Adenosine acts as a chemoprotective agent by stimulating G-CSF production: a role for A1 and A3 adenosine receptors.

Fishman P, Bar-Yehuda S, Farbstein T, Barer F, Ohana G. Source Laboratory of Clinical and Tumor Immunology, The Felsenstein Medical Research Center, Tel-Aviv University, Rabin Medical Center, Petach-Tikva, Israel. pnina@mor-research.com

Abstract

Adenosine, a ubiquitous nucleoside, is released into the extracellular environment from metabolically active or stressed cells. It binds to cells through specific A1, A(2A), A(2B), and A3 G-protein-associated cell-surface receptors, thus acting as a signal-transduction molecule by regulating the levels of adenyl cyclase and phospholipase C. In this study, we showed that adenosine stimulates the proliferation of murine bone marrow cells in vitro. Pharmacological studies, using antagonists to the adenosine receptors, revealed that this activity was mediated through the binding of adenosine to its A1 and A3 receptors. This result was further corroborated by showing that the two selective A1 and A3 receptor agonists, N-cyclopentyladenosine (CPA) and 1-deoxy-1-[6-[[3-iodophenyl)methyl]amino]-9H-purin-9-yl]-N-methyl-beta-D-ribofuranuronamide (IB-MECA) respectively, induced bone marrow cell proliferation in a manner similar to adenosine. Adenosine's interaction with its A1 and A3 receptors induced G-CSF production, which led to its stimulatory effect on bone marrow cells. These results were confirmed in vivo when we demonstrated that low-dose adenosine (0.25 mg/kg) acted as a chemoprotective agent. When administered after chemotherapy, it restored the number of leukocytes and neutrophils to normal levels, compared with the decline in these parameters after chemotherapy alone. It is suggested that low-dose adenosine, already in clinical use, may also be applied as a chemoprotective agent.

Copyright 2000 Wiley-Liss, Inc. PMID:10797314 [PubMed - indexed for MEDLINE]

Differential effect of adenosine on tumor and normal cell growth: focus on the A3 adenosine receptor.

Ohana G, Bar-Yehuda S, Barer F, Fishman P. Source Laboratory of Clinical and Tumor Immunology, The Felsenstein Medical Research Center, Tel-Aviv University, Petach-Tikva, Israel.

Abstract

Adenosine is an ubiquitous nucleoside present in all body cells. It is released from metabolically active or stressed cells and subsequently acts as a regulatory molecule through binding to specific A1, A2A, A2B and A3 cell surface receptors. The synthesis of agonists and antagonists to the adenosine receptors and their cloning enabled the exploration of their physiological functions. As nearly all cells express specific adenosine receptors, adenosine serves as a physiological regulator and acts as a cardioprotector, neuroprotector, chemoprotector, and as an immunomodulator.

At the cellular level, activation of the receptors by adenosine initiates signal transduction mechanisms through G-protein associated receptors. Adenosine's unique characteristic is to differentially modulate normal and transformed cell growth, depending upon its extracellular concentration, the expression of adenosine cell surface receptors, and the physiological state of the target cell.

Stimulation of cell proliferation following incubation with adenosine has been demonstrated in a variety of normal cells in the range of low micromolar concentrations, including mesangial and thymocyte cells, Swiss mouse 3T3 fibroblasts, and bone marrow cells. Induction of apoptosis in tumor or normal cells was shown at higher adenosine concentrations (>100 microM) such as in leukemia HL-60, lymphoma U-937, A431 epidermoid cells, and GH3 tumor pituitary cell lines. It was further noted that the A3 adenosine receptor (A3AR) plays a key role in the inhibitory and stimulatory growth activities of adenosine. Modulation of the A3AR was found to affect cell growth either positively or negatively depending on the concentration of the agonist, similar to the effect described for adenosine.

At nanomolar concentrations, the A3AR agonists possess dual activity, i.e., antiproliferative activity toward tumor cells and stimulatory effect on bone marrow cells. In vivo, these agonists exerted anti-cancer effects, and when given in combination with chemotherapy, they enhanced the chemotherapeutic index and acted as chemoprotective agents.

Taken together, **activation of the A3AR, by minute concentrations of its natural ligand or synthetic agonists, may serve as a new approach for cancer therapy.**


**A3 adenosine receptor as a target for cancer therapy.**

Fishman P, Bar-Yehuda S, Madi L, Cohn I. Source Laboratory of Clinical and Tumor Immunology, The Felsenstein Medical Research Center, Tel-Aviv University, Rabin Medical Center, Petach Tikva 49100, Israel. pfishman@post.tau.ac.il

**Abstract**

Targeting the A3 adenosine receptor (A3AR) by adenosine or a synthetic agonist to this receptor (IB-MECA and CI-IB-MECA) results in a differential effect on tumor and on normal cells. Both the adenosine and the agonists inhibit the growth of various tumor cell types such as melanoma, colon or prostate carcinoma and lymphoma. **This effect is specific and is exerted on tumor cells only.**

Moreover, exposure of peripheral blood mononuclear cells to adenosine or the agonists leads to the induction of granulocyte colony stimulating factor (G-CSF) production. When given orally to mice, the agonists suppress the growth of melanoma, colon and prostate carcinoma in these animals, while inducing a myeloprotective effect via the induction of G-CSF production. The deregulation of the Wnt signaling pathway was found to be involved in the anticancer effect. Receptor activation induces inhibition of adenyl cyclase with a subsequent decrease in the level of protein kinase A and protein kinase B/Akt leading to activation of glycogen synthase kinase-3beta, a key element in the Wnt pathway. The oral bioavailability of the synthetic A3AR agonists, and their induced systemic anticancer and myeloprotective effect, renders them potentially useful in three different modes of treatment: as a stand-alone anticancer treatment, in combination with chemotherapy to enhance its therapeutic index and myeloprotection. It is evident that use of the A3AR agonist for increasing the therapeutic index of chemotherapy may also invariably give rise to myeloprotection and vice versa. The A3AR agonists are thus a promising new class of agents for cancer therapy.

