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Short Communication

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Use of murine L1210 and P388 lymphocytic leukemia cells in cytotoxic studies of flavonoids

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Abstract

In studies of antileukemic properties of flavonoids two murine cellular systems (L1210 and P388) are widely used beside the various established human leukemia lines. Differently from conventional clinically used chemotherapeutics, P388 cells reveal somewhat lower susceptibility towards plant polyphenolic agents than L1210 cells. Moreover, based on the cytotoxic analysis of different flavonoids, we provide some novel evidence of Burkitt`s lymphoma as a human equivalent of L1210 mouse model, enabling thus the use of L1210 murine model in development of novel antilymphoma drugs proceeding from natural polyphenols. At that, structurally different flavones can be considered as potential lead compounds; however, the most potent flavonoid described so far in B-cell lymphoma cells is the rotenoid deguelin, certainly requiring further *in vitro* and *in vivo* investigation.

Keywords: B-cell lymphoma; Mouse model; Human cell lines; Cytotoxic action.

Introduction

It is well proven to date that numerous natural products can be considered as a promising source of clinically useful anticancer compounds [1]. Indeed, up to 70% of current drugs used for fighting against cancer are originally derived from nature whereas several agents, including vinca alkaloids, camptothecin, paclitaxel, and etoposide are of plant origin [1, 2]. Much attention of studies of novel anticancer agents has recently focused on the plant secondary metabolites, flavonoids. These heterocyclic compounds constitute a major chemopreventive part of human diet and exhibit different pharmacological and biological activities both *in vitro* as well as *in vivo* systems [3-6]. Depending on their structure, flavonoids can display antiinflammatory, antioxidant and anticancer properties by inducing inactivation of carcinogens, modulating the cell cycle, promoting apoptosis, or suppressing angiogenesis [6-8].

However, it is also generally accepted that cytotoxic action of flavonoids measured in different cellular model systems may not necessarily predict their biological behavior in humans due to the rapid metabolism and conversion of these polyphenols *in vivo* conditions [9-11]. It means that established cell lines could not always mimic the action of flavonoids *in vivo* and therefore, the choice of appropriate model systems for studies of anticancer properties of plant secondary metabolites is especially important.

In the current short communication, we compiled the data published about antileukemic activity of flavonoids in murine cells and compared them with the respective activity constants in corresponding human cell lines. As a result, we indicate that L1210 mouse model could be considered as a human equivalent of Burkitt`s lymphoma cells in studies of various flavonoids. In addition, we also bring forth some characteristic features of cytotoxic profiles of flavonoids in B-cell lymphoma cells.

Mouse leukemia models L1210 and P388

L1210 and P388 are extensively used models for investigation of processes related to leukemogenesis both *in vitro* and *in vivo* [12]. Indeed, most anticancer agents nowadays in clinical use were first identified and screened by murine leukemias [13]. Both leukemia models were induced in mouse of DBA/2 strain following painting their skin with methylcholanthrene; L1210 and P388 cell lines were established from murine ascitic fluid [13, 14].

Compared to human tumor xenografts, L1210 and P388 models have some advantages but also certain limitations. Although, these murine models are rapidly growing, homogenous and well reproducible, neither of them is considered satisfactory to discover novel drugs for human cancers [13, 14]. Moreover, a relatively poor correlation was described when comparing the efficacy of different drugs in these two mouse models as only 15% of 1564 agents active against P388 were also active against L1210 [13]. In general, P388 leukemia has been shown to be more sensitive than L1210 leukemia in studies of clinically useful compounds, including chlorambucil, mitomycin C, carboplatin, floxuridine, actinomycin D, daunorubicin, teniposide, doxorubicin, vinca alkaloids and some other drugs, producing a certain overprediction of their activity [13]. Despite these disadvantages, the L1210 and P388 models are still in use today, although to a lesser extent than in previous decades, to evaluate the anticancer activity and develop new agents but also to study the mechanisms of drug resistance [12, 13].

L1210 and P388 leukemia cells are widely used also in studies of anticancer effects of flavonoids (Table 1). However, differently from

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the clinically used conventional chemotherapeutics [13], P388 leukemia cells exert somewhat lower sensitivity towards flavonoids than L1210 leukemia cells. This tendency becomes evident in the examination of cytotoxicity of flavones luteolin [7, 15] and chrysin [7, 16, 17], but also of flavonol galangin [7, 16] enabling some comparative analysis (Table 1). These data further emphasize the necessity to clarify the specific events of intracellular signaling mechanisms triggered by flavonoids in leukemia cells.

