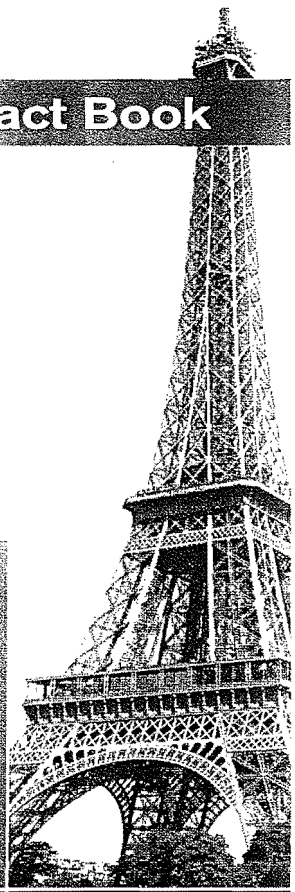
ICACT

23rd International Congress on Anti-Cancer Treatment

Abstract Book



Paris, France
Palais des Congrès
31st January - 2nd February 2012

PRESIDENTS

David KHAYAT, MD, PhD - *Paris, France*

Gabriel N. HORTOBAGYI, MD, PhD - *Houston, USA*

GENERAL SECRETARIES

Robert S. Benjamin, MD, PhD - *Houston, USA*

Daniel G. Haller, MD - *Philadelphia, USA*

Peter Harper, MD - *London, UK*

Moïse Namer, MD, PhD - *Nice, France*

Paris

This Meeting has been accredited By the ESMO, UEMS and ACOE

www.icact.com

Académie Nationale de Chirurgie

POSTER

IC/AB235

■ *THE EFFECTS OF UKRAIN (NSC631570) IN AN IN VITRO MODEL OF FIBROID TUMOR CELLS (ERL)*

KAROLY Stefan
NOWICKY Askold

Medical University Of Vienna, Ukrainian Cancer Institute, OBGYN, Vienna - Austria
Ukrainian Cancer Institute, Vienna - Austria

Introduction
Ukrain (NSC631570) is a semisynthetic compound derived from alkaloids of the plant Greater Celandine. Recent studies suggest that NSC631570 has a cytolytic and cytostatic effect on various human cancer cell lines including pancreatic, melanoma, renal and glioblastoma 1,2,3,4,5,6 while displaying no toxicity on healthy human cells7. It was shown1,6, that NSC631570 inhibits MMP-2 and MMP-9 in glioblastoma (human cultured glioblastoma cells) and pancreatic (HPAF-II, PL45, HPAC) cancer cell lines. However, this effect has not been investigated in fibroid tumor.

Materials and Methods
To examine the in vitro toxicity of NSC631570 in fibroid tumor, we used ERL, a human fibroid tumor cell line containing luciferase. The cells were cultivated in a 48 well plate at a concentration of 5000 cells/ well, and were treated with different concentrations of NSC631570 (25μg/ml in 10% media, 50μg/ml in 10% media, 75 μg/ml in 10% media and 100 μg/ml in 10% media). Following 24, 48 and 72 hours of treatment with NSC631570, cells were trypsinized and manually counted. After 72 hours of treatment with NSC631570, MTT-assay8 was used to determine cell viability.

Conclusions
We observed a time and dose-dependent inhibition of cell growth in the ERL cell line. In conclusion, NSC631570 seems to have cytolytic and cytotoxic effects in an fibroid tumor in vitro setting. Further studies should be conducted to examine the effect in an in vivo model.