**Virol J.** 2011 Feb;15;8:72

**Long-term nucleos(t)ide analogues therapy for adults with chronic hepatitis B reduces the risk of long-term complications: a meta-analysis.**


**Abstract**

**BACKGROUND:** The effect of antiviral therapy in chronic hepatitis B (CHB) on reducing the risk of long-term complications (LTCs) remains unclear so far. **To study whether long-term nucleos(t)ide analogues therapy can reduce the risk of long-term complications.**

**METHODS:**

We searched MEDLINE, EMBASE, OVID, the Cochrane Central Register of Controlled Trials. Relative risks (RRs) of long-term complications with or without treatment were studied. Also subgroup analyses including the status of drug-resistance, HBeAg and pre-existing compensated cirrhosis were done using relative risks of long-term complications either with or without treatment or among nucleos(t)ide analogues treatment groups.

**RESULTS:**

Six eligible studies (3644 patients in all) were included. Data showed the incidence of long-term complications in treatment groups was reduced by 74%(RR:0.26, 95% CI: 0.15-0.47) compared with no treatment. Whether drug-resistant happened or not during the long-term therapy, the incidence of long-term complications was still significantly induced respectively by 45%(RR: 0.55,95%CI:0.40-0.76) and 78% (RR:0.22, 95%CI: 0.13-0.36). For both different status of HBeAg and pre-existing compensated cirrhosis, there was significant lower incidence of long-term complications in treatment groups compared with no treatment, too. Moreover, among the NA treatment groups, patients with drug-resistance had 2.64 times (RR:2.64, 95%CI: 1.58-4.41) higher chance of developing to long-term complications, and patients with pre-existing compensated cirrhosis also had 3.07 times (RR:3.07, 95%CI: 1.04-9.11) higher chance of
Long-term nucleos(t)ide analogue therapy for adults with CHB prevents or delays the development of long-term complications including decompensated cirrhosis, CHB-related death or CHB-related HCC in patients with CHB. The patients who need take antiviral drugs should receive the antiviral therapy as soon as possible.

**CONCLUSIONS:**

Long-term nucleos(t)ide analogue therapy for adults with CHB prevents or delays the development of long-term complications including decompensated cirrhosis, CHB-related death or CHB-related HCC in patients with CHB. The patients who need to take antiviral drugs should receive the antiviral therapy as soon as possible.

PMID:21324130 [PubMed - indexed for MEDLINE]
PMCID:PMC3046930


**Nucleotide deficiency promotes genomic instability in early stages of cancer development.**

Bester AC1, Roniger M, Oren YS, Im MM, Sarni D, Chaoat M, Bensimon A, Zamir G, Shewach DS, Kerem B. Author information

1Department of Genetics, The Life Sciences Institute, Edmond J. Safra Campus, The Hebrew University, Jerusalem, Israel.

Abstract

Chromosomal instability in early cancer stages is caused by stress on DNA replication.

The molecular basis for replication perturbation in this context is currently unknown. We studied the replication dynamics in cells in which a regulator of S phase entry and cell proliferation, the Rb-E2F pathway, is aberrantly activated. Aberrant activation of this pathway by HPV-16 E6/E7 or cyclin E oncogenes significantly decreased the cellular nucleotide levels in the newly transformed cells.

Exogenously supplied nucleosides rescued the replication stress and DNA damage and dramatically decreased oncogene-induced transformation.

Increased transcription of nucleotide biosynthesis genes, mediated by expressing the transcription factor c-myc, increased the nucleotide pool and also rescued the replication-induced DNA damage. Our results suggest a model for early oncogenesis in which uncoordinated activation of factors regulating cell proliferation leads to insufficient nucleotides that fail to support normal replication and genome stability.


**Immunonutrition improves functional capacities in head and neck and esophageal cancer patients undergoing radiochemotherapy: A randomized clinical trial.**


Abstract

**BACKGROUND & AIMS:**

Malnutrition is frequent in head and neck (HN) and esophageal cancer patients and aggravated by radiochemotherapy (RCT), increasing morbi-mortality and treatment toxicity. Our goal was to investigate the effect of immunonutrition consisting of an arginine, omega-3 fatty acid, nucleotides-enriched diet on nutritional status, and functional capacity in HN or esophageal cancer patients undergoing RCT.

METHODS:

37 patients were randomized in a double-blind clinical trial. 5 days before and until the end of RCT (5-7 weeks), they received either an Immunomodulating Enteral Nutrition (IEN) or an isonitrogenous, isenergetic Standard Enteral Nutrition (SEN). Anthropometrical parameters, nutritional risk index (NRI), serum albumin, plasma antioxidant capacity, and functional capacity were recorded between the beginning and the end of RCT.