Evaluation of human equivalent of L1210 mouse model by cytotoxic action of flavonoids

Although L1210 and P388 are not considered as completely satisfactory models to identify novel drugs for human malignancies these models have a long history of use in pharmacological screening and they are still used in numerous studies devoted to finding of new and potent anticancer agents. At that, it has been previously suggested that L1210 mouse model can be equivalent to human Burkitt`s lymphoma [18]. The works performed with flavonoids also corroborate this position as the values of half maximal inhibitory concentrations (IC_{50}) of isoflavone genistein are rather similar in mouse L1210 cells [19] and various human B-cell lymphoma lines GA10, Raji, and Toledo [19, 20], revealing at the same time somewhat stronger potency in human B-cell acute lymphoblastic leukemia (ALL) RS4;11 cells [20] (Tables 1 and 2). The human cell lines used in the respective studies are well representative of their malignant categories [20]. Likewise, flavonol quercetin is cytotoxically active in similar micromolar doses both in murine L1210 cells [7, 21] as well as in human Burkitt`s lymphoma cell lines Daudi and Namalwa [22] (Tables 1 and 2). Again, prenylated chalcone xanthohumol is considerably more sensible in B-cell ALL lines 697, ALL-PO, Nalm-6, and RS4;11 than in L1210 model [2] (Tables 1 and 2). These data show that L1210 cells may reflect the processes occurring following exposure of human Burkitt`s lymphoma cells to flavonoids and therefore, L1210 could be used as a model to study the potential anticancer action of polyphenolic plant secondary metabolites in B-cell lymphoma, both *in vitro* as well as *in vivo*.

Burkitt`s lymphoma is an aggressive and very rapidly growing malignancy in lymphatic system and its incidence is steadily increasing. However, treatment of this cancer by using different modalities (i.e., chemotherapy, molecularly targeted therapy, immunotherapy) has revealed varied success rates showing that an effective antilymphoma treatment is still highly needed [3, 23]. Therefore, identification of novel drugs for prevention and treatment of this non-Hodgkin lymphoma is presently a major challenge [24] and possession of appropriate model systems is a critical prerequisite for this task.

Nevertheless, there are still not enough experimental data available to propose or draw any clear conclusions about the human equivalent of P388 model based on the cytotoxic action of flavonoids.

Characteristic features of anticancer effects of flavonoids in B-cell lymphoma cells

The data compiled in Tables 1 and 2 clearly show that different flavones are cytotoxically active in Burkitt`s lymphoma models, both in mouse L1210 cells as well as human B-cell lymphoma lines. Indeed, apigenin, chrysin and luteolin display antiproliferative effects in micromolar range in murine L1210 cells (Table 1).

Table 1. Anticancer effects of flavonoids on murine lymphocytic leukemia cells *in vitro* measured by cell counting or cell viability assay (MTT assay).

Flavonoid	L1210 cells			P388 cells		
	Time, h	IC_{50}, μM	Reference	Time, h	IC_{50}, μM	Reference
Apigenin	24	~10	[7]			
(+)-Catechin	~72	51.7	[4]			
(-)-Catechin				48	>345	[27]
Chrysin	24	>20	[7]	72	28.0±3.0 15.3	[16] [17]
(-)-Epicatechin	~72	51.7	[4]			
Galangin	24	>20	[7]	72	>185.0	[16]
Genistein	24	18.5	[19]			
Hesperidin	~72	NA at 82	[4]			
Luteolin	24	~10	[7]		>34.9	[15]
Morin	24	44±5	[21]			
Quercetin	24 24	47±6 ~10	[21] [7]			
Sulfuretin				48	120.2	[1]
Xanthohumol	48	4	[2]			

NA, not active

and micromolar doses of baicalein, chrysin, luteolin, and wogonin reveal cytotoxic activity in different human Burkitt`s lymphoma lines (Table 2). At that, it is interesting that both the flavone aglycones (baicalein) as well as their glycosides (baicalin, scutellarin) express anticancer effects at relatively similar concentration ranges (Table 2), despite the well-known fact that sugar moieties generally decrease the biological activity of flavonoids by impeding their penetrating of cellular membranes as well as enhancing the steric hinderance of molecules [21, 25, 26]. The specific signaling pathways triggered by flavone aglycones and their glycosides in Burkitt`s lymphoma models certainly need further investigation.

