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The Role of Antioxidants in Platelet Aggregation and their Stimulation of NK Cells Against Cancer Cells Line K562

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Atherosclerosis and cancer are two very common diseases that concern the global scientific community because of high lethal rate worldwide. Platelet aggregation is a process, which can be evaluated clinically and with laboratorial methods by measuring the platelet receptors [1]. On the other hand, the role of natural killer cells (NKCs, a population of Lymphocytes with shorter half-life compared to B and T cells) [2], which is the backbone of the immune system seemed to play a major role for the defense of the immune system against tumors. The major immunoregulatory role of these God given cells had been demonstrated especially when administered by plant extracts or vitamin supplements (antioxidants) in numerous in vitro as wells as in vivo scientific studies [3]. Recently, Huntington et al. reported a series of studies that clinically prove that NK cells are a key immune constituent in the protective antitumour immune, and provide an overview of the prognostic value of NK cell gene expression in 25 tumour types [2].

According to the process of platelet aggregation, it is well known that the major glycoprotein, which activated called GpIIb/IIIa, and it's a platelet receptor. The formation of the so-called thrombus (coagulation) is the phenomenon of the binding of fibrinogen with GpIIb/IIIa receptor and other functionally activated receptors [1]. Several studies have been published in the past, and they have demonstrated that antiplatelet drugs have potential action in patients with conditions such as angiogenesis, tumor development, and metastasis [4]. Moreover, the past years, several studies have been performed and proved the stimulation of NKCs on tumor cells by administration of antioxidants both *in vitro* and *in vivo* [3,5].

The major issue for Platelet aggregation for evaluation and treatment of the associated diseases is the selection of the receptors. A wide variety of mobile transmembrane receptors covers the platelet membrane, including many integrins, leucine-rich repeated (LRR) receptors, G-protein coupled seven transmembrane receptors (GPCR) (PAR-1 and PAR-4 thrombin receptors, proteins belonging to the immunoglobulin superfamily (GP VI, FcγRIIA), C-type lectin receptors (P-selectin), tyrosine kinase receptors and and a miscellaneous of other types (CD63, CD36, P-selectin ligand 1, TNF receptor type, etc). However, the most important receptor in platelet aggregation is the GpIIb/IIIa, which is at the forefront of recent research [6]. The basic methodology for platelet aggregation was fully described by Theoxaris et al, and was induced by three different platelet stimulators (PAF, platelet activating factor, ADP, adenosine phosphate, ARA, arachidonic acid). In this study, three antioxidant substances (apigenin, genistein, quercetin) were demonstrated to inhibit platelet aggregation majory in PAF. All three flavonoids showed the same ability to decrease the number of GpIIb/IIIa receptors per platelet [7]. Moreover, apigenin, genistein, and quercetin showed to decrease the production of TXA2 suggesting that each compound may act as an anti-inflammatory agent. Having as basal concept that TXA2 is crucial for every platelet aggregation agonist pathway, it is also demonstrated that flavonoid inhibiting effects are launched by the inhibition of TXA2 production [7]. Another antioxidant such as resveratrol showed in vitro anti-platelet properties, where it completely inhibited platelet aggregation at a concentration of 3 X 10^{-3} M, decreased TXB2 levels, and inhibited the expression of receptor GpIIb/IIIa in non-stimulated platelets [1]. Also, Toliopoulos et al, in another study demonstrated the anti-platelet action of ascorbic acid with five stimulators and various concentrations, by evaluating in vitro its effectiveness in the receptor of GpIIb/IIIa. The ascorbic acid in

3X 10^{-3} M concentration completely inhibited platelet aggregation, decreased thromboxane B2 levels, and inhibited the expression of protein membranic receptor GpIIb/IIIa in non-stimulated platelets [2]. Results in this study showed also ascorbic acid acts also as an anti-inflammatory inhibiting COX (cycloxygenase) action. In other words, COX inhibition by antioxidant substances is caused by the scavenging of oxygen free radicals, which significantly results in decrease of TXA2 production, inhibition of platelet aggregation, and expression of the receptor GpIIb/IIIa [3]. This glycoprotein is considered a basic biological marker in vascular immunological actions and therefore, there is a great clinical interest in development of bioactive compounds that can bind to platelet GpIIb/IIIa, block fibrinogen binding and can be used in the prevention and management of thrombotic diseases affairs [3].

Another major health issue where the global scientific community is still controversial in the daily practice is the management of the immune system in cancer patients and their quality of life. NKCs are the first line of defense even against malignancies without the need of recognition of MHC (major histocompatability complex), which enables them to directly kill tumors or by their activating receptors or by granzymes. Many antioxidants have been administered in patients and have been proved for the increasing stimulation on NKCs against tumors where cancer patients have been benefited clinically [1]. In an in vitro study, Toliopoulos et al. evaluated the precise stimulation of resveratrol on NKCs against K562 cell line by flow cytometric analysis. It was proven that concentration of resveratrol in 3X 10^{-3} M significantly increased the cytotoxicity of NKCs against K563 cancer cell line, where when it was incubated only with target cells without NKCs present it didn't cause significant apoptotic effect. Therefore, resveratrol can be a prominent regulator of cellular toxic events, apoptosis, tumor progression, cell cycle, and many other cellular functions [1]. Other antioxidants such as apigenin, genistein, powerfully reinforce cytotoxic activity of NKCs against tumor cells, where the action of quercetin was statistically not important [6]. Further studies demonstrated in vitro and in vivo the effects of vitamin C and Aloe Vera on NKCs. Vitamin C showed the highest stimulatory effects on NKCs at a concentration of 3X 10^{-3} M [7], where Aloe vera juice at 50μl in vitro. This result was also verified in vivo, when administered to 15 healthy individuals for 45 days, and increase in NKCs activity was observed in all of them and specific immune response was recorded individually.[3] Moreover, a promising study performed by Evangelou et al. was conducted and assisted in management of quality of life in end stage cancer patients by enhancing NKCs by exposure to coherent electromagnetic fields (EMFs), and increasing the levels of NKCs and NK T cells, which means that patients' bone marrow was stimulated during haematopoesis (Table 1) [8]. In this study, patients had completed their chemotherapy, radiation, and/or adjuvant antioxidant treatment at least 4 weeks before participation to the exposure of EMFs. Exposure to EMF at RF has been shown to increase antioxidant system in lymphocytes (NKCs) in patients with other serious diseases, which is in consistent with the data of this study showing that plant extracts, such as resveratrol, enhance significantly NK T-lymphocytes' cytotoxicity against cancer cells [8].

