Potentiation of Natural Killer Cell Activity with Myricetin

CHRISTER LINDQVIST1, MAŁGORZATA BOBROWSKA-HÄGERSTRAND1, LUCYNA MRÓWCZYŃSKA2, CHRISTINE ENGBLOM1 and HENRY HÄGERSTRAND1

1Department of Biosciences, Åbo Akademi University, Turku, Finland; 2Department of Cell Biology, A Mickiewicz University, Poznań, Poland

Abstract. Background/Aim: Myricetin is a flavanoid that can be found in a variety of food sources, including red wine. Several reports have indicated that flavonoids may reduce the disease risk of cancer. The aim of the present study was to investigate the effect of some flavonols on natural killer (NK) cell activity. Materials and Methods: A time-resolved fluorometric assay (TDA-labeled K562 target cells) was used for measuring the cytotoxic activity of NK-cells pre-treated with different flavonoids. Results: A limited number of flavanoids was tested for their ability to enhance the NK activity. Pre-treating NK cells with myricetin, could potentiate their ability to kill K562 erythroleukemia cells. This enhancement of the NK activity was observed in a dose-dependent manner. Similar treatments with the structurally similar molecule quercetin, that lacks one hydroxyl group, did not have any impact at all on NK activity. Conclusion: The enhanced cytotoxic activity observed with myricetin-pretreated-NK cells might shed some light on human studies indicating a preventive role of flavonols against cancer.

Polyphenols belong to a family of molecules characterized by the presence of several phenol groups, each of them having at least one hydroxyl group. They can be found in many types of foods, such as vegetables, fruits and plant-derived beverages (especially red wine), and are further divided into flavonoids and non-flavonoids. Furthermore, many polyphenols share a characteristic color and flavour (1). There are several epidemiological studies indicating that intake of flavonoids may reduce the incidence of breast, colon, lung, prostate and pancreatic cancer (2-4). However, their mechanisms of action are to some extent diffuse, although both anti-oxidant and anti-radical properties have been reported (4, 5).

Natural killer (NK) cells, discovered in 1975 by Kiessling et al. and Herberman et al., are critical mediators of host immunity against cancer and infection (6, 7). In peripheral blood they are characterized as large granular lymphocytes (LGLs) expressing CD3–, CD16+, CD56+ and CD94+ surface markers (8).

NK cell activity is regulated by surface structures that can be broadly categorized as either activating or inhibitory receptors. Inhibitory receptors, that in the human system are killer cell immunoglobulin-like receptors (KIRs), immunoglobulin-like transcripts (ILTs) and C-type lectin inhibitory receptors such as CD94/NKG2A, are used to gauge surrounding cells for their expression of self-molecules (9). Cells having low or no expression levels of MHC class I type of self molecules will, due to this absence, be efficiently lysed (10). The activating types of receptors present on NK cells are even more complex, but some of them are used for detecting molecules that under normal conditions are scarcely expressed, but will be induced upon various forms of stress (11). At least three activating receptor types have been identified, namely the C-type lectin homodimer NKG2D, CD16 and the natural cytotoxicity receptors (NCRs). Totally, three different NCRs have been observed, namely NKp46, NKp44 and NKp30 (12).

Since the balance between the activating and the inhibitory signals will determine the outcome of the NK-cell killing process (13), we have focused our interest on this, by screening for molecules that may alter this balance. The role of flavonols in this process will be discussed.

Materials and Methods

Reagents. Bovine serum albumin (BSA, A2153) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Human rIL-2 was purchased from PeproTech Inc. (Rocky Hill, NJ, USA). Genistein was purchased from Sigma-Aldrich, myricetin from Fluka (Buchs, Switzerland) and quercetin from Merck (Darmstadt, Germany). All compounds were dissolved in DMSO.
Cell lines. The natural killer cell line NK-92 (ImmuneMedicine, Inc.) and the erythroleukemia cell line K562, were cultured at 37˚C in RPMI 1640 with 5% Fetal Calf Serum (GIBCO™, Invitrogen Corp., Paisley, Scotland) supplemented with 2 mM L-glutamine, 10 U/ml penicillin G sodium salt and 10 μg/ml streptomycin sulfate (GIBCO™, 14, 15). The culture medium for the NK-92 cell line was always supplemented with 20 U/ml IL-2.

Pre-treatment of NK-92 cells. 2×10⁶ NK-92 cells/sample were washed once (306 × g, 5 min) in phosphate-buffered saline (PBS, pH 7.4) supplemented with 1 mM CaCl₂, 0.5 mM MgSO₄, and 10 mM glucose. Cells (5×10⁶ cells/ml) were then mixed and incubated for one hour at 37˚C with indicated concentrations of flavonoids. DMSO alone was used as a control (=untreated cells).

Labelling of target cells with BATDA. 1×10⁶ K562 cells in 1 ml RPMI 1640 with 5% FCS were first pre-incubated for 15 min at 37˚C before incubation with 20 μM BATDA (PerkinElmer, Inc. Wellesley, MA, USA) for 25 min at 37˚C. The cells were then washed three times (306 × g, 2 min) in RPMI 1640 with 5% FCS and adjusted to 5×10⁴/ml before mixed with the effector cells.