RESULTS:

A significant gain in total body weight (+2.1 ± 3.1 kg) was observed in IEN patients. Albuminemia and NRI were improved concomitantly in IEN malnourished patients. Plasma antioxidant capacity was improved (+100 ± 13 µM EqTrolox) in IEN patients. Functional capacity measured by WHO...
Performance Status and Karnofsky index was maintained in IEN patients but significantly reduced in SEN patients.

CONCLUSIONS:

These preliminary data show that immunonutrition could improve the nutritional status together with functional capacity in HN and esophageal cancer patients undergoing RCT.

CLINICAL TRIAL REGISTRATION:
This clinical trial promoted by the University Hospital Center of Clermont-Ferrand has been registered at ClinicalTrial.gov website under the following reference: NCT00333099. Copyright © 2013 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.


Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t)ide therapy: a systematic review.
Papatheodoridis GV, Lampertico P, Manolakopoulos S, Lok A. Source 2nd Department of Internal Medicine, Athens University Medical School, Athens, Greece. gepapath@med.uoa.gr

Abstract

BACKGROUND & AIMS:
Chronic hepatitis B patients are at increased risk for hepatocellular carcinoma (HCC). The effect of medium-term nucleos(t)ide analogue therapy on HCC incidence is unclear; therefore, we systematically reviewed all the data on HCC incidence from chronic hepatitis B patients treated with nucleos(t)ide analogues.

METHODS:
We performed a literature search to identify studies with chronic hepatitis B patients treated with nucleos(t)ide analogues for > or = 24 months.

RESULTS:
Twenty-one studies including 3881 treated and 534 untreated patients met our inclusion criteria. HCC was diagnosed in 2.8% and 6.4% of treated and untreated patients, respectively, during a 46 (32-108) month period (p=0.003), in 10.8% and 0.5% of nucleos(t)ide naive patients with and without cirrhosis (p<0.001) and in 17.6% and 0% of lamivudine resistance patients with and without cirrhosis (p<0.001). HCC developed less frequently in nucleos(t)ide naive patients compared to those without virological remission (2.3% vs 7.5%, p<0.001), but there was no difference between lamivudine resistance patients with or without virological response to rescue therapy (5.9% vs 8.8%, p=0.466).

CONCLUSIONS:
Chronic hepatitis B patients receiving medium-term nucleos(t)ide analogue therapy had a significantly lower incidence of HCC compared to untreated patients but treatment does not completely eliminate the risk of HCC.

Among the treated patients, cirrhosis, HBeAg negative at baseline and failure to remain in virological remission were associated with an increased risk of HCC.

Copyright 2010 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved. PMID:20483498[PubMed - indexed for MEDLINE]

Adenosine acts as an inhibitor of lymphoma cell growth: a major role for the A3 adenosine receptor.

Fishman P, Bar-Yehuda S, Ohana G, Pathak S, Wasserman L, Barer F, Multani AS. Laboratory of Clinical and Tumor Immunology, The Felsenstein Medical Research Center, Tel-Aviv University, Rabin Medical Center, Petach-Tikva, Israel. pnina@mor-reseach.com

**Abstract**

In this study, we demonstrated several mechanisms exploring the inhibitory effect of low-dose adenosine on lymphoma cell growth. Adenosine, a purine nucleoside present in plasma and other extracellular fluids, acts as a regulatory molecule, by binding to G-protein associated cell-surface receptors, A1, A2 and A3. Recently we showed that low-dose adenosine released by muscle cells, inhibits tumour cell growth and thus attributes to the rarity of muscle metastases. In the present work, a cytostatic effect of adenosine on the proliferation of the Nb2-11C rat lymphoma cell line was demonstrated. This effect was mediated through the induction of cell cycle arrest in the G0/G1 phase and by decreasing the telomeric signal in these cells. Adenosine was found to exert its antiproliferative effect mainly through binding to its A3 receptor. The **cytostatic anticancer activity, mediated through the A3 adenosine receptor**, turns it into a potential target for the development of anticancer therapies.

**PMID:** 10899660 [PubMed - indexed for MEDLINE]

---


Cyclic adenosine monophosphate differentiated beta-endorphin neurons promote immune function and prevent prostate cancer growth.

Sarkar DK, Boyadjieva NL, Chen CP, Ortiguela M, Reuhl K, Clement EM, Kuhn P, Marano J. Endocrine Program and Neurotoxicology Laboratories, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, USA. sarkar@aesop.rutgers.edu

**Abstract**

Pituitary adenylate cyclase-activating peptide (PACAP), a cAMP-activating agent, is highly expressed in the hypothalamus during the period when many neuroendocrine cells become differentiated from the neural stem cells (NSCs). Activation of the cAMP system in rat hypothalamic NSCs differentiated these cells into beta-endorphin (BEP)-producing neurons in culture. When these in vitro differentiated neurons were transplanted into the paraventricular nucleus (PVN) of the hypothalamus of an adult rat, they integrated well with the surrounding cells and produced BEP and its precursor gene product, proopiomelanocortin (POMC). Animals with BEP cell transplants demonstrated remarkable protection against carcinogen induction of prostate cancer. Unlike carcinogen-treated animals with control cell transplants, rats with BEP cell transplants showed rare development of glandular hyperplasia, prostatic intraepithelial neoplasia (PIN), or well differentiated adenocarcinoma with invasion after N-methyl-N-nitrosourea (MNU) and testosterone treatments. Rats with the BEP neuron transplants showed increased natural killer (NK) cell cytolytic function in the spleens and peripheral blood mononuclear cells (PBMCs), elevated levels of anti-inflammatory
cytokine IFN-gamma, and decreased levels of inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha) in plasma. These results identified a critical role for cAMP in the differentiation of BEP neurons and revealed a previously undescribed role of these neurons in combating the growth and progression of neoplastic conditions like prostate cancer, possibly by increasing the innate immune function and reducing the inflammatory milieu.