The last important study in NKCs issue, which can open up scientific roads to homeopathic doctors, is the one performed by Toliopoulos et al. and was designed in order to evaluate the effects of five homoeopathic complex preparations on functional activity (NKCs) in advanced cancer patients [9]. Both trials, one performed in volunteers in vitro and one in cancer patients in vivo, data showed significant increase in NKCs activation by the stimulation of the five preparations and gives a hope in the future that they can be used as an adjuvant immunotherapy in advanced cancer

Table 1: The number of NK, NKT cells and their cytotoxicity in 15 end stage cancer patients before and after treatment with EMF at RF [7].

							Cytotoxicity % of NK cells before treatment			Cytotoxicity % of NK cells after treatment			Variation in Cytotoxicity % of NK cells after treatment		
No/Sex	Age	NK	NKT cells before treatment	%NK	NKT cells after treatment	Variation % of total NK cells after treatment	12.5:1	25:1	50:1	12.5:1	25:1	50:1	12.5:1	25:1	50:1
1/F	38	10.57	1.17	21.41	6.12	þ134	26	48	89	32	28	72	"	#	#
2/F	52	6.16	5.54	10.16	20.43	þ161	3	89	84	43	59	72	"	#	#
3/F	66	20.14	3.14	19.25	4.15	þ 1.1	5	15	27	25	38	46	"	"	"
4/F	75	2.48	3.45	5.45	6.78	þ 97	15	35	45	18	42	48	"	"	"
5/F	79	3.55	1.50	8.60	3.50	þ100	2	15	28	5	25	32	"	"	"
6/F	28	5.89	1.70	17.69	16.55	þ351	16	43	38	32	39	79	"	#	"
7/M	36	6.27	3.23	10.62	15.46	þ 174.5	24	49	90	32	39	79	"	#	#
8/M	52	6.80	5.40	10.20	6.35	þ 36	25	46	68	35	52	72	"	"	"
9/M	68	5.25	4.35	8.55	12.70	þ121	18	45	70	28	32	85	"	#	"
10/M	49	15.29	2.45	16.80	4.60	þ 4,3	22	48	55	31	45	72	"	#	"
11/M	55	6.45	4.25	12.70	10.25	þ124	12	32	45	25	57	73	"	"	"
12/F	52	8.30	3.70	15.20	6.70	þ119	15	36	49	20	38	55	"	"	"
13/F	70	3.60	5.25	9.20	12.80	þ122	7	19	39	25	32	48	"	"	"
14/F	47	6.70	3.60	14.55	10.25	þ170	12	42	72	32	38	67	"	#	#
15/F	54	5.95	3.45	17.36	8.58	þ173	14	35	79	23	32	71	"	#	#
Mean	54.7	7.6	3.5	13.2	9.7		14.4	14.4	39.8	58.5	27.1	39.7			
S.D.	14.6	4.6	1.4	4.7	5.0		7.8	7.8	17.9	21.7	8.8	10.0			

patients [9]. A recent encouraging detailed study by Lee et al. demonstrated the anticancer effects of resveratrol as antioxidant in vivo mediated by NK cell activation. Moreover, they proved that resveratrol appeared to activate NK cells most effectively among the substances tested and synergistically increased IFN-γ secretion and NK cell cytotoxicity with interleukin-2 (IL-2).[10]

Overall, it would be significant for most health professionals who care for the benefit of their patients who suffer from a variety of diseases such as cardiovascular, prothrombotic ones such as diabetes, sepsis, thrombocytopenia, sickle cell disease, and vasculitis (Behcet's syndrome), to test them individually for platelet aggregation methodologies, and specifically for the activity of receptor GPIIb-IIIa, which can be specifically evaluated by flow cytometry even before the expression of the disease [10]. Therefore, individual evaluation of specific anti-platelet bioactive substances can be tested by activation of GPIIb-IIIa in relation to other markers (CD9, CD29, CD31, CD36, CD41, CD42a, CD42b, CD61, CD63, CD107a, CD154), and may contribute majorly in daily clinical practice in platelet immunology in both health and disease [11].

Nowadays Huntington et al reported that NK studies are targeted to specific cellular pathways, which can offer a significant value in evolvement of tumor progression, and evaluate cancer immunotherapy response [2]. In conclusion, cancer immunotherapy is a unique methodology, which can be performed by specialized doctors daily by stimulating NKCs and testing their functionality with different cancer cell lines of solid tumors. This can lead to the future of evaluation of clinical therapies and can include biological markers, which may include metabolism, multiplication, EMT (epithelial mesenchymal transition), metastasis, adhesion, and survival of the cancer cell [12]. All the above marker selection and can bring a new era in the specific diagnostic approach, which will absolutely will lead to the elongation of the life span and quality of life of the cancer patient.

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