POSTER

IC/AB264

■ *PAIN EVALUATION IN AN ONCOLOGY DEPARTMENT: PREVALENCE, CHARACTERIZATION AND TREATMENT*

CUSTODIO Sandra
JOAQUIM Ana, PEIXOTO Vânia, ESPIGA
MACEDO Joana, FARIA Ana Luísa, MACIAS Emílio,
REGO Sonia, ARAUJO António

Centro Hospitalar De Entre O Douro E Vouga (Chedv), Medical Oncology, Santa Maria Da Feira - Portugal
Chedv, Santa Maria Da Feira - Portugal
Chedv, Santa Maria Da Feira - Portugal
Chedv, Santa Maria Da Feira - Portugal
Chedv, Santa Maria Da Feira - Portugal
CHEDV, Santa Maria Da Feira - Portugal
Chedv, Santa Maria Da Feira - Portugal
Chedv, Santa Maria Da Feira - Portugal

Introduction
Pain is one of the symptoms most feared by cancer patients. It is estimated that 50% of cancer patients have at least one episode of severe pain. Studies show that the majority are under-treated. Objectives: Determine the prevalence of pain in a sample of cancer patients followed in the Oncology Department of Centro Hospitalar de Entre o Douro e Vouga (CHEDV), and make their characterization (demographic, clinical and pain data).

Methods
Cross-sectional study using a convenience sample of patients preformed between April 2010 and June 2011. Collection of data was made through the application of a questionnaire. For registration and data analysis Microsoft Excel 2007 and SPSS were used.

Results
Were included 224 patients, 50% female, with an average age of 65 years. Ninety-three patients (42%) reported pain in the preceding 24 hours. Most of these had a metastatic or advanced disease (stages III / IV); lung cancer was the most prevalent. With regard to pain intensity: 33% of patients had moderate baseline pain and 10% severe, 48% breakthrough moderate pain and 33% severe. Of the 93 patients with pain, 42% were treated with paracetamol and/or anti-inflammatory

increase, respectively). In relation to IDO, levels were elevated in a clinical stage-dependent fashion (meanSD: 1,28±0,35 in stage I; 1,56±0,33 in stage II; 1,76±0,34 in stage III; 1,94±0,26 in stage IV) being the increase statistically significant in patients with stage III and IV (1.4- and 1.5-fold increase, respectively; $p<0.005$). In addition, there was a significant difference between the patients with (n=30) and without (n=80) lymphatic dissemination ($p<0,001$). Furthermore, IDO levels in recurrent patients (n=13) were significantly increased with respect to IDO levels performed at the time of diagnosis ($p=0,001$).

Conclusions

Our results suggest that IDO could be a good prognostic marker for malignant melanoma and for the early detection of recurrences when IDO levels increase is detected. This study has been supported by grants from the University of the Basque Country, SACYL, Caja Burgos and Mutua Madrileña.

POSTER

IC/AB 309

■ *ANTITUMOR ACTION OF CANCER-SELECTIVE DRUG NSC-631570 IS COMPLEX AND MULTIDIRECTIONAL*

SKIVKA Larysa
FEDORCHUK Olexander, BEZDENEZNIKH Natalia, LYKHOVA Olexandra, SEMESIUK Nadija, KUDRYAVETS Yuri, SUSAK Mychailo

Taras Shevchenko National Kyiv University, immunology, Kyiv - Ukraine
R. E. Kavetsky Institute Of Experimental Pathology, Oncology And Radiobiology, National Academy Of Sciences Of Ukraine, Kyiv - Ukraine
R. E. Kavetsky Institute Of Experimental Pathology, Oncology And Radiobiology, National Academy Of Sciences Of Ukraine, Kyiv - Ukraine
R. E. Kavetsky Institute Of Experimental Pathology, Oncology And Radiobiology, National Academy Of Sciences Of Ukraine, Kyiv - Ukraine
R. E. Kavetsky Institute Of Experimental Pathology, Oncology And Radiobiology, National Academy Of Sciences Of Ukraine, Kyiv - Ukraine
O.Bogomolets National Medical University, Kyiv - Ukraine

Background

NSC631570 is a greater celandine (Chelidonium majus) alkaloid derivative with cytostatic activities. The preparation has ability to be selectively accumulated in cancer cells. It has been confirmed due to its unique property to autofluorescence under UV light. The mechanisms of action of the preparation have not been yet extensively investigated. It is known that NSC631570 induces tumor cell apoptosis independently of death receptor signalling via a pathway involving mitochondrial damage, cytochrome c release in the cytoplasm and caspase-activation that is partly sensitive to overexpression of Bcl-2, Bcl-xL and a dominant negative caspase-9. Therapeutic effect of NSC631570 is accompanied by immune responses stimulation. The aim of our study was to investigate mechanisms of this phenomenon.

