Cytotoxic, antiradical activity and limited stability of anthocyanidins in human cell cultures

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Introduction

Anthocyanins (ACs) are molecules in which a sugar is bound to another non-sugar functional group – aglycone - anthocyanidin (ACdn). Numerous studies have shown that both forms are biologically active. The ACdn are limited to a few structure variants such as delphinidin, cyanidin, pelargonidin, peonidin and malvidin. Although effects of ACdn in antioxidant, cell proliferation and stability assays are studied, their metabolism in biological fluids and stability in different cell line cultures in vitro assays are still little investigated.

The aim of this study was to compare biological activity of three ACdn – malvidin (M), delphinidin (D) and cyanidin (C) in different human cell lines, as well as to study metabolism of the ACdn in cell culture environment.

Methods

Influence on cell proliferation of ACdn (Sigma-Aldrich, USA) at concentration of 25, 50 and 100 μM was investigated by using ViaCount and CCK-8 tests, and antiradical activity by DPPH assay. Stability of ACdn in cell media after 24 h was evaluated by UHPLC-TOF-MS/MS method. Studied human commercial cell lines (ATCC, USA) were: monocytic leukemic cell line (THP-1), adipose mesenchymal stem cells (aMSCs), breast adenocarcinoma cell line (MCF-7) and metastatic breast adenocarcinoma cell line (MDA-MB-231).

Results

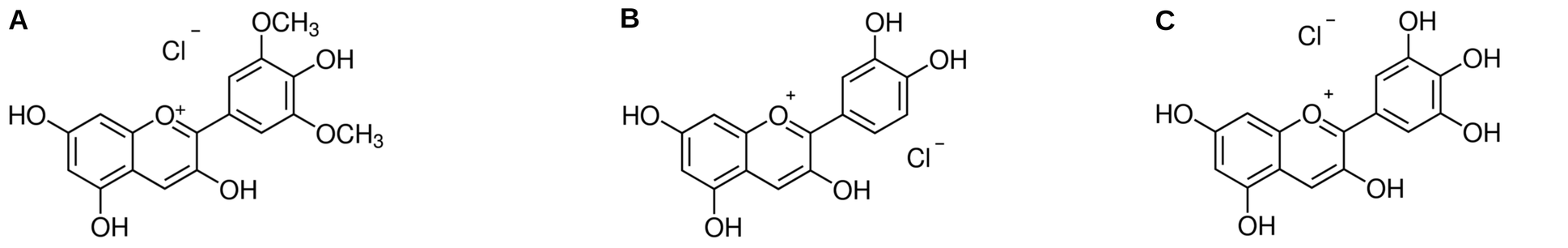

Fig. 1. **Structural formulas of malvidin chloride (A), cyanidin chloride (B) and delphinidin chloride (C).**

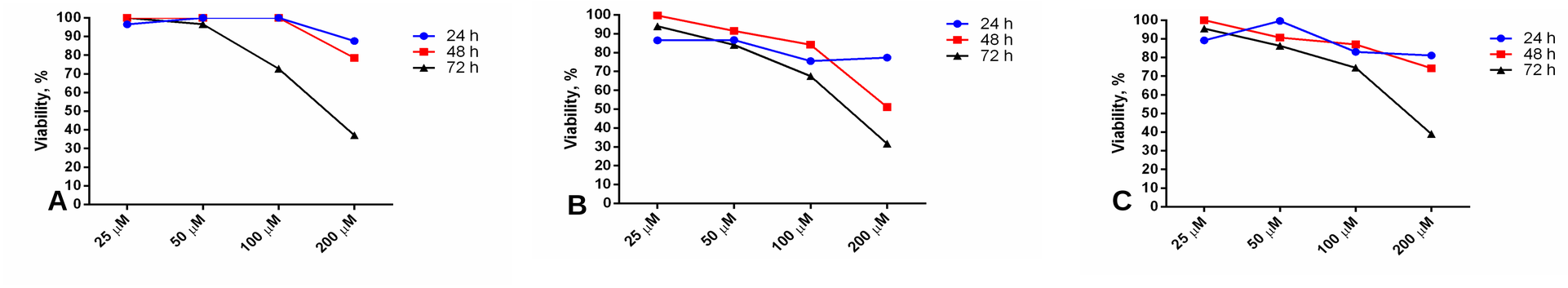

Fig. 2 . Effects of **malvidin (A), cyanidin (B) and delphinidin (C) on THP-1 cell viability in CCK-8 assay.**

	IC50, μM		
Cell lines	Malvidin,	Cyanidin	Delphinidin
THP1	70 ± 6	60 ± 12	30 ± 3
aMSCs	90 ± 14	54 ± 15	40 ± 9
MCF-7	> 100	> 100	> 100
MDA-MB-231	> 100	> 100	> 100

Table 1. Effects of **ACdns on cell line proliferation. Data analysed with GraphPad Prism 5 software.**

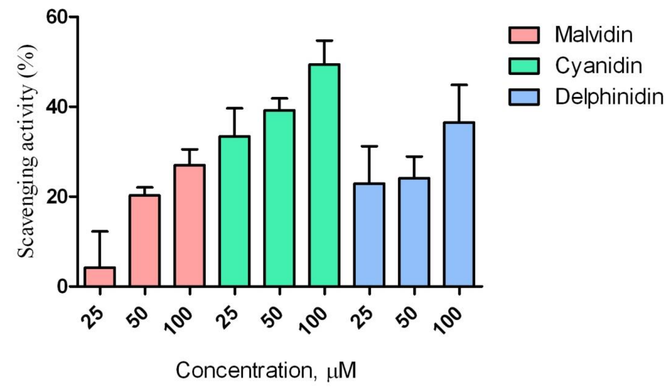

Fig. 3 **Antiradical activity by DPPH assay.**

ACdn	Conc., μg/mL	Cell lines			
		MDA	aMSC	THP-1	MCF 7
		SA concentration μg/mL			
Malvidin	25	3.51	2.95	7.15	7.44
	50	10.98	6.5	10.3	13.61
	100	22.17	15.25	17.69	27.94

ACdn	Conc., μg/mL	Cell lines			
		MDA	aMSC	THP-1	MCF 7
		PA concentration μg/mL			
Cyanidin	25	0.38	0.48	0.35	0.67
	50	1.05	0.95	0.81	1.73
	100	3.18	2.39	1.41	3.68

ACdn	Conc., μg/mL	Cell lines			
		MDA	aMSC	THP-1	MCF 7
		GA concentration μg/mL			
Delphinidin	25	0.48	-	3.13	2.84
	50	0.89	-	1.89	2.47
	100	3.18	-	2.65	2.51

Table 2. **Average concentrations of main metabolites of ACdns in cell media after 24 h incubation with corresponding ACdns. Abbreviations: syringic acid – SA, protocatechuit acid (PA), gallic acid (GA).**

Conclusions

ACdns possess cell line-selective cytotoxicity and limited life time in cell culture. Delphinidin showed the most obvious anti-proliferative effect. Cyanidin exerted stronger antiradical activity. ACdns were not detected in the cell supernatants after 24 h. Concentration of PA and SA increased accordingly to the added concentration of ACdns with the exception of GA. GA was not identified in aMSCs cell medium, but in THP-1 and MCF-7 cells the level of GA did not reflect added amount of delphinidin. GA was further metabolized to various other phenolic acids.

Acknowledgements

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