The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Adenosine Overcomes Triple Negative Breast Cancer Resistance to Platin-Derived Chemotherapeutic Drugs

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

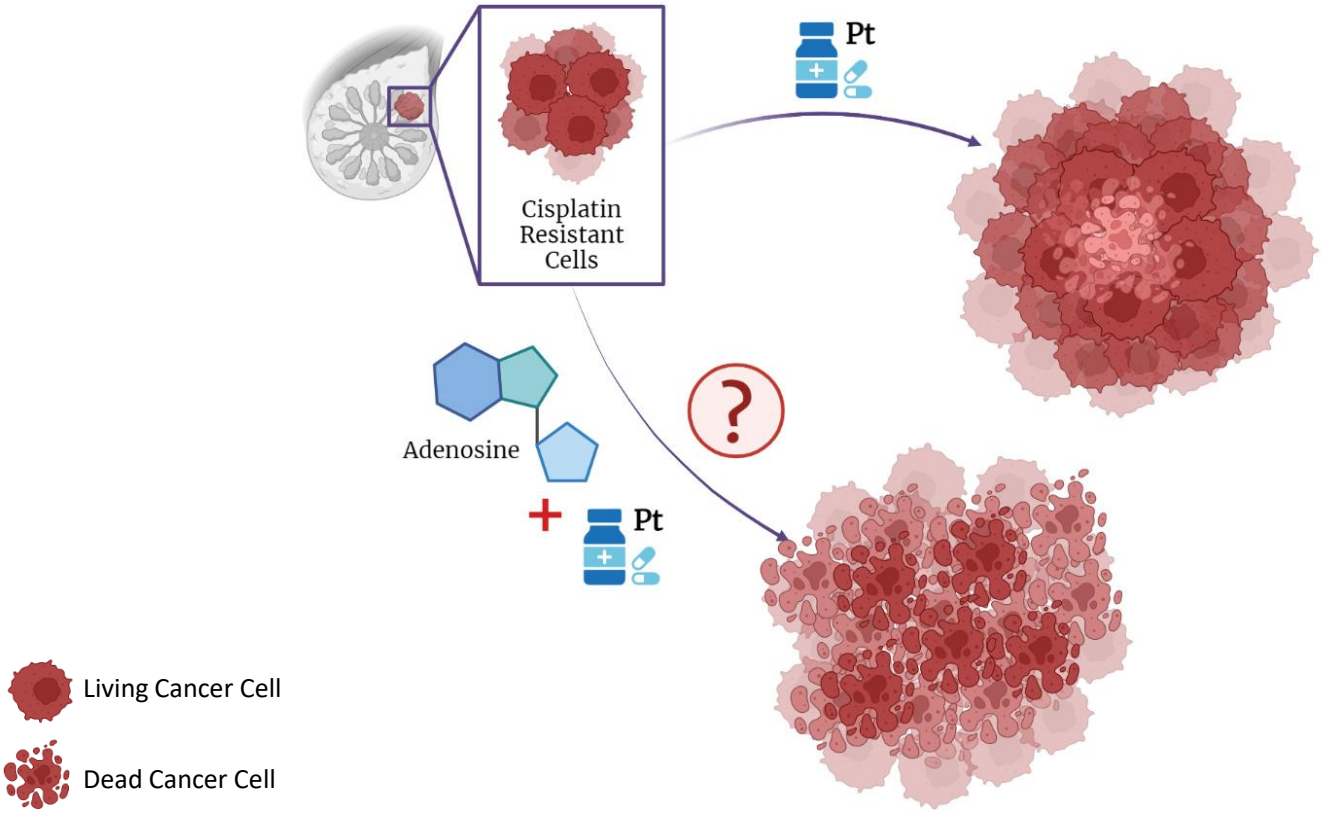
Ana Cerquido[1,*], Martin Vojtek[1], Olga Viegas[2,3], Patricia Dias-Pereira[4] and Carmen Diniz[1,*]

[1] LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal;
[2] LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[3] Faculty of Nutrition and Food Science, University of Porto, 4150-180 Porto, Portugal
[4] ICBAS – Instituto de Ciências Biomédicas de Abel Salazar, University of Porto, 4050-313 Porto, Portugal

* Corresponding author: anacerquido99@gmail.com (A.C.); cdiniz@ff.up.pt (C.D.)