Methods

Low- and high-metastatic variants of B16 melanoma cells (MM-4 and MM-4M2 respectively) were used. NSC631570 (Nowicky Farma, Austria) was use at the apoptogenic and nonapoptogenic concentrations. TAP1 and TAP2 mRNA expression was evaluated in RT-PCR. Tumor cell death by apoptosis was assessed by flow cytometry. To investigate tumor cell death immunogenicity HMGB1 expression was determined in cell culture medium using a specific anti-HMGB1 ELISA (Shino Test Corporation). Cis-platinum was used in these experiments as a positive control. To estimate the effect of NSC631570 on functional polarization of tumor associated macrophages adherent murine macrophage population (BMDMs) were generated in the presence of M-CSF from precursors isolated from the femurs of intact mice. For hypoxia induction experiments, the mouse BMDMs were treated under either normoxia (21% O2) or hypoxia (3% O2) for 48 h. Nitrite assay was performed using the Greiss reaction. Arginase activity was determined by colorimetric method.

Results

Treatment of MM-4M2 cells with NSC-631570 at the non-apoptogenic concentration increased expression of mRNA encoding TAP2 by ~3-fold. At

the apoptogenic concentrations both cis-platinum and NSC631570 exert comparable cytotoxicity towards melanoma cells. HMGB1 level in the culture supernatant of cells treated with NSC631570 was significantly higher than that after treatment with platinum compound. The levels of HMGB1 in cell probes treated with NSC631570 exhibited strong correlation with the levels of cell death. Unlike in the cell probes treated with platinum compound the correlation between HMGB1 level and cell death level was absent. The metabolism of L-arginine through arginase by BMDMs was greater and nitric production was lower in cells cultured in hypoxia compared with those cultured under normoxic conditions. Treatment of hypoxia-polarized BMDMs with NSC631570 resulted in increase of nitric production and decrease of arginase activity of phagocytes.

Conclusion

NSC-631570 induces immunogenic death of B16 melanoma cells and could restore antitumor activity of hypoxia-polarized macrophages. It suggests that NSC-631570 can be used for multimodal tumor therapy not only to kill the tumor cells, but also to stimulate a specific immune response to keep residual tumor (stem) cells and metastases under control.

POSTER

IC/AB337

■ *METASTATIC SQUAMOUS CELL CARCINOMA FROM OCCULT PRIMARY TUMOR: A CASE REPORT*

VALLINOTO Rosa
SHVETS Yuliya, GODINHO Joana, AFONSO Mariana, SOARES Marta

Instituto Português De Oncologia, Porto, Oncology, Porto - Portugal
Instituto Português De Oncologia, Porto, Porto - Portugal
Instituto Português De Oncologia, Porto, Porto - Portugal
Instituto Português De Oncologia, Porto, Porto - Portugal
Instituto Português De Oncologia, Porto, Porto - Portugal

Introduction

Malignant neoplasms of unknown primary origin are defined histologically as tumors whose primary site of metastatic disease can not be identified. They have a wide variety of clinical presentation and a poor prognosis. Clinical absence of the primary tumor, early dissemination, aggressiveness and unpredictability of metastatic pattern are characteristic of these tumors. Most of these patients are refractory to systemic treatments and chemotherapy is only palliative and does not increase the survival. Life expectancy is very short, with a median survival of about 6-9 months.