Cytotoxicity assay. The cytotoxicity assay was performed as described earlier, with minor modifications (16). In brief, serial dilutions of pre-treated NK-92 cells in 100 μl/well (in triplicate) were added to v-bottomed 96-well microtiter plates (Sarstedt Inc) in order to give effector to target ratios ranging from 5:1 to 1.25:1 after addition of 100 μl (5×10⁵) target cells to each well. The plates were then, after a short centrifugation (34 × g, 1 min), incubated for 2 h at 37˚C in a humidified (95%) air atmosphere with 5% CO₂. The plates were thereafter centrifuged for 5 min (688 × g), followed by a transfer of 20 μl supernatant from each well to 100 μl Europium solution (PerkinElmer, Inc. Wellesley, MA, USA) present in flat-bottomed 96-well Costar RIA/EIA plates (Corning Inc., Corning, NY, USA). The spontaneous release was determined by incubating the target cells with culture medium instead of effector cells and maximum release was determined similarly by incubating the target cells in the presence of 0.5% Triton X-100. Plates containing the Europium solution and supernatants were finally shaken for 15 min and fluorescence of the EuTDA chelates formed was measured with a 1420 Victor multilabel counter (PerkinElmer, Inc. Wellesley, MA, USA). The percent specific release was calculated using the following formula:

\[
\text{percent specific release} = \left(\frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}}\right) \times 100\%
\]

Results

A panel of five different flavonoids (quercitin (Figure 1A), myricetin (Figure 1B), genistein (Figure 1C), euchrestaflavone A (Figure 1D) and sophoraflavone H (Figure 1E), were analyzed for their ability to potentiate NK-cell activity using the NK cell line NK-92. They are all structurally different...
from each other, with the exception of myricetin and quercitin, that differs only in one hydroxyl residue. This latter “pair” was selected since out of all molecules tested, only myricetin could clearly enhance the NK-cell activity compared to the close to identical compound-quercetin (Table I). As it can be seen in Figure 2, there is an increase in percent lysis from 56.4±1.6% to 66.1±1.2% for the 1:5 effector:target (E:T) ratio, compared to untreated cells. A similar pattern was also observed for the other E:T ratios. This difference in percent lysis was even higher, when 165 μM myricetin and quercetin pre-treated NK-92 cells were compared.

Pre-treating NK-92 cells with higher concentrations of myricetin increased the NK-cell activity even more (Figure 3). Just 82.5 μM myricetin potentiated the NK-cell activity of the treated NK-92 cells from 36.2±4.2% lysis (untreated) up to 45.8±4.9% (1:2.5 E:T ratio). Pre-treating NK-92 effector cells with 495 μM of myricetin stimulated their NK activity even more. For an E:T ratio of 1:5 one could see an increase from 60.1±2.5% lysis, for the control cells, compared to 91.8±3.85% lysis for the 495μM myricetin-treated effector cells.

**Discussion**

The limited interface between the NK cell and its target cell is referred to as an “NK cell immunological synapse” (NKIS) and serves to link them so close to each other that an efficient delivery of lytic granules can occur (17,18). NKIS can be further divided into two forms, namely non-cytolytic NKIS and cytolytic NKIS, depending on outcome of the recognition process. The non-cytolytic NKIS contains, in addition to adhesion molecules, inhibitory receptors, whereas cytolytic NKIS are built from activating receptors. Lipid rafts are also thought to be involved in the formation of the final NKIS. We have recently demonstrated, using NK sensitive and insensitive target cells, that manipulation of monosialo ganglioside GM1 per se do not affect their sensitivity to NK cell-mediated cytotoxicity (19). The actual presence or absence of the GM1-associated molecules seems to be more important.
Here we have screened different types of flavonoids for their ability to potentiate the NK activity of the NK-92 cell line. One of the molecules analyzed, namely myricetin, could efficiently potentiate the NK activity of these effector cells (Figure 2). Interestingly, quercetin that lacks one hydroxyl residue compared to myricetin did not affect NK activity at all.

A recent article by Koch et al. pinpoints the importance of NCRs in order to combat cancer and infections (20). The NK-92 cell line is in this respect an interesting model to use, since its high-endogenous cytotoxic potential is likely to be solely due to cytolytic NKIS, since all inhibitory receptors, with the exception of KIR2DL4, are absent (21). Whether myricetin, that could potentiate NK activity in a dose-dependent manner (Figure 3), acts by manipulating the formation of cytolytic NKIS remains to be solved. However, the additional hydroxyl residue in myricetin, makes it less hydrophobic, which in turn might have an effect on the assembly of the NKIS.

Furthermore, myricetin has been reported to affect a number of different signaling pathways (22). Recent data by Büchter et al. shows that myricetin can also reduce accumulation of reactive oxygen species in a dose-dependent manner (23). This observation, combined with findings that oxidative stress mediates a reduced expression of the activating receptor NKG2D in NK cells (24), might also favor the idea that myricetin, via reduced oxidative stress, increases the expression of activating receptors. Such a scenario would also result in an enhanced NK-cell activity.

In summary, the data presented herein might shed some light on human studies indicating a preventive role of flavonols against cancer (25). Furthermore, they will hopefully provide a framework for further clarification of the relationship between flavonols and the immune system in general, and NK-cells in specific.

Conflicts of Interest

The Authors declare that no conflicts of interest exist.

Acknowledgements

This study was supported by grants from the Research Institute of Åbo Akademi University.

References


Received May 14, 2014
Revision June 5, 2014
Accepted June 6, 2014