Molecular mechanisms of A3 adenosine receptor-induced G1 cell cycle arrest and apoptosis in androgen-dependent and independent prostate cancer cell lines: involvement of intrinsic pathway.

Aghaei M, Panjehpour M, Karami-Tehrani F, Salami S. School of Medical Science, Tarbiat Modares University, P.O. Box: 14115-331, Tehran, Iran.  

**Abstract**

**PURPOSE:**
A3 adenosine receptor has shown several physiological and pathological activities, including cell proliferation and apoptosis in various cancer cell lines. This study is designed to investigate molecular mechanism and apoptotic pathway of A3 adenosine receptor in DU-145, PC3 and LNcap-FGC10 human prostate cancer cells.

**METHODS:**
The expression level of A3 adenosine receptor was examined using real-time RT-PCR. cAMP concentration was also measured. MTI viability, cell counting and BrdU incorporation tests were used to study the cell proliferation effect of IB-MECA. Cell cycle analysis, Annexin V-FITC staining, Hoechst 33258 staining, mitochondrial membrane potential (ΔΨM), caspase-3 activity, Bcl-2 and Bax protein expression were used to detect apoptosis.

**RESULT:**
A3 adenosine receptors mRNAs were detected at different levels. IB-MECA inhibited forskolin-stimulated cAMP. IB-MECA at (1 μM) suppressed cell proliferation and induced G1 cell cycle arrest. Indeed, IB-MECA down-regulated the expression of CDK4, cyclin D1 and up-regulated p53 expression. IB-MECA at (10-100 μM) induced apoptosis. The activity of caspase-3 was also increased. Expression of Bcl-2 was decreased in response to IB-MECA, while the expression of Bax protein was increased. The results showed a significant loss of ΔΨM, in a dose-dependent manner.

**CONCLUSION:**
This study introduces a possible mechanism through A3 adenosine receptor activation. IB-MECA inhibited prostate cancer cells proliferation and induced G1 cell cycle arrest through p53, Cdk4/cyclinD1 pathway. Apoptosis determined by characteristic morphological changes and increased in sub-G1 population. Loss of MMP, activation of caspase-3 and down-regulation of Bcl-2 expression indicated mitochondrial signaling pathway that involved in the apoptosis.


Adenosine induces cell-cycle arrest and apoptosis in androgen-dependent and -independent prostate cancer cell lines, LNcap-FGC-10, DU-145, and PC3.

Aghaei M, Karami-Tehrani F, Panjehpour M, Salami S, Falihani F. School of Medical Science, Tarbiat Modares University, Tehran, Iran.  

**Abstract**

**BACKGROUND:**
Adenosine has been shown to inhibit cell growth and induce apoptosis in the several cancer cells via intrinsic and extrinsic pathway. The present study was designed to understand the mechanism underlying adenosine-induced apoptosis in the DU-145, PC3, and LNcap-FGC10 human prostate cancer cells.

**METHODS:**
To observe cell viability and proliferation, MTT assay, cell counting, and BrdU assay were carried out in DU-145, PC3, and LNcap-FGC10 cells. Apoptosis was assessed with the analysis of cell cycle, Hoechst 33258 staining, propidium iodide and annexin-V staining, reactive oxygen species (ROS) formation, mitochondrial membrane potential (ΔΨM) measurement, caspase-3 activity assay, Bcl-2 and Bax protein expression. Moreover, the expression of adenosine receptors and the effects of adenosine receptor (A(1), A(2a), and A(3)) antagonists were examined.

RESULT:
Adenosine significantly reduced cell proliferation in a dose-dependent manner in DU-145, PC3, and LNcap-FGC10 cell lines. Adenosine induced arrest in the cell-cycle progression in G0/G1 phase through Cdk4/cyclinD1-mediated pathway. Adenosine induced apoptosis, which was determined by morphological changes and increased sub-G1 population. Furthermore, increase of ROS, loss of MMP, activation of caspase-3, and down-regulation of Bcl-2 expression was observed. A(1), A(2a), A(2b), and A(3) adenosine receptors mRNA are expressed in the cell lines. Moreover, adenosine-induced apoptosis was inhibited by MRS1220. A(3) adenosine receptor antagonist.

CONCLUSION:
Our results suggest that adenosine induced apoptosis in prostate cancer cells via the mitochondrial pathway and is related to the adenosine receptors. These data might suggest that adenosine could be used as an agent for the treatment of prostate cancer.

---


Adenosine induces cell cycle arrest and apoptosis via cyclinD1/Cdk4 and Bcl-2/Bax pathways in human ovarian cancer cell line OVCAR-3.

Shirali S1, Aghaei M, Shabani M, Fathi M, Sohrabi M, Moeinifard M. 1Department of Laboratory Sciences, Chalous Branch, Islamic Azad University, Chalous, Iran.