Case report

Woman, 46 years old, with a personal history of hypertension and deep vein thrombosis, with symptoms in the iliac region since December 2010, to walk with limited, requiring of strong opioids (Morphine 120mg/day). The Investigations showed a tomography (CT) abdominal-pelvic and thoracic with a mass in the left iliac bone with 12 cm, which conditioned thrombosis of the left external iliac vein, with blood invasion,and in addition to 2 nodules in the liver suspected metastasis. Endoscopy showed only hemorrhoids. PET-CT showed a tumor in the pelvic region adjacent to the left iliac bone and a conglomerate adenopathy in the left supraclavicular region. Gynecological tumors was excluded. Biopsy of this mass, as well as the conglomerate of supraclavicular region revealed that it was metastatic squamous cell carcinoma. The liver metastasis was not confirmed. The further investigation did not identify the location of the primary tumor. The patient had a good general condition and underwent palliative chemotherapy protocol with docetaxel, cisplatin and 5-fluorouracil in tri-weekly cycles in a total of 6 cycles, with good tolerability. Showed near complete response of the disease in the the iliac region and stable disease of the supraclavicular conglomerate adenopathy. The patient remains alive 10 months after diagnosis, with a good quality of life, pain control without the need for opioids and will undergo radiation therapy to the pelvic and supraclavicular region.

Conclusion

The approach of metastatic tumors of unknown primary origin, requires extensive knowledge on the pathology of malignant tumors. Imaging tests are associated with pathologic analysis such as immunohistochemistry and molecular genetics are key to identifying the primary tumor. This case revealed that although we have not found the primary tumor, the correct histology allowed direct systemic treatment with good response. Although there

SOMPS POSTER AWARD

Given To Dr. SKIVKA Larysa

In recognition of the quality of the work presented at the

23^{b} International Congress On Anti-Cancer Treatment
Held in Paris, February 2012

Pr. David Khayat, *MD, PhD*
S.O.M.P.S. CHAIRMAN

ORDRE : OBJET :

N° 1368088 DATE : MONTANT :

2011010720 10934

1368088075000066908
016910132301

Crédit Industriel et Commercial

Payez contre ce chèque en euros € Cinq cent euro
non endossable sauf au profit d'un établissement bancaire ou assimilé.

à SKIVKA Lausa € 500,00 €

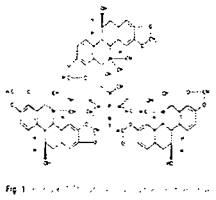
Payable en France. 30088 10934 00010132301
CIC PARIS ST HONORE ENTREPRISES EQUATOUR
11 RUE D'AGUESSEAU
75008 PARIS
Tél 01-40-07-44-30 124 BOULEVARD EXELMANS
75016 PARIS

A Paris
LE 30/01/2012
Signature

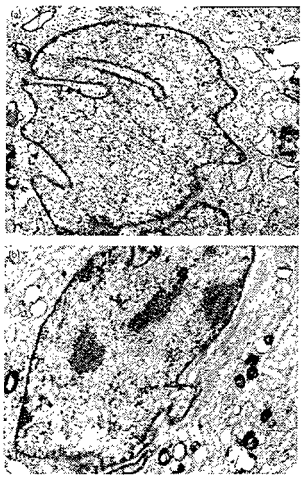
N° 1368088 (21)

1368088 5075000066908 016910132301

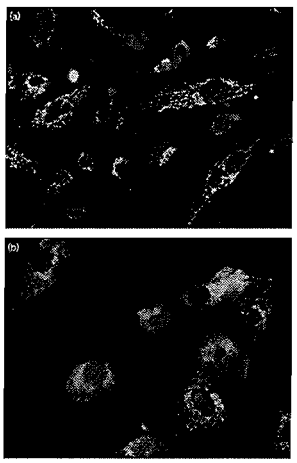
The antitumoral properties of NSC 631570



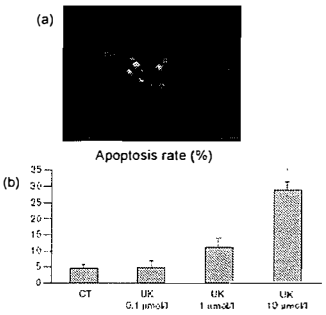
NOWICKY PHARMA, University of Milan, Italy



Representative electron photomicrographs of ultrathin sections of glioblastoma cells (T60) showing nuclear ultrastructure.
(a)Untreated cells (original magnification, 10 000).
(b)Glioblasloma cells after 10 mmol/l of NSC 631570, showing nuclear morphology changes after drug administration (original magnification, 7200).