U.PORTO FACULDADE DE FARMÁCIA UNIVERSIDADE DO PORTO requimte rede de química e tecnologia LAQV

Adenosine Overcomes Triple Negative Cancer Resistance to Platin-Derived Chemotherapeutic Drugs



ECMC 2022
The 8th International Electronic Conference on Medicinal Chemistry
01–30 NOVEMBER 2022 | ONLINE

Abstract: Triple negative breast cancer (TNBC), a poor survival cancer has high resistance to therapy, with low drug efficacy. Adenosine is present in high concentrations in tumor microenvironment. Recently, adenosine was found to sensitize ovarian cisplatin-resistant cancer. This work aims at addressing if adenosine can sensitize TNBC resistance to platin drugs. Concomitant/preincubation of adenosine with cisplatin or carboplatin induced cell proliferation in TNBC cisplatin-sensitive (MDA) and -resistant (MDA/R) cells (using Lionheart-FX microscope). Phosphorylation of ERK or NF-κB pathways and cAMP production were evaluated (AlphaScreen assays). Data analyzed with One-way ANOVA *t*-test. Results: concomitant or preincubation of adenosine (300, 600, 700 μM) with cisplatin reduced resistance in MDA/R, with proliferation levels approaching those observed in MDA. In MDA, endogenous and exogenous adenosine have no effect over ERK phosphorylation; in MDA/R, exogenous adenosine lowers ERK phosphorylation. NF-κB phosphorylation was induced by A_3R and $A_{2B}R$ tonic activation in MDA and MDA/R, respectively, increasing survival - exogenous adenosine inactivates this via. Tonically cAMP production was altered in MDA and MDA/R, revealing inhibitory and stimulatory effects in cAMP production by A_1R and $A_{2B}R$, respectively, in MDA/R. By contrast, exogenous adenosine revealed that adenosine receptors in MDA contribute differently while in MDA/R all receptor subtypes have a similar contribution to cAMP production. Thus, adenosine contributes to overcome platin-derived resistance in TNBC, involving the inactivation of NF-κB pathway and decrease of ERK phosphorylation (partially mediated by A_3R).

Keywords: Adenosine; Cancer Resistance; Carboplatin; Cisplatin; TNBC

Introduction

TNBC presents few pharmacological therapy options and the development of drug resistance further aggravates treatment. As a result, new therapy regiments capable of restoring drug efficiency are required. Recently, adenosine has been found to sensitize ovarian cisplatin-resistant cancer cells. However, the impact of adenosine over TNBC cisplatin-resistant cancer cells has never been addressed.

1. How does cisplatin resistance promote TNBC cells survival?
2. Is the treatment of TNBC cells with adenosine capable of overcoming cisplatin-resistance?
3. What is the best treatment strategy: concomitant or pre-incubation of adenosine with platin-drugs?

Results and discussion

Impact of endogenous adenosine

- Endogenous adenosine has no effect over ERK phosphorylation.
- In cisplatin-sensitive TNBC cells, NF-κB phosphorylation is dependent on $A_{2A}R$, $A_{2B}R$ and A_3R.
- In cisplatin-resistant TNBC cells, NF-κB phosphorylation is dependent on $A_{2B}R$.
- cAMP production is dependent of A_1R and $A_{2B}R$ in both cisplatin-sensitive and -resistant TNBC cells.

Cisplatin-resistance of TNBC cells seems to be a result of a tonic cAMP increase and NF-κB activation.