**Abstract**

Adenosine is a regulatory molecule with widespread physiological effects in almost every cells and acts as a potent regulator of cell growth. Adenosine has been shown to inhibit cell growth and induce apoptosis in the several cancer cells via caspase activation and Bcl-2/Bax pathway. The present study was designed to understand the mechanism underlying adenosine-induced apoptosis in the OVCAR-3 human ovarian cancer cells. MTT viability, BrdU and cell counting assays were used to study the cell proliferation effect of adenosine in presence of adenosine deaminase inhibitor and the nucleoside transporter inhibitor. Cell cycle analysis, propidium iodide and annexin V staining, caspase-3 activity assay, cyclinD1, Cdk4, Bcl-2 and Bax protein expressions were assessed to detect apoptosis. Adenosine significantly inhibited cell proliferation in a concentration-dependent manner in OVCAR-3 cell line. Adenosine induced cell cycle arrest in G0/G1 phase via Cdk4/cyclinD1-mediated pathway. Adenosine induced apoptosis, which was determined by Annexin V-FITC staining and increased sub-G1 population. Moreover, down-regulation of Bcl-2 protein expression, up-regulation of Bax protein expression and activation of caspase-3 were observed in response to adenosine treatment. The results of this study suggest that extracellular adenosine induced G1 cell cycle arrest and apoptosis in ovarian cancer cells via cyclinD1/ Cdk4 and Bcl-2/Bax pathways and caspase-3 activation. These data might suggest that adenosine could be used as an agent for the treatment of ovarian cancer.

PMID:23345014[PubMed - indexed for MEDLINE]

---


Intracellularly transported adenosine induces apoptosis in [corrected] MCF-7 human breast cancer cells by accumulating AMID in the nucleus.
Extracellular adenosine induced apoptosis of MCF-7 human breast cancer cells in a concentration (10μM-10mM) - and treatment time (24-72h)-dependent manner, and the effect was inhibited by the adenosine transporter inhibitor dipyridamole, but not an inhibitor of adenosine kinase, an inhibitor of AMP-activated protein kinase, or inhibitors for A(1), A(2a), A(2b), and A(3) adenosine receptors. No significant activation of caspase-7, -8, or -9 was obtained with adenosine. Adenosine promoted translocation of apoptosis-inducing factor (AIF)-homologous mitochondrion-associated inducer of death (AMID) from the cytosol into the nucleus, although the total amount of AMID was not affected. Adenosine-induced MCF-7 cell death was abrogated by knocking-down AMID. The results of the present study indicate that intracellularly transported adenosine induces MCF-7 cell apoptosis by accumulating AMID in the nucleus in a caspase-independent manner.

Copyright © 2012 Elsevier Ireland Ltd. All rights reserved. PMID:22388174 [PubMed-indexed for MEDLINE]
Abstract

Extracellular adenosine induced apoptosis in HepG2 cells, a human hepatoma cell line, by tuning apoptosis-mediator gene transcription. The present study aimed at identifying the responsible adenosine receptor and clarifying the signaling pathway underlying adenosine-induced HepG2 cell apoptosis. Adenosine and CGS21680, an A(2a) adenosine receptor agonist, induced HepG2 cell apoptosis, and the effect was inhibited by DMPX, an A(2a) adenosine receptor antagonist, or by knocking-down A(2a) adenosine receptors. Adenosine reduced expression of Bcl-X(L) mRNA and protein but otherwise increased expression of the Bid mRNA and protein in HepG2 cells, and those effects were also prevented by knocking-down A(2a) adenosine receptors. Adenosine caused disruption of mitochondrial membrane potentials and stimulated cytochrome c efflux from the mitochondria in HepG2 cells. Adenosine activated caspases-3 and -9 in HepG2 cells, which was significantly inhibited by knocking-down A(2a) adenosine receptors. The results of the present study indicate that extracellular adenosine downregulates Bcl-X(L) expression and upregulates Bid expression, thereby disrupting mitochondrial membrane potentials to allow cytochrome c efflux from the mitochondria, and then causing activation of caspase-9 and the effector caspase-3, as mediated via A(2a) adenosine receptors.

Copyright © 2011 Wiley Periodicals, Inc.

Extracellular adenosine induces apoptosis in Caco-2 human colonic cancer cells by activating caspase-9/-3 via A(2a) adenosine receptors.

Yasuda Y1, Saito M, Yamamura T, Yaguchi T, Nishizaki T.1 Department of Physiology, Hyogo College of Medicine, 1-1 Mukogawa, Nishinomiya, 663-8501, Japan.

Abstract

BACKGROUND: Extracellular adenosine has been shown to induce apoptosis in a variety of cells via an intrinsic pathway linked to adenosine uptake into cells and the ensuing signaling cascades and an extrinsic pathway linked to adenosine receptors. The present study was designed to understand the mechanism underlying adenosine-induced apoptosis of Caco-2 human colonic cancer cells.

METHODS: To observe cell viability, an MTT assay was carried out in Caco-2 cells untransfected or transfected with the A(2a) adenosine receptor pcDNA3.1. Apoptotic cell death was assessed with flow cytometry using propidium iodide and annexin V and internucleosomal DNA fragmentation analysis. Activities of caspase-3, -8, and -9 were measured using a caspase fluorometric assay kit. Mitochondrial membrane potentials were monitored using a DePsipher kit. Expression of adenosine receptors was examined with the reverse transcription-polymerase chain reaction (RT-PCR) method.

