Derivation of Cinnamon Blocks Leukocyte Attachment by Interacting with Sialosides

Wei-Ling Lin², Shih-Yun Guu¹, Chan-Chuan Tsai³, Ekambaramello Prakash⁴, Mohan Viswaraman⁴, Hsing-Bao Chen⁵, Chuan-Fa Chang¹,6,7*  

¹ Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan, ² Department of Biochemistry and Molecular Biology, College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan, ³ Department of Pathology, Pingtung Christian Hospital, Pingtung 900, Taiwan, ⁴ Indus Biotech Private Limited, Kondhwa, Pune 411048, India, ⁵ Division of Colorectal Surgery, Department of Surgery, E-DA Hospital, Kaohsiung 82445, Taiwan, ⁶ Center of Infectious Disease and Signaling Research, College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan, ⁷ Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, 1 University Road, Tainan 70101, Taiwan  

* affa@mail.ncku.edu.tw  

Abstract  
Molecules derived from cinnamon have demonstrated diverse pharmacological activities against infectious pathogens, diabetes and inflammatory diseases. This study aims to evaluate the effect of the cinnamon-derived molecule IND02 on the adhesion of leukocytes to host cells. The anti-inflammatory ability of IND02, a pentameric procyanidin type A polyphenol polymer isolated from cinnamon alcohol extract, was examined. Pretreatment with IND02 significantly reduced the attachment of THP-1 cells or neutrophils to TNF-α-activated HUVECs or E-selectin/ICAM-1, respectively. IND02 also reduced the binding of E-, L- and P-selectins with sialosides. Furthermore, IND02 could agglutinate human red blood cells (RBC), and the agglutination could be disrupted by sialylated glycoprotein. Our findings demonstrate that IND02, a cinnamon-derived compound, can interact with sialosides and block the binding of selectins and leukocytes with sialic acids.

Introduction  
Cinnamon, a traditional Chinese herb, has been widely used as a spice in India, China, and many Asian countries. Cinnamon extracts, which contain several bioactive components, have also been studied for their anti-cancer [1–4], anti-infectious [5, 6], anti-diabetic [7–11] and anti-inflammatory responses [12–17]. For example, cinnamon extracts suppressed tumor progression and promoted tumor cell death through different signal pathways [1, 4]; crude cinnamon extracts showed significant anti-bacterial activities against foodborne pathogenic bacteria [5]; cinnamon extracts can also regulate glucose transporters, improve glucose uptake and fasting blood glucose, inhibit α-glucosidase activity and increase insulin sensitivity in diabetes or obesity [7, 8, 18–20]. Although the biological functions of cinnamon extracts have been widely
studied, specific pharmaceutical applications and mechanisms