Microphotographs showing immunofluorescence detection of cytochrome c in controls (a) and 10 mmol/l NSC 631570 -treated glioblastoma (GBM) cells (b).
Untreated control cells showed a punctate cytoplasmic staining pattern typical for mitochondrial localization.
Original magnification, 20.



(a) Representative apoptotic cells determined by Annexin V assay. Flourescent-labeled Annexin V binds to the cell membrane. This pattern, characterized by the absence of blebs, suggests that NSC 631570 (UK)-treated cells are in the early phases of apoptosis. Original magnification, 20. (b) Bar graphs showing apoptosis rates in glioblastoma (GBM), T60 and T63 glioblastoma cells. Mean±SEM. *$P<0.05$ for 10 vs. 0.1 mm NSC 631570. *CT. control.*

NSC 631570 modulates glial fibrillary acidic protein, but not connexin 43 expression, and induces apoptosis in human cultured glioblastoma cells
Nicoletta Gaglianoa, Claudia Moschenia, Carlo Torria, Elena Donettia, Ivana Magnanib, Francesco Costaa, Wassil Nowickyc and Magda Gioiaa

Aim

- **to research new mechanisms of action of NSC 631570**

Methods

-On the cell lines Caki-1, Caki-2, and ACHN it was investigated how NSC 631570 modulates the malignant phenotype of clear cell renal cell carcinoma (ccRCC).

Results

NSC 631570 did not induce E-cadherin/β-catenin immunoreactivity at the cell-cell boundary, although it determined the actin cortical expression in Caki-2 and ACHN, and did not affect vimentin organization; however, in some Caki-1 and ACHN cells the perinuclear concentration of vimentin was consistent with its downregulation. MMP-2 and MMP-9 activity was significantly downregulated 48 h after NSC 631570 administration. At this time point, NSC 631570 significantly decreased migration and invasion, and downregulated SPARC levels in cell supernatants at all doses in Caki-2, and at 20 μmol/l in Caki-1 and ACHN cells. Concomitantly, SPARC was upregulated in all ccRCC cells, suggesting that NSC 631570 could also affect cell proliferation by cell cycle inhibition, as supported by the cell cycle analysis, as SPARC also acts as a cell cycle inhibitor.

CONCLUSION

These results suggest that NSC 631570 can switch the epithelial-to-mesenchymal transition-related phenotype of ccRCC cells, and targets the two major aspects involved in RCC progression: tumor invasion/microenvironment remodelling and cell proliferation.

Background: mechanisms of action of NSC-631570 in cancer cells

-selectively accumulates in cancer cells and causes the dose-dependant inhibition of DNA, RNA and protein synthesis with growth inhibition between 50% and 100%

-causes the disruption of the microtubule network in the investigated malignant cell lines with subsequent accumulation of these cells in G2/M phase

-induces depolarisation of the mitochondrial membrane potential with following reduction of oxygen consumption and caspases activation leading to apoptotic cell death

-caused changes in the concentrations of mitotic cyclin A and B1 as well as Cdk1 and Cdk2. Increased expression of Cdk inhibitor p27 has been also observed in the cancer cell lines. This can promote accumulation of the cancer cells in the G2/M phase

-inhibits the formation of new vessels nutrifying the tumor, i.e. it possesses antiangiogenic properties

-modulates the radiation toxicity against human cancer cell lines and protects normal human fibroblasts from radiation damage

-causes the down-regulation of the secreted protein acidic and rich in cysteine (SPARC) expression. This protein is involved into the regulation of the cell-matrix interactions and tumor invasiveness

-enhances macrophage tumoricidal activity, increases number of monocytes, T-helpers and NK-cells and reduces the number of T-suppressors. Fulfils all criteria of a biological response modifier

All these effects are related to the cancer cells and do not involve the normal cells.

