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Polishchuk N., Chornyi S.

THE IMPACT OF ARGININE LIMITATION IN COMBINATION WITH CANAVANINE TREATMENT ON CELL VIABILITY AND PI3K/AKT/MTORC1 SIGNALING PATHWAY IN COLON CARCINOMA CELLS

Institute of Cell Biology, National Academy of Sciences of Ukraine
14/16, Drahomanova Str., 79005 Lviv, Ukraine
e-mail: nikitathewhale@gmail.com

The cells of many aggressive cancer types in course of malignant transformation become auxotrophic for arginine. This cellular inferiority has been used to design selective anticancer therapy based on the enzymatic arginine depletion. Reduction of arginine availability alone is not sufficient to eradicate malignant cells in an organism, but combinatory treatment with additional anticancer drugs is a promising way to increase cytotoxicity. The goal of the project was to investigate how co-application of mTOR inhibitor Torin-1 and an arginine analogue of plant origin – canavanine will impact PI3K/Akt/mTORC1 signalling and cell viability of colon carcinoma cells under arginine starvation.

We used colon carcinoma cell model with hyperactivated mTORC1 – cell line SW480 with knock out of TSC2 and wild-type SW480 cell line in experiments. Cell lines were incubated in control (arginine-replete) medium with all sufficient components for cell growth or in arginine-depelete medium for 0–72 hour. Canavanine was used in previously determined, minimal toxic for the cell lines concentration of 100 μM. mTOR inhibition was induced with 10 μM Torin-1. Cell viability was determined using MTT assay and protein level was determined with Western blotting.

As was expected, the cell line with TSC2 knock out showed higher level of sensitivity to mTOR inhibition in compare to wild-type cell line. Co-application of canavanine with arginine deprivation led to significant decrease of cell viability in all analyzed cell lines. Torin-1 and canavanine in combination in arginine-deplete medium significantly decreased cytotoxicity of canavanine. Mechanism of canavanine toxicity decreasing by Torin-1 probably associated with inhibition of translation caused by mTORC1 inhibition. Unfortunately, no significant difference in sensitivity to arginine deprivation with/without the addition of canavanine or Torin-1 between cell line with hyperactivated mTORC1 and wild-type cell line was detected. Also, we found interesting specific changes in Akt signalling under arginine deprivation (48–72 h) and canavanine treatment in TSC2 knock out cell lines but not in the wild-type cell line.

Rarok Y.[1], Khoma V.[1], Gnatyshyna L.[1, 3], Horyn O.[1], Druvietis I.[2], Ozoliņš D.[2], Parele E.[2], Skuja A.[2], Kokorīte I.[2], Falfushynska H.[1], Spriņģe G.[2], Stoliar O.[1]

DIFFERENT VULNERABILITY OF INDIGENOUS AND INVASIVE BIVALVE MOLLUSKS TO ENVIRONMENTAL IMPACT DETECTED FROM THEIR RESPONSES OF STRESS AND TOXICITY

[1] Ternopil National Pedagogical University
2, M. Kryvonosa Str., 46027 Ternopil, Ukraine
[2] University of Latvia, Institute of Biology
3, Miera Str., Salaspils, LV 2169, Latvia
[3] I. Ya. Horbachevsky Ternopil State Medical University
Maidan Voli 1, 46001 Ternopil, Ukraine
e-mail: Oksana.Stolyar@tnpu.edu.ua

Bivalve molluscs are known by the sensitive responses to changes in environmental conditions. Due to these dentary nature, filter-feeding behaviour, ability to accumulate pollutants and sensitivity to environmental temperature, their molecular and biochemical activities represent the examples of particular adaptation to chemical pollution and temperature changes in certain limits. In the current study, we focused on stress and toxicity responses of two species of bivalve mollusks, *Unio tumidus* and *Dreissena polymorpha* depending on their settlement in the artificial reservoirs associated with hydropower plants (HPPs). Biochemical responses of the indigenous and invasive bivalves inhabiting the reservoirs of the hydropower plants (HPPs) in Ukraine (Dniester basin) and Latvia (Daugava basin) were compared with responses of the mussels from the pristine (Latvia) or after dam (Ukraine) sites. We hypothesised that zebra mussel *D. polymorpha* as an invasive species can adapt easily to environmental impact.

In the indigenous mussels from the reservoir of HPP, typical responses to pollution by pesticides and personal care products were indicated. They demonstrated the depletion of choline esterase activity typical for effect of thiocarbamate pesticides. The level of vitellogenin, determined as alkali labile phosphates (ALP), was the highest in this group. They were distinguished also by high glutathione *S*-transferase (GST) activity that can be induced by organic substances pollution. The highest cathepsin D lysosomal activity and efflux in group of *U. tumidus* from the reservoir attested the response of autophagy. In *D. polymorpha* from the reservoir, the same as in *U. tumidus*, depletion of GSH/GSSG and signs of the metabolic depression due to low pyruvate concentration and, consequently, higher in 1.4 times lactate/pyruvate ratio was found compare to specimens from native pond. ALP level was higher in zebra mussels from reservoir the same as in *U. tumidus*. However, GST activity was the same in zebra mussels from two sites of comparison. Moreover, choline esterase activity was higher in *D. polymorpha* from the reservoir than in mollusks from the pristine site, demonstrating neurological abnormalities. Lysosomal cantepsin D efflux in zebra mussels from the reservoir was 1.6 times lesser than in their counterparts from the pristine site. The concentration of metallothioneins was low in comparison with known examples of exposed to metals mollusks, and did not differ significantly between the groups from different sites for both species. This feature indicated low level of pollution by trace metals in the areas of investigation. Unexpectedly, mollusks from the reservoirs, both unionidae and dreissenidae, did not demonstrate

signs of lipid peroxidation, unlike mollusks from pristine sites. In mollusks from the reservoirs, superoxide dismutase (Cu, Zn-SOD and Mn-SOD) activities were even higher than in mollusks from groups of comparison.

