

The histology and immunoreactivity of skin specimens taken from SCID mice injected with HT168 (tumour-bearing control mice) and those injected with HT168 and treated with cimetidine plus DPPE show that this combination enhances the infiltration of IFN-γ-producing macrophages, and that their distribution within the tumour is different in treated and control animals. A similar tendency, but with much fewer cells, was found when natural killer (NK) cells were examined.

V. DISCUSSION

Our in vitro results proved the H2 receptor expression of melanoma cells. It was shown that the H2 receptor antagonist cimetidine when combined with DPPE inhibits the in vitro proliferation of HT168 and M1 melanoma cells. Our in vivo studies are in accordance with this, suggesting that treatment with the H2 histamine receptor blocker cimetidine in combination with DPPE, a ligand of intracellular P450 moieties, effectively reduces the in vivo growth of xenografted human HT168

melanoma in immunodeficient mice. Furthermore this combination of antihistamines elevates the survival of human melanoma-grafted immunodeficient mice, although there is no obvious correlation between tumour growth/size and survival of animals.

HT168 melanoma cells showed lower tumour growth potential than M1 cells in in vivo studies. For all further experiments the HT 168 cell line was used, which showed longer survival time.

Our immunohistochemical findings suggest that results of the cimetidine and DPPE combined treatment are associated with enhanced infiltration of IFN-γ-producing mouse macrophages and (to a much smaller extent) NK cells into the tumour tissue. Elevated concentrations of INF-γ result in the inhibition of cell proliferation by direct stimulation of natural killer cells.

The previously described effect of DPPE with low anti-oestrogen capacity is based on the interference of DPPE with the binding of histamine to P450 molecules. DPPE causes significant inhibition of the proliferation in melanoma cells. DPPE increased the efficiency of cimetidine in combination. Details of this effect are not as yet clear.

Cimetidine has been shown to inhibit the growth of several types of tumours both in vitro and in vivo in animal models. Our findings suggest that ranitidine alone or a combined application of cimetidine and DPPE both reduce tumour growth in immunodeficient mice in vivo, although with different kinetics, as ranitidine alone decreased the growth of melanoma injected to SCID mice less. These data can be explained by results showing that cimetidine, but not ranitidine, dramatically elevates the INF-γ production by human lymphocytes via a possibly histamine-independent pathway, likely through cytochrome P450 moieties. This would also explain why cimetidine but not ranitidine was effective in our experimental system using HT168 human melanoma cells. Some researchers claim that increased histamine level can help develop tumour proliferation while having an immunosuppressive effect, which helps tumour cell growth by switching off the activity of NK (natural killer) cells. Cimetidine however may inhibit cell proliferation by stimulating NK cells.

Since SCID mice possess a defective T cell system, in addition to a direct inhibitory effect on melanoma cells, suggested by the in vitro findings, one possible explanation of our findings could be that cimetidine and DPPE locally activate mouse macrophages and NK cells, and these cells reverse tumour formation by HT168 cells. This complex interaction is further complicated by the fact that both IFN-α and IFN-γ suppress HDC expression in melanoma cells in vitro. However, in the present system antagonists did not greatly influence the expression of HDC, which was lower than that in in vivo conditions. It is possible that there is a mutually inhibiting interaction between endogenous histamine in melanoma and the cells of the local immune system. In contrast to IFN-γ at least in the advanced stages, histamine-inducible interleukin-6 has been shown to act as an autocrine supporting factor in melanoma, suggesting that a highly complex network is involved in the in

situ regulation of melanoma growth.

Our data suggest that the melanoma cell line HT168 expresses H2 receptors and that blocking by cimetidine combined with DPPE inhibits the growth of melanoma. Since HDC is strongly expressed in melanoma cells and tissue, autocrine enhancement of melanoma growth through H2 histamine receptor is suggested. These findings suggest that two different mechanisms are probably acting concordantly: direct inhibition of tumour cell proliferation by the H2 receptor antagonists, and activation of the local immune response characterized by interferon-γ production. Our work may help elucidate the possibility of a rationally designed antihistamine strategy in melanoma therapy.