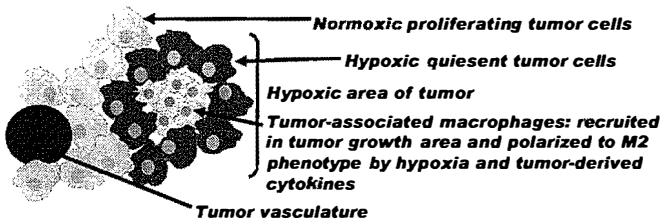
-NCI, Bethesda, Maryland, USA;
-Memorial University of Newfoundland, St. John's, Canada
-University of Rochester School of Medicine, USA;
-University of Ulm, Germany
-Eberhard Karl University, Tubingen, Germany;
-Instituto de Cancerologia, Mexico;
-Emory University, Atlanta, Georgia, USA;
-Kennesaw State University, Kennesaw, Georgia, USA
-Institute of Biochemistry and Molecular Cell Biology, Vienna University, Austria
-Institute of Radiobiology, German Armed Forces Medical Academy, Germany
-University of Milan and University of Pisa, Italy
-University of Miami School of Medicine, Florida, USA;

Antitumor action of cancer-selective drug NSC-631570 is complex and multidirectional

SKIVKA L[1], Fedorchuk O[2], Bezdeneznikh N[2], Lykhova O[2], Semesiuk N[2], Kudryavets Yu[2], Susak M.[3]
[1]Taras Shevchenko Kyiv National University, Kyiv, Ukraine
[2]R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv, Ukraine
[3]O.Bogomolets National Medical University, Kyiv, Ukraine

Background: NSC631570 is a greater celandine (Chelidonium majus) alkaloid derivative with cytostatic activities. The mechanisms of action of the preparation have not been yet extensively investigated. It is known that NSC631570 induces tumor cell apoptosis independently of death receptor signalling via the pathway involving mitochondrial damage, cytochrome c release in the cytoplasm and caspases activation that is partly sensitive to over expression of Bcl-2, Bcl-xL and a dominant negative caspase-9. Therapeutic effect of NSC631570 is accompanied by immune responses stimulation. The aim of our study was to investigate reasons and mechanisms of this phenomenon.

Study design: NSC631570 has ability to be selectively accumulated in cancer cells. It has been confirmed due to its unique property to autofluorescence under UV light. We have made an assumption that the immune responses stimulation had to begin in the tumor tissue. The selective targets of NSC631570 are tumor cells. In addition the preparation could affect the microenvironment cells, including macrophages. We have investigated the immunogenicity of tumor cells after treatment with NSC631570 at the apoptogenic and non-apoptogenic concentrations. We have also estimated the effect of NSC-631570 on functional polarization of macrophages exposed to hypoxia.

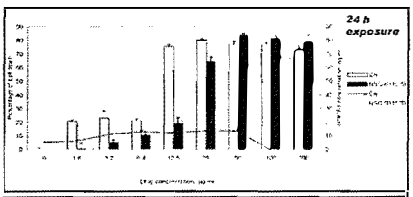


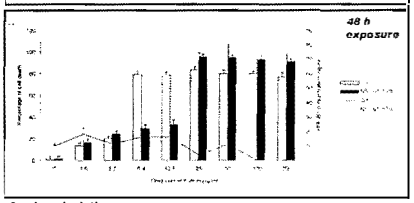
Methods: Low- and high-metastatic variants of B16 melanoma cells (MM-4 and MM-4M2 respectively) were used. TAP1 and TAP2 mRNA expression was evaluated in RT-PCR. Tumor cell death by apoptosis was assessed by flow cytometry. To investigate tumor cell death immunogenicity HMGB1 expression was determined in cell culture medium using a specific anti-HMGB1 ELISA. To estimate the effect of NSC631570 on functional polarization macrophages exposed to hypoxia nitrite assay was performed using the Greiss reaction and arginase activity was determined by colorimetric method.