RESULTS: Extracellular adenosine induced Caco-2 cell apoptosis in a concentration-dependent (1-20 mM) and treatment time-dependent (24-72 h) manner. The adenosine effect was inhibited by DMPX, an inhibitor of A(2a) adenosine receptors and SQ22536, an inhibitor of adenylate cyclase. CGS21680, an agonist of A(2a) adenosine receptors, and forskolin, an adenylate cyclase activator, mimicked the adenosine action. Caco-2 cell death was still induced by overexpressing A(2a) adenosine receptors, and adenosine further promoted the cell death. Adenosine disrupted mitochondrial membrane potentials and activated caspase-9 and -3, but not caspase-8.

CONCLUSIONS: Extracellular adenosine induces apoptosis in Caco-2 cells by activating caspase-9 and the downstream effector caspase caspase-3 in association with mitochondrial damage via A(2a) adenosine receptors.

PMID:19159073[PubMed - indexed for MEDLINE]

Otsuki T, Kanno T, Fujita Y, Tabata C, Fukuoka K, Nakano T, Gotoh A, Nishizaki T. Division of Bioinformation, Department of Physiology, Hyogo College of Medicine, Nishinomiya, Japan.

Abstract

BACKGROUND/AIMS: A(3) adenosine receptor mediates apoptosis in cancer cells via diverse signaling pathways. The present study examined A(3) adenosine receptor-mediated apoptosis in Lu-65 cells, a human giant cell lung carcinoma cell line.

METHODS: MTT assay, TUNEL staining, real-time RT-PCR, Western blotting, and assay of caspase-3, -8, and -9 activities were carried out in Lu-65 cells, and A(3) adenosine receptor or p53 was knocked-down by transfecting each siRNA into cells.

RESULTS: Extracellular adenosine induces Lu-65 cell apoptosis in a concentration (0.01-10 mM)- and treatment time (24-72 h)-dependent manner, and the effect was inhibited by the A(3) adenosine receptor inhibitor MRS1191 or by knocking-down A(3) adenosine receptor or p53. Like adenosine, the A(3) adenosine receptor agonist 2-Cl-IB-MECA also induced Lu-65 cell apoptosis. Adenosine upregulated expression of p53 and Noxa mRNAs and activated caspase-3 and -9, but not caspase-8. Those adenosine effects were still inhibited by knocking-down A(3) adenosine receptor or p53.

CONCLUSION: The results of the present study show that adenosine upregulates p53 expression via A(3) adenosine receptor, to promote p53-dependent Noxa gene transcription, causing activation of caspase-9 and the effector caspase-3 to induce Lu-65 cell apoptosis.

Adenosine induces apoptosis in SBC-3 human lung cancer cells through A(3) adenosine receptor-dependent AMID upregulation.

Kanno T, Nakano T, Fujita Y, Gotoh A, Nishizaki T. Division of Bioinformation, Department of Physiology, Hyogo College of Medicine, Nishinomiya, Japan.

Abstract

BACKGROUND/AIMS: We have shown that A(3) adenosine receptor mediates apoptosis in human lung cancer cells such as A549 cells, an epithelial adenocarcinoma cell line, and Lu-65 cells, a giant cell cancer cell line, via each different signaling pathway. AMID, a pro-apoptotic protein, induces caspase-independent apoptosis by accumulating in the nucleus. The present study investigated AMID-dependent apoptosis through A(3) adenosine receptor in SBC-3 cells, a human small cell lung cancer cell line.

METHODS: MTT assay, TUNEL staining, flow cytometry using propidium iodide and annexin V-FITC, and Western blotting were carried out in SBC-3 cells transfected with and without the siRNA to silence the A(3) adenosine receptor-targeted gene or the AMID-targeted gene.

RESULTS: Adenosine induced SBC-3 cell apoptosis in a concentration (0.01-10 mM) and treatment time (24-72 h)-dependent manner, and a similar effect was obtained with the A(3) adenosine receptor agonist 2-Cl-IB-MECA. Adenosine-induced SBC-3 cell death was inhibited by the A(3) adenosine receptor inhibitor MRS1191, knocking-down A(3) adenosine receptor, or knocking-down AMID. Adenosine upregulated expression of the AMID mRNA and protein in SBC-3 cells, that is suppressed by knocking-down A(3) adenosine receptor. In addition, adenosine increased nuclear AMID localization in concert with decreased cytosolic AMID localization.

CONCLUSION:
The results of the present study show that adenosine induces SBC-3 cell apoptosis by upregulating AMID expression and promoting AMID translocation into the nucleus via A(3) adenosine receptor.


Kanno T, Gotoh A, Fujita Y, Nakano T, Nishizaki T. Division of Bioinformation, Department of Physiology, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Japan.

Abstract

BACKGROUND/AIMS:
A(3) adenosine receptor mediates apoptosis in a variety of cancer cells via diverse signaling pathways. The present study was conducted to assess A(3) adenosine receptor-mediated apoptosis in human bladder cancer cell lines and to understand the underlying mechanism.

METHODS:
Human bladder cancer cell lines such as 253J, 5637, KK-47, TCCSUP, T24, and UMUC-3 cells were cultured. The siRNA to silence the A(3) adenosine receptor-targeted gene was constructed and transfected into cells. MTT assay, TUNEL staining, Western blotting, and real-time RT-PCR were carried out.