Increase in cell survival

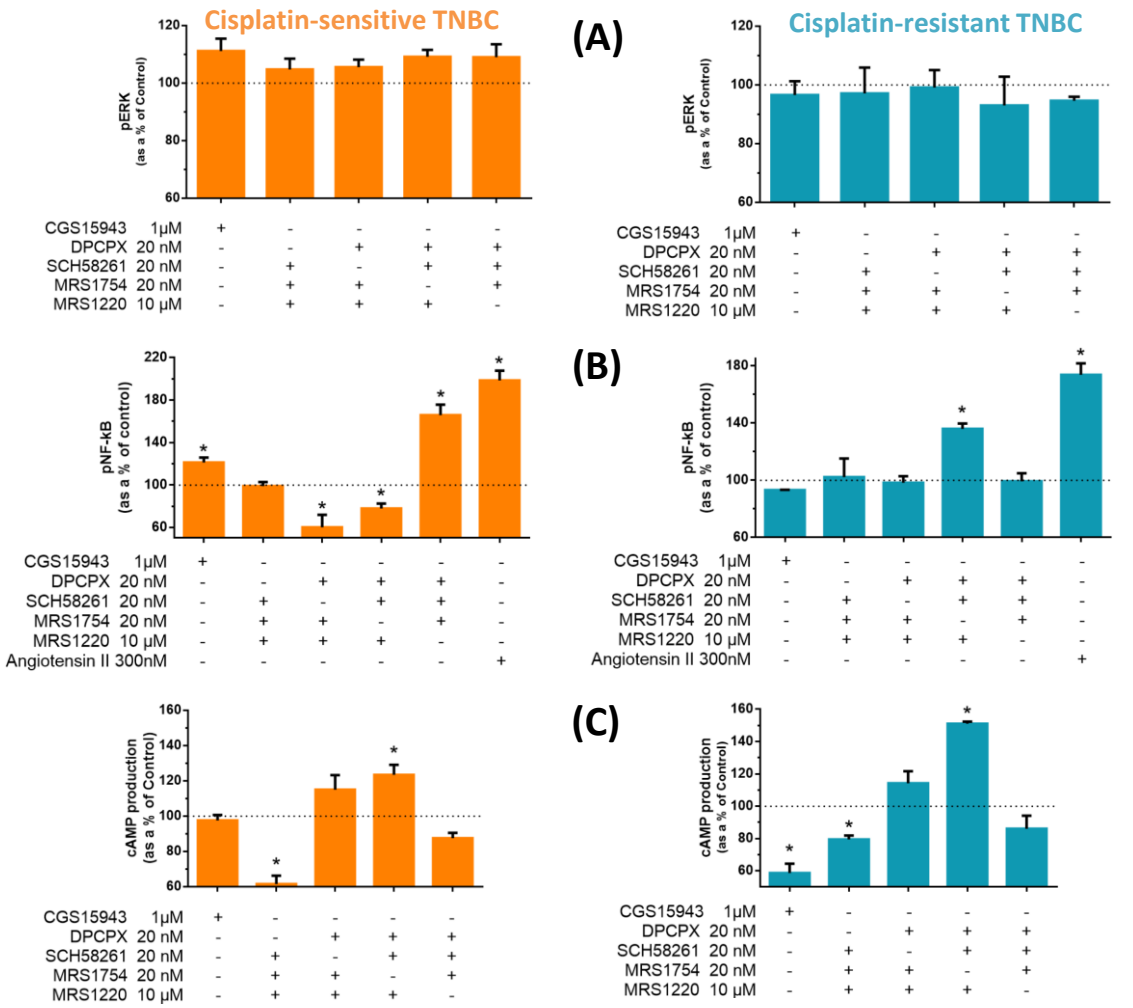


Figure 1 – Impact of endogenous adenosine on TNBC cells. Effects of endogenous adenosine on TNBC cisplatin-sensitive (orange) and -resistant (blue) cells on (A) ERK and (B) NF-κB phosphorylation and (C) cAMP production, after treatment with CGS15943, DPCPX, SCH58261, MRS1754 and MRS1220 (a non-selective, A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R antagonists, respectively) repreented as a % of control. Data represented as mean ± SEM, n=3. Data analyzed with One-Way ANOVA *t*-test followed by Dunnett's multiple comparison test; *p<0.05, differences from control.

Results and discussion

Impact of exogenous adenosine

- In cisplatin-resistant TNBC cells, exogenous adenosine causes a decrease in ERK phosphorylation.
- Exogenous adenosine leads to an inactivation of NF-κB pathway.
- Exogenous adenosine increases cAMP production in adenosine receptor dependent and independent ways.
- In cisplatin-sensitive TNBC cells, cAMP production is modulated by A_1R and $A_{2B}R$. While in cisplatin-resistant TNBC cells, cAMP production seems to be modulated similarly by adenosine receptors.

Exogenous adenosine contributes to NF-κB inactivation and reduction of ERK phosphorylation.