RESULTS

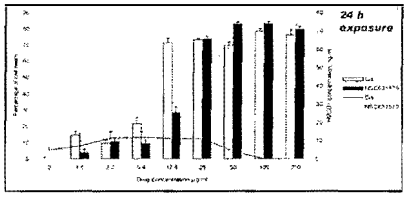
Effect of NSC631570 on tumor cell immunogenicity

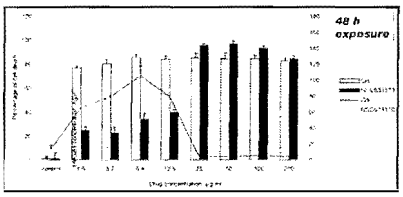
Low-metastatic MM-4 cells





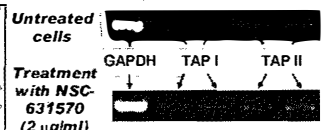
High-metastatic MM-4M2 cells



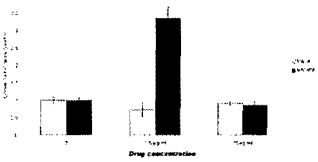


* - cis - cisplatin

Treatment of melanoma B16 cells with NSC631570 at the apoptogenic concentrations induces dose-dependent tumor cell death accompanied by dose-dependent HMGB1 release in vitro, more in MM-4M2 cells than in MM-4 cells.



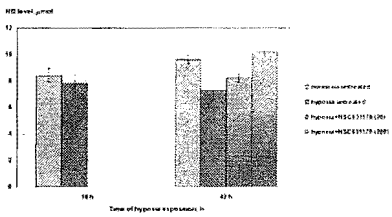
Effect of NSC631570 on TAP mRNA expression in MM-4M2 cells



Effect of NSC631570 on TAP I mRNA expression

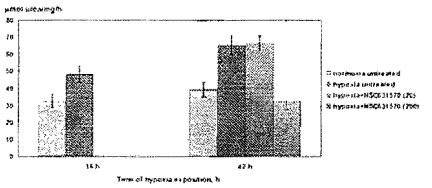
Quantitative RT-PCR demonstrated that treatment of high-metastasizing MM-4M2 cells with NSC-631570 at the non-apoptogenic concentration increased expression of mRNA encoding TAP2 by ~3-fold ($p < 0.05$) and at the apoptogenic concentration did not increase TAP2 mRNA. Unlike, treatment with NSC-631570 at the different concentrations did not influence TAP2 mRNA expression in low-metastatic MM-4 cells. TAP1 mRNA expression in MM-4 cells was also not affected by NSC-631570 treatment (data not presented). Surprisingly, we found mRNA for TAP1 in high-metastatic low-immunogenic MM-4M2 cells after treatment with NSC-631570 at the concentration of 1.6 μg/ml, whereas in untreated cells it was absent.

Effect of NSC631570 on hypoxic macrophage functional polarization



Effect of NSC631570 on NO production in hypoxic macrophages

Arginase activity in hypoxic macrophage lysates was increased over values found in normoxic cell lysates. Treatment of hypoxic macrophages with NSC631570 at the concentration of 200 μg/ml (apoptogenic for tumor cells) for 18h downregulated arginase activity. NO production was slightly decreased in hypoxic macrophages over 42-hour incubation period. Treatment with NSC631570 caused upregulation of NO production. Interestingly, no significant difference in NO levels has been observed between untreated hypoxic macrophages and hypoxic macrophages treated with the preparation at the low concentration.



Effect of NSC631570 on arginase activity of hypoxic macrophages

CONCLUSION

All the results taken together suggest that the application of NSC631570 to a target that encompasses the tumor could elicit some effects that exceed cell killing per se, and include specific and effective signals to the immune system of the host. The preparation induces tumor cell death accompanied by release of alarmins. Alarmins are the potent activators of dendritic cell maturation. In addition, NSC631570 is able to repolarize hypoxic macrophages in tumor microenvironment and thus to restore an antitumor activity of those cells. Therefore, we suggest that NSC631570 not only selectively kills tumor cells but also ideally recovers the role of the dying tumor as an effective immunogenic hub.