RESULTS:
For all the investigated cell types, adenosine induced apoptosis in a concentration (0.01-10 mM)-and treatment time (24-48 h)-dependent manner. Adenosine-induced 5637 cell death was significantly inhibited by the A(3) adenosine receptor inhibitor MRS1191 or knocking-down A(3) adenosine receptor, and the A(3) adenosine receptor agonist 2-Ch-IB-MECA mimicked the adenosine effect. The adenosine effect was prevented by GF109203X, an inhibitor of protein kinase C (PKC), but it was not affected by forskolin, an activator of adenylate cyclase. Adenosine-induced 5637 cell death, alternatively, was not inhibited by the pan-caspase inhibitor Z-VAD. Adenosine upregulated expression of apoptosis-inducing factor (AIF), that is suppressed by knocking-down A(3) adenosine receptor, and accumulated AIF in the nucleus.

CONCLUSION:
The results of the present study show that adenosine induces 5637 cell apoptosis by upregulating AIF expression via an A(3) adenosine receptor-mediated G(q) protein/PKC pathway.


Yaguchi T, Saito M, Yasuda Y, Kanno T, Nakano T, Nishizaki T. Division of Bioinformation, Department of Physiology, Hyogo College of Medicine, Nishinomiya, Japan.

Abstract

BACKGROUND/AIMS:
Adenosine 5'-triphosphate (ATP) mediates a variety of signal transductions via ATP receptors such as P2X and P2Y receptors. The present study aimed at understanding the mechanism underlying extracellular ATP-induced suppression of Caco-2 human colonic cell proliferation.

METHODS:
Caco-2 cells were cultured. To examine cell viability and cell cycling, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, fluorescent cytochemistry, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, and flow cytometry were carried out. To see mRNA expression of ATP receptors, reverse transcription-polymerase chain reaction (RT-PCR) was performed. To examine PKC activity and mitogen-activated protein (MAP) kinase activity, in situ PKC assay and Western blotting using an anti-extracellular signal-regulated kinase 1 (ERK1)-antibody and an anti-phospho-ERK antibody were carried out.

RESULTS:
Extracellular ATP or the unhydrolyzed ATP analogue 5'-adenylylimido-diphosphate (AMP-PNP) reduced Caco-2 cell viability in a concentration (10 microM-10 mM)-dependent manner at 48-h treatment, and the effect was not affected by caspase inhibitors. Caco-2 cells were little reactive to propidium iodide and Hoechst 33342 or little positive to TUNEL after 48-h treatment with ATP (1 mM). In the flow cytometry, 48-h treatment with ATP (1 mM) arrested cell cycling at the
S phase in Caco-2 cells. P(2) purinoceptor agonists reduced Caco-2 cell viability with the order of potency: 2-methylthio ATP > UTP > beta, gamma-methylene ATP, and the ATP effect was partially inhibited by suramin, a non-selective inhibitor of P(2) purinoceptors. The PKC inhibitor GF109203X or the MAP kinase kinase inhibitor PD98059 reduced Caco-2 cell viability to an extent similar to that achieved by ATP (1 mM), and no further reduction was obtained with co-treatment with ATP. ATP and its ATP analogues such as AMP-PNP and ATPgammaS, at higher concentrations (1-10 mM), inhibited PKC activation in Caco-2 cells in a fashion that mimics the effect of GF109203X, but PD98059 exhibited no effect on PKC activation. The inhibitory effect of ATP on PKC activation was not found with SK-N-SH cells, a human neuroblastoma cell line, but the cells expressed all the mRNAs for P2X and P2Y receptors that Caco-2 cells did. ATP (10 mM) or GF109203X inhibited activation of ERK, a MAP kinase, in Caco-2 cells.

CONCLUSION:
Extracellular ATP, at higher concentrations, suppresses Caco-2 cell proliferation at the S phase of cell cycling by inhibiting PKC, possibly as mediated via an unknown ATP receptor, followed by MAP kinase.

Adenine nucleotides inhibit proliferation of the human lung adenocarcinoma cell line LXF-289 by activation of nuclear factor kappaB1 and mitogen-activated protein kinase pathways.
Schäfer R1, Hartig R, Sedehizade F, Weite T, Reiser G.1Institut für Neurobiochemie, Otto-von-Guericke-Universität, Medizinische Fakultät, Magdeburg, Germany.

Abstract
Extracellular nucleotides have a profound role in the regulation of the proliferation of diseased tissue. We studied how extracellular nucleotides regulate the proliferation of LXF-289 cells, the adenocarcinoma-derived cell line from human lung bronchial tumor. ATP and ADP strongly inhibited LXF-289 cell proliferation. The nucleotide potency profile was ATP = ADP = ATPgammaS > > UTP, UDP, whereas alpha,beta-methylene-ATP, beta,gamma-methylene-ATP, 2',3'-O-(4-benzoylbenzoyl)-ATP, AMP and UMP were inactive. The nucleotide potency profile and the total blockade of the ATP-mediated inhibitory effect by the phospholipase C inhibitor U-73122 clearly show that P2Y receptors, but not P2X receptors, control LXF-289 cell proliferation. Treatment of proliferating LXF-289 cells with 100 micromol ATP or ADP induced significant reduction of cell number and massive accumulation of cells in the S phase. Arrest in S phase is also indicated by the enhancement of the antiproliferative effect of ATP by coapplication of the cytostatic drugs cisplatin, paclitaxel and etoposide. Inhibition of LXF-289 cell proliferation by ATP was completely reversed by inhibitors of extracellular signal related kinase-activating kinase/extracellular signal related kinase 1/2 (PD98059, U0126), p38 mitogen-activated protein kinase (SB203580), phosphatidylinositol-3-kinase (wortmannin), and nuclear factor kappaB1 (SN50). Western blot analysis revealed transient activation of p38 mitogen-activated protein kinase, extracellular signal-related kinase 1/2, and nuclear factor kappaB1 and possibly new formation of p50 from its precursor p105. ATP-induced attenuation of LXF-289 cell proliferation was accompanied by transient translocation of p50 nuclear factor kappaB1 and extracellular signal-related kinase 1/2 to the nucleus in a similar time period. In summary, inhibition of LXF-289 cell proliferation is mediated via P2Y receptors by activation of multiple mitogen-activated protein kinase pathways and nuclear factor kappaB1, arresting the cells in the S phase.