Increase in cell death

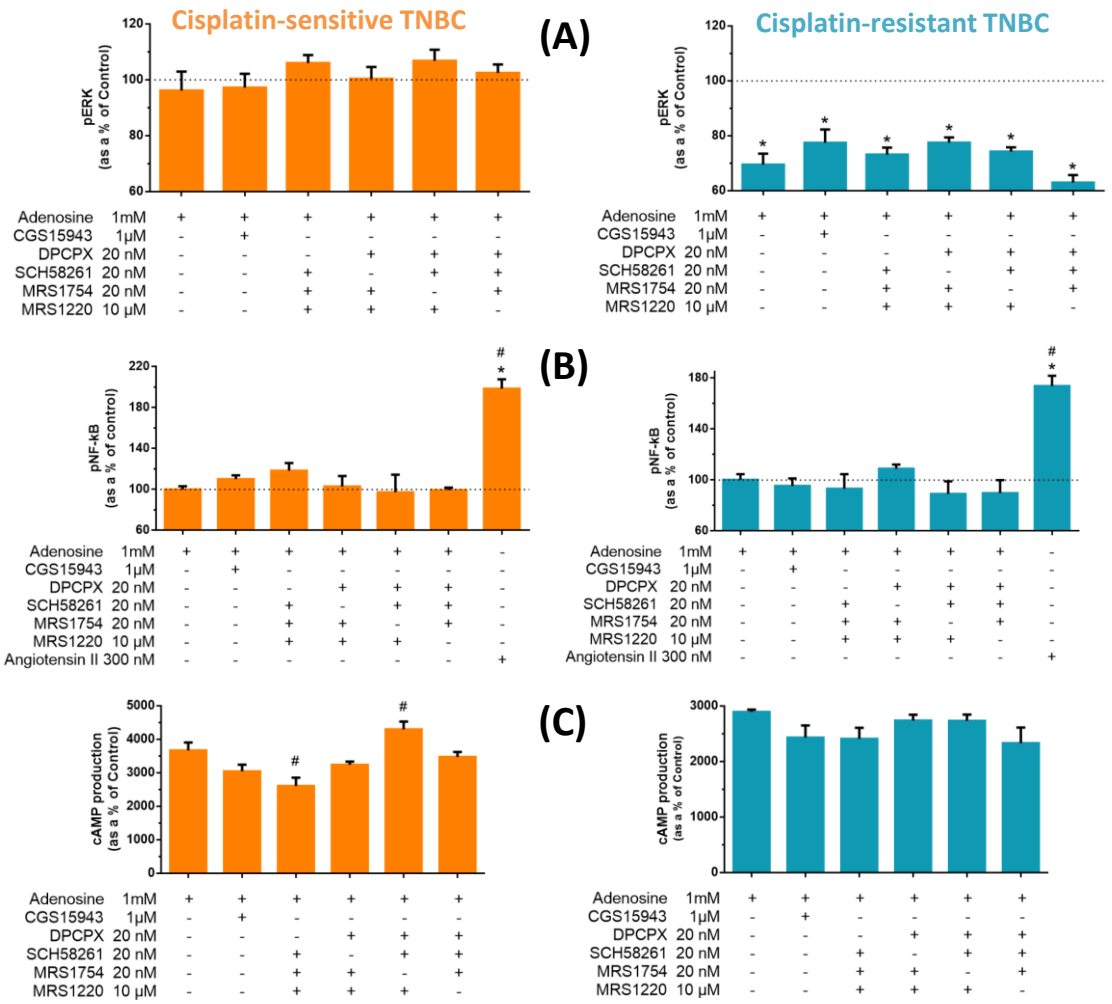


Figure 2 – Impact of exogenous adenosine on TNBC cells. Effects of exogenous adenosine on TNBC cisplatin-sensitive (orange) and -resistant (blue) cells on (A) ERK and (B) NF-κB phosphorylation and (C) cAMP production, after treatment with CGS15943, DPCPX, SCH58261, MRS1754 and MRS1220 (a non-selective, A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R antagonists, respectively), followed by adenosine represented as % of control. Data represented as mean ± SEM, n=3. Data analyzed with One-Way ANOVA *t*-test followed by Dunnett's multiple comparison test; *p<0.05, differences from control.

Results and discussion

Impact of exogenous adenosine

- Combination treatment of adenosine and cisplatin is capable of overcoming cisplatin resistance in TNBC cells, both in pre-incubation and concomitant regiments.
- Only pre-incubation with adenosine followed by carboplatin was able to overcome cisplatin-resistance in TNBC cells.

When used in a pre-incubation regiment, adenosine increases sensitivity of resistant TNBC cells to platin-derived chemotherapeutic drugs.