These data reflect that up-regulation of ALP and antioxidant response but depletion of glutathione are common responses on two molluscan species. The sensitivity of responses to specific kinds of pollution (cholinesterase and GST activities) were more developed in the indigenous mollusks, whereas the invasive mollusks are more tolerate to environment of HPP reservoir. This comparison gives a new prove to regularity that invasive species have higher adaptive depending on environment.

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Revka O., Patalakh I., Misztal T., Marcińczyk N., Gołaszewska A., Grinenko T.

PLATELETS AS COORDINATION CENTERS OF FIBRIN FORMATION, STRUCTURING OF FIBRIN NETWORK AND INITIATION OF FIBRINOLYSIS

Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine
9, Leontovicha Str., 01601 Kyiv, Ukraine
e-mail: sedrickedel@gmail.com

Fibrin formation and its subsequent lysis are complex and related processes, accurately regulated to maintain hemostatic balance. Platelets play an essential role in such regulation as an interconnection point of both coagulation and fibrinolysis. The aim of the present study was to investigate possible molecular mechanisms underlying the coordination of these processes by platelets.

Clot waveform analysis assay (CWA) was used as a tool to study the net effect of platelets in platelet rich plasma (PRP) on local hemostatic balance reached due to concerted rates of fibrin polymerization/degradation processes. Using confocal microscopy method, under flow and static conditions, formation of platelet-fibrin microaggregates on collagen surface and binding of t-PA to microaggregates obtained from the whole blood in real time as well as fibrin clot structures obtained from PRP was analyzed.

The initiation of coagulation by calcium chloride or calcium chloride with thrombin was performed in our modification of CWA assay to distinguish the influence of platelets on the intrinsic coagulation pathway or terminal reactions of coagulation cascade, subsequently. Platelets stimulated coagulation in direct proportion to the cell number (in particular, lag phase and time to peak were shortened), but after addition of t-PA, platelets also potentiated fibrinolysis, ultimately shifting the overall balance to a profibrinolytic state (and shortening the lifetime of the clot).

Using flow chamber model system to visualize the fibrin formation processes in real time after platelets adhesion on collagen, we observed that platelets' microaggregates served as centers of the fibrin polymerization initiation. Formation of fibrin network continued in a space between microaggregates. These findings were also confirmed in static conditions with clotted PRP, where fibrin network was structured around clusters of platelets that played a role of clot architecture "organization centers". In comparison with platelet free plasma-derived clots fibrin fibers packing in PRP were not homogenous, being the densest on clustered platelets, thus presumably creating the structure to simplify the access of fibrinolytic factors and to make more beneficial milieu for lysis. Indeed, under flow conditions we observed t-PA binding to platelet microaggregates. Moreover, t-PA binding (presumably through fibrin) was higher on platelets, stimulated by additional agonists, for instance, by thrombin receptor-activating peptide (TRAP) or ionomycin (ionophore used to evoke secretion). At the same time, binding of t-PA was retained also in a presence of ε-aminocaproic acid (blocker of lysin-binding sites). Furthermore, when platelets where stimulated by both collagen and thrombin (generated *in situ*), there was a colocalization between bound labeled t-PA and labeled FX(a) antibodies, which can be an evidence of the role of prothrombinase in binding t-PA and potential plasminogen activation. Together with the observation of t-PA binding also in a presence of tirofiban (glycoprotein IIb/IIIa inhibitor) these data suggest another, fibrin – independent mechanisms of t-PA binding.

In conclusion, the evidence from this study suggests that platelets regulate both fibrin formation and it's subsequent lysis, and can coordinate coagulation and fibrinolysis processes through the components of the intrinsic pathway and plasminogen/plasmin system, particularly by structuring fibrin network and creating areas with different properties within clot, and by binding of t-PA through fibrin-dependent and -independent mechanisms, therefore regulating local hemostatic balance.

Rożek P.[1], Kluz M.[2]

ANTIMICROBIAL EFFECT OF DIFFERENT CHOCOLATES

[1] Students Scientific Group of Food Technologists "FERMENT", University of Rzeszów
2, Ćwiklińskiej Str., 35-601 Rzeszów, Poland
[2] Department of Bioenergetics and Food Analysis, University of Rzeszow
Zelwerowicza 4, 35-601 Rzeszow, Poland
email: piotrek1998.80@gmail.com

Apart from a good source of energy and great taste, chocolate has other less well-known applications. One of them is its antimicrobial activity. The effectiveness of antibacterial activity depends mainly on the type of chocolate. The antiseptic ingredient is cocoa. It constitutes 30 to 99 % of the chocolate composition.