The cytotoxically most potent natural flavonoid described to date in Burkitt`s lymphoma cells is the rotenoid deguelin possessing the IC_{50} value of only about 50 nM [3, 5] (Table 2).

Table 2. Cytotoxicity of flavonoids on human B-cell lymphocytic leukemia and lymphoma lines measured by cell counting or cell viability assays (MTT, XTT or Alamar blue assay).

Flavonoid	Cell line	Characteristics	Time, h	IC_{50}, μM	Reference
Baicalein	Daudi	Burkitt lymphoma	48	7.0	[22]
	Daudi	Burkitt lymphoma	96	NA at 1.7 μM	[28]
	Nalm-6	B-cell ALL*	96	NA at 1.7 μM	[28]
	Namalwa	Burkitt lymphoma	48	23.7	[22]
	P3HR-1	Burkitt lymphoma	72	Between 39.2…75.9	[29]
	Raji	Burkitt lymphoma	72	Between 39.2…75.9	[29]
Baicalin	CA46	Burkitt lymphoma	48	10	[23]
	Daudi	Burkitt lymphoma	96	Active at 23.3 μM	[28]
	Nalm-6	B-cell ALL*	96	Active at 23.3 μM	[28]
	P3HR-1	Burkitt lymphoma	72	Between 23.7…45.9	[29]
	Raji	Burkitt lymphoma	72	Between 23.7…45.9	[29]
Chrysin	Daudi	Burkitt lymphoma	48	40.5	[22]
	Namalwa	Burkitt lymphoma	48	71.8	[22]
Deguelin	Daudi	Burkitt lymphoma	24	0.05	[3]
	Raji	Burkitt lymphoma	24	0.05	[5]
5,7-dimethoxy-flavone	YCUB-2	B-cell ALL*	96	22.7±6.7	[6]
	YCUB-4	B-cell ALL*	96	10.6±1.1	[6]
	YCUB-5	B-cell ALL*	96	24.8±9.2	[6]
	YCUB-6	B-cell ALL*	96	12.8±9.6	[6]
	YCUB-8	B-cell ALL*	96	9.9±12.4	[6]
Fisetin	Daudi	Burkitt lymphoma	48	30.8	[22]
	Namalwa	Burkitt lymphoma	48	51.2	[22]
Genistein	GA10	Lymphoma	72	13.1±3.5	[20]
	Raji	Burkitt lymphoma	48	18.6	[19]
	RS4;11	B-cell ALL*	72	4.9±4.3	[20]
	Toledo	Lymphoma	72	16.9±3.9	[20]
Hesperetin	Daudi	Burkitt lymphoma	48	NA up to 100 μM	[22]
	Namalwa	Burkitt lymphoma	48	NA up to 100 μM	[22]
Kaempferol	CCRF-SB	B leukemia	48	NA to 175 μM	[30]
	JIYOYE	B leukemia	48	NA to 175 μM	[30]
	Namalwa	Burkitt lymphoma	48	NA to 175 μM	[30]
Luteolin	Daudi	Burkitt lymphoma	48	20.0	[22]
	Namalwa	Burkitt lymphoma	48	31.6	[22]
	P3HR-1	Burkitt lymphoma	72	66.4	[31]
Myricetin	RPMI 8226	B leukemia	48	NA at 10 nM-100 μM	[30]
Naringenin	Nalm-6	B-cell ALL*	48	157±16	[8]
Quercetin	Daudi	Burkitt lymphoma	48	14.0	[22]
	Namalwa	Burkitt lymphoma	48	58.1	[22]
Scutellarin	Daudi	Burkitt lymphoma	24	118.1	[24]
	Namalwa	Burkitt lymphoma	24	16.7	[24]
	Raji	Burkitt lymphoma	24	70.1	[24]
Wogonin	P3HR-1	Burkitt lymphoma	72	Between 37.3…72.1	[29]
	Raji	Burkitt lymphoma	72	Between 37.3…72.1	[29]
Xanthohumol	697 line	B-cell ALL*	72	More sensible than L1210	[2]
	ALL-PO	B-cell ALL*	72		[2]
	Nalm-6	B-cell ALL*	72		[2]
	RS4;11	B-cell ALL*	72		[2]

*ALL, acute lymphoblastic leukemia; NA, not active

Its antiproliferative effects in human malignant B-cell lymphoma cells is related to the action on nuclear factor NF-κB [5], however, the effects of this compound in L1210 mouse model have not been studied so far, neither *in vitro* nor *in vivo*, offering thus an interesting further step in antilymphoma research of flavonoids.