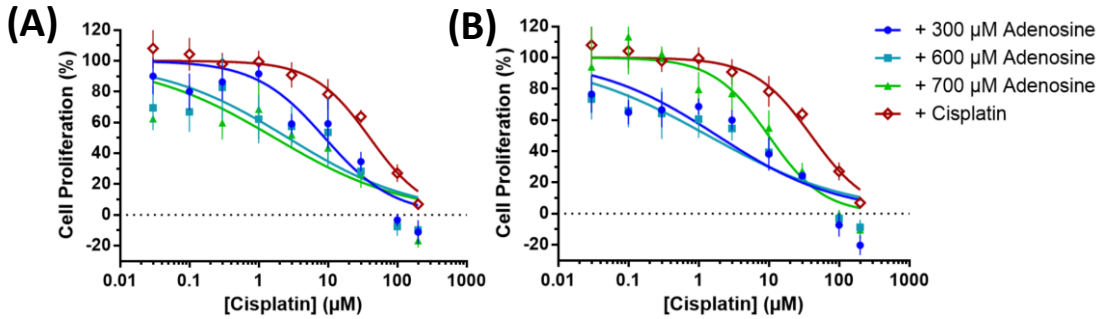


Figure 3 – Impact of adenosine on cisplatin treatment of cisplatin-resistant TNBC cells. Dose-response curves of cisplatin (red) in cisplatin-resistant TNBC cells after (A) 48h pre-incubation treatment and (B) concomitant treatment with adenosine 300 μM, 600 μM and 700 μM (blue, turquoise, and green, respectively) at 48h of incubation. Data is expressed as mean ± SEM, n=3 Data points with no visible error bars have errors smaller than the size of the symbol.

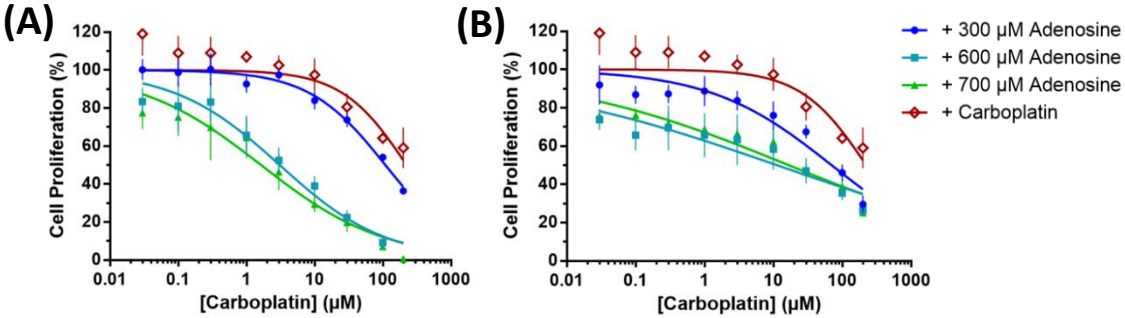


Figure 4 – Impact of adenosine on carboplatin treatment of cisplatin-resistant TNBC cells. Dose-response curves of carboplatin (red) in cisplatin-resistant TNBC cells after (A) 48h pre-incubation treatment and (B) concomitant treatment with adenosine 300 μM, 600 μM and 700 μM (blue, turquoise, and green, respectively) at 72h of incubation. Data is expressed as mean ± SEM, n=3 Data points with no visible error bars have errors smaller than the size of the symbol.

Conclusions

1. Cisplatin-resistance of TNBC cells seems to be a result of a tonic cAMP increase and NF-κB activation.

2. Addition of exogenous adenosine to cisplatin-resistant TNBC cells leads to NF-κB inactivation and reduction of ERK phosphorylation.

3. When utilized in a pre-incubation regiment followed by cisplatin or carboplatin treatment, adenosine increases sensitivity of resistant TNBC cells to platin-derived chemotherapeutic drugs.

Adenosine is a natural occurring compound capable of overcoming platin-derived chemotherapeutic drug resistance in resistant TNBC cells.

Acknowledgments

This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020. M.V. thanks the Portuguese Foundation for Science and Technology (FCT) and PhD Program in Medicines and Pharmaceutical Innovation (i3DU) for PhD Grant PD/BD/135460/2017 funded by the European Social Fund of the European Union and national funds FCT/MCTES.