MEDLINE

PMID:16911524

The adenosine A3 receptor agonist CI-IB-MECA induces cell death through Ca²⁺/ROS-dependent down regulation of ERK and Akt in A172 human glioma cells.
Kim TH1, Kim YK, Woo JS.1Department of Physiology, School of Medicine, Pusan National University, Beomeo-ri, Mulgeum-eup, Yangsan 626-870, Gyungsangnam-do, Republic of Korea.

Abstract
Adenosine A(3) receptor (A3AR) is coupled to G proteins that are involved in a variety of intracellular signaling pathways and physiological functions. 2-Chloro-N(6)-(3-iodobenzyl) adenosine-5'-N-methylcarboxamide (CI-IB-MECA), an agonist of A3AR, has been reported to induce cell death in various cancer cells. However, the effect of CI-IB-MECA on glioma cell growth is not clear. This study was undertaken to examine the effect of CI-IB-MECA on glioma cell viability and to determine its molecular mechanism. CI-IB-MECA inhibited cell proliferation and induced cell death in a dose- and time-dependent manner. Treatment of CI-IB-MECA resulted in an increase in intracellular Ca(2+) followed by enhanced reactive oxygen species (ROS) generation. EGTA and N-acetylcysteine (NAC) blocked the cell death induced by CI-IB-MECA, suggesting that Ca(2+)}
and ROS are involved in the CI-IB-MECA-induced cell death. Western blot analysis showed that CI-IB-MECA induced the down-regulation of extracellular signal-regulated kinases (ERK) and Akt, which was prevented by EGTA, NAC, and the A3AR antagonist MRS1191. Transfection of constitutively active forms of MEK, the upstream kinase of ERK, and Akt prevented the cell death. CI-IB-MECA induced caspase-3 activation and the CI-IB-MECA-induced cell death was blocked by the caspase inhibitors DEVD-CHO and z-VAD-FMK. In addition, expression of XIAP and Survivin were decreased in cells treated with CI-IB-MECA. Collectively, these findings demonstrate that CI-IB-MECA induce a caspase-dependent cell death through suppression of ERK and Akt mediated by an increase in intracellular Ca(2+) and ROS generation in human glioma cells. These suggest that A3AR agonists may be a potential therapeutic agent for induction of apoptosis in human glioma cells.

PMID:22878643 [PubMed - indexed for MEDLINE]

A3 adenosine receptor mediates apoptosis in in vitro RCC4-VHL human renal cancer cells by up-regulating AMID expression.

Nagaya H, Gotoh A, Kanno T, Nishizaki T. Laboratory of Cell and Gene Therapy, Institute for Advanced Medical Sciences and Division of Bioinformation, Department of Physiology, Hyogo College of Medicine, Mukogawa-cho, Nishinomiya, Japan.

Abstract

PURPOSE:
Accumulating studies have shown that extracellular adenosine induces apoptosis in various cancer cells via diverse signaling pathways. We sought to understand adenosine induced apoptosis in human renal cancer cells and the underlying pathway.

MATERIALS AND METHODS:
RCC4-VHL (European Collection of Animal Cell Cultures, Salisbury, United Kingdom), ACHN (Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University, Aoba-ku, Sendai, Japan) and 786-O (ATCC®) human renal cancer cells were cultured. MTT assay, TUNEL staining, reverse transcriptase-polymerase chain reaction and Western blot were done in cells untransfected and transfected with siRNA silencing the A(3) adenosine receptor targeted gene or the AMID targeted gene.

RESULTS:
Adenosine induced apoptosis in all cell types used in a concentration (1 to 10 mM) dependent manner. A similar effect was obtained with the A(3) adenosine receptor agonist 2-Cl-IB-MECA. Adenosine induced RCC4-VHL cell death was inhibited by the A(3) adenosine receptor inhibitor MRS1191 or by knocking down A(3) adenosine receptor or AMID. Adenosine up-regulated the expression of AMID mRNA and protein in RCC4-VHL cells, which was suppressed by A(3) adenosine receptor knockdown. Moreover, adenosine promoted AMID translocation from cytosol to nucleus.

CONCLUSIONS:
Adenosine induces RCC4-VHL cell apoptosis by up-regulating AMID expression and accumulating AMID in the nucleus via A(3) adenosine receptor.

Copyright © 2013 American Urological Association Education and Research, Inc. Published by Elsevier Inc. All rights reserved.

PMID:23174235[PubMed - indexed for MEDLINE]