Conclusions

In this short communication we indicate that mouse L1210 lymphocytic leukemia model is an appropriate equivalent of human Burkitt`s lymphoma in anticancer studies of various flavonoids and therefore, the respective model can be used for development of novel effective polyphenolic antilymphoma agents against this aggressive and rapidly growing lymphatic malignancy. Based on the data currently available in the literature, structurally different flavones can be considered as promising lead compounds, however, the most potent natural flavonoid described so far is the rotenoid deguelin, certainly requiring further *in vitro* and *in vivo* studies. Possession of appropriate model systems for these investigations is a critical step in the antilymphoma drug development process.

References

[1]. Lee KW, Chung KS, Seo JH, Yim SV, Park HJ, Choi JH, Lee KT. Sulfuretin from heartwood of Rhus verniciflua triggers apoptosis through activation of Fas, Caspase-8, and the mitochondrial death pathway in HL-60 human leukemia cells. *J Cell Biochem*. 2012;113(9):2835-2844.

[2]. Benelli R, Vene R, Ciarlo M, Carlone S, Barbieri O, Ferrari N. The AKT/NF-kB inhibitor xanthohumol is a potent anti-lymphocytic leukemia drug overcoming chemoresistance and cell inflitration. *Biochem Pharmacol*. 2012;83(12):1634-1642.

[3]. Liu HL, Chen Y, Cui GH, Wu QL, He J. Regulating expressions of cyclin D1, pRb, and anti-cancer effects of deguelin on human Burkitt`s lymphoma Daudi cells in vitro. *Acta Pharmacol Sin*. 2005;26(7):873-880.

[4]. Grace MH, Wilsonb GR, Kandil FE, Dimitriadis E, Coates RM. Characteristic flavonoids from Acacia burkittii and A. acuminata heartwoods and their differential cytotoxicity to normal and leukemia cells. *Nat Prod Commun*. 2009;4(1):69-76.

[5]. Chen WH, Chen Y, Cui GH. Deguelin inhibits expression of IkappaBalpha protein in Raji and U937 cells. *Acta Pharmacol Sin*. 2006;27(4):485-490.

[6]. Goto H, Yanagimachi M, Goto S, Takeuchi M, Kato H, Yokosuka T, Kajiwara R, Yokota S. Methylated chrysin reduced cell proliferation, but antagonized cytotoxicity of other anticancer drugs in acute lymphoblastic leukemia. *Anticancer Drugs* 2012;23(4):417-425.

[7]. Cipak L, Novotny L, Cipakova I, Rauko P. Differential modulation of cisplatin and doxorubicin efficacies in leukemia cells by flavonoids. *Nutr Res*. 2003;23(8):1045-1057.

[8]. Kanno S, Tomizawa A, Hiura T, Osanai Y, Shouji A, Ujibe M, Ohtake T, Kimura K, Ishikawa M. Inhibitory effects of naringenin on tumor growth in human cancer cell lines and sarcoma S-180-implanted mice. *Biol Pharm Bull*. 2005;28(3):527-530.

[9]. Ciesielska E, Wolszczak M, Gulanowski B, Szulawska A, Kochman A, Metodiewa D. In vitro antileukemic, antioxidant and prooxidant activities of Antoksyd S (C/E/XXI): a comparison with baicalin and baicalein. *In Vivo* 2004;18(4):497-503.

[10]. Zhang J, Harrison JS, Uskokovic M, Danilenko M, Sludzinski GP. Silibinin can induce differentiation as well as enhance vitamin D3-induced differentiation of human AML cells ex vivo and regulates the levels of differentiation-related transcription factors. *Hematol Oncol*. 2010;28(3):124-132.

[11]. Ludwig-Müller J, Tokalov SV, Franz A, Gutzeit HO. Quercetin metabolism in vital and apoptotic human leukaemia cells. *Biol Chem*. 2005;386(3):279-283.

[12]. Aldred AJ, Cha MC, Meckling-Gill KA. Determination of a human endpoint in the L1210 model of murine leukemia. *Contemp Top Lab Anim Sci*. 2002;41(2):24-27.

[13]. Dykes D, Waud W. Murine L1210 and P388 leukemias. In: Teicher B, ed. Tumor Models in Cancer Research. Totowa, NJ: Humana Press, Inc., 2002, pp.23-40.

[14]. Donnou S, Galand C, Touitou V, Sautes-Fridman C, Fabry Z, Fisson S. Murine models of B-cell lymphomas: promising tools for designing cancer therapies. *Adv Hematol*. 2012;2012:701704.