VI. SUMMARY

1. The H2 receptor antagonist cimetidine when combined with N, N-diethyl-2-[4-(phenylmethyl)phenoxy]-ethanamine-HCl (DPPE), a tamoxifen derivate, significantly inhibits the in vitro proliferation of both HT168 cells and M1 melanoma cells. Ranitidine however alone caused weaker inhibition.
2. The presence of H2 receptor mRNA in HT168 cells is proved and the presence of active H2 receptors on HT168 cells is demonstrated.
3. The mean tumour volume and in vivo tumour growth in mice injected with the M1 line was higher than that in mice injected with the HT168 line.
4. Cimetidine, a H2 histamine receptor antagonist combined with DPPE considerably reduce the growth of HT168 melanoma cells in SCID mice in vivo.
5. When the effect of antihistamines on the tumor development potential of melanoma cells was examined, we concluded that growth potential was lower in mice receiving DPPE and cimetidine together, than that of control and differentially treated mice.
6. The survival of mice treated with cimetidine and DPPE significantly exceeds that of controls and other treatment groups.
7. The HDC expression in HT168 cells increases under in vivo conditions compared with that of in vitro cultivated cells; however, the various in vivo treatments did not influence the amount of HDC protein in the tissue extracts as the Western blot analysis shows.
8. The histology and immunoreactivity of skin specimens taken from SCID mice injected with HT168 and treated with cimetidine plus DPPE in contradiction to the control group show that this combination enhances the infiltration of IFN-γ producing macrophages and natural killer cells (NK cells) less.

VIII. PUBLICATIONS:

Publications on the subject of the thesis:

1. **Szincsák N**, Hegyesi H, Hunyadi J, Martin G, Lázár-Molnár E, Kovács P, Rivera E, Falus A, Juhász I. Cimetidine and a tamoxifen derivate reduce tumour formation in SCID mice xenotransplanted with a human melanoma cell line. *Melanoma Res.* 2002 Jun; 12(3):231-240.

IF: 1,773

2. **Szincsák N**, Hegyesi H, Hunyadi J, Falus A, Juhász I. Different H2 receptor antihistamines dissimilarly retard the growth of xenografted human melanoma cells in immunodeficient mice. *Cell Biol. Int.* 2002; 26(9):833-836.

IF: 1,017

Other publications:

1. Nagy T, Glavinas H, **Szincsák N**, Hunyadi J, Jánossy T, Duda E, Vizler C, Juhász I. Tumor cells expressing membrane-bound tumor necrosis factor activate macrophages and have a compromised growth in immunosuppressed and immunodeficient mice. *Cancer Lett.* 2003; 196(1):49-56.

IF: 2,614

2. Márián T, Szabó G, Goda K, Nagy H, **Szincsák N**, Juhász I, Galuska L, Balkay L, Mikecz P, Trón L, Krasznai Z. In vivo and in vitro multitracer analyses of P-glycoprotein expression-related multidrug resistance. *Eur. J. Nucl. Med. Mol. Imaging*. 2003 Aug; 30(8):1147-1154.

IF: 3,324

Abstracts:

1. Juhász I, Veres I, **Szincsák N**, Simics E, Hunyadi J. Introduction of a novel transplantation model for experimental studies of the human pilosebaceus unit. *J. Invest. Dermatol.* 1997; 109(3):457

2. Győry I, Nagy T, **Szincsák N**, Kusz E, Falus A, Juhász I, Hunyadi J, Duda E. Cytokine gene therapy of tumors in animal model systems. *J. Invest. Dermatol.* 1998; 110(4):523

3. Juhász I, **Szincsák N**, Nagy T, Kusz E, Jánossy T, Glavinas H, Hunyadi J, Duda E. Investigation of tumor necrosis faktor-α induced tumor rejection in mice with severe combined immunodeficiency. *J. Invest. Dermatol.* 1999; 113(3):496.

4. Krasznai Z, **Szincsák N**, Rubovszky B, Goda K, Szabó G, Juhász I, Galuska L, Bálint K, Dezső B, Trón L, Márián T. In vivo visualization of multidrug resistance

by parallel administration of 18FDG and 99mTc-MIBI. *Eur. J. Nucl. Med.* 2000; 27(8):1181.
5. Hegyesi H, **Szincsák N**, Martin G, Lázár-Molnár E, Kovács P, Rivera E, Hunyadi J, Falus A, Juhász I. Reduction of tumor formation in SCID mice xenotransplanted with human melanoma cell line by cimetidine and a tamoxifene derivate. *J. Invest. Dermatol.* 2001; 117(3):782.
6. **Szincsák N**, Márián T, Nagy H, Galuska L, Trón L, Balkay L, Krasznai Z, Hunyadi J, Juhász I. Visualization of multidrug resistance with radiotracers in scid mice. *J. Eur. Acad. Dermatol. Venerol.* 2004; 18(1):99.