[15]. Pettit GR, Hoard MS, Doubek DL, Schmidt JM, Pettit RK, Tackett LP, Chapuis JC. Antineoplastic agents 338. The cancer cell growth inhibitory. Constituents of Terminalia arjuna (Combretaceae). *J Ethnopharmacol*. 1996;53(2):57-63.

[16]. Chen JJ, Lee HH, Duh CY, Chen IS. Cytotoxic chalcones and flavonoids from the leaves of Muntingia calabura. *Planta Med*. 2005;71(10):970-973.

[17]. Nshimo CM, Pezzuto JM, Kinghorn AD, Farnsworth NR. Cytotoxic constituents of Muntingia calabura leaves and stems collected in Thailand. *Int J Pharmacog*. 1993;31(1):77-81.

[18]. Mills AE, Sullivan JR. Malignant disease. In: Cook GC, Zumla AI, eds. Manson`s Tropical Diseases.

Philadelphia Saunders, 2009, pp.635-.660.

[19]. Raynal NJ, Momparler L, Charbonneau M, Momparler RL. Antileukemic activity of genistein, a major isoflavone present in soy products. *J Nat Prod*. 2008;71(1):3-7.

[20]. Li W, Frame LT, Hoo KA, Li Y, D`Cunha N, Cobos E. Genistein inhibited proliferation and induced apoptosis in acute lymphoblastic leukemia, lymphoma and multiple myeloma cells in vitro. *Leuk Lymphoma* 2011;52(12):2380-2390.

[21]. Krol W, Dworniczak S, Pietsz G, Czuba ZP, Kunicka M, Kopacz M, Nowak D. Synthesis and tumoricidal activity evaluation of new morin and quercetin sulfonic derivatives. *Acta Pol Pharm*. 2002;59(1):77-79.

[22]. Sak K, Lust H, Everaus H. Cytotoxic action of flavonoids in human Burkitt`s lymphoma cell lines and its antiandrogenic modulation. *Int J Toxicol Pharmacol Res*. 2005; accepted.

[23]. Huang Y, Hu J, Zheng J, Li J, Wei T, Zheng Z, Chen Y. Down-regulation of the PI3K/Akt signaling pathway and induction of apoptosis in CA46 Burkitt lymphoma cells by baicalin. *J Exp Clin Cancer Res*. 2012;31:48.

[24]. Feng Y, Zhang S, Tu J, Cao Z, Pan Y, Shang B, Liu R, Bao M, Guo P, Zhou Q. Novel function of scutellarin in inhibiting cell proliferation and inducing cell apoptosis of human Burkitt lymphoma Namalwa cells. *Leuk Lymphoma* 2012;53(12):2456-2464.

[25]. Sak K. Cytotoxicity of dietary flavonoids on different human cancer types. *Pharmacogn Rev*. 2014;8(16):122-146.

[26]. Namgoong SY, Son KH, Chang HW, Kang SS, Kim HP. Effects of naturally occurring flavonoids on mitogen-induced lymphocyte proliferation and mixed lymphocyte culture. *Life Sci*. 1994;54(5):313-320.

[27]. El-Gamal AA, Takeya K, Itokawa H, Halim A, Amer MA, Saad HE, Awad SA. Studies on the chemical constituents of Atraphaxis spinosa L. var. sinaica BOISS. *Nat Med*. 1994;48(4):304-306.

[28]. Kumagai T, Muller CI, Desmond JC, Imai Y, Heber D, Koeffler HP. Scutellaria baicalensis, a herbal medicine: anti-proliferative and apoptotic activity against acute lymphocytic leukemia, lymphoma and myeloma cell lines. *Leuk Res*. 2007;31(4):523-530.

[29]. Shieh DE, Cheng HY, Yen MH, Chiang LC, Lin CC. Baicalin-induced apoptosis is mediated by Bcl-2-dependent, but not p53-dependent, pathway in human leukemia cell lines. *Am J Chin Med*. 2006;34(2):245-261.

[30]. Dimas K, Demetzos C, Mitaku S, Marselos M, Tzavaras T, Kokkinopoulos D. Cytotoxic activity of kaempferol glycosides against human leukaemic cell lines in vitro. *Pharmacol Res*. 2000;41(1):85-88.

[31]. Chiang LC, Chiang W, Chang MY, Ng LT, Lin CC. Antileukemic activity of selected natural products in Taiwan. *Am J Chin Med*. 2003;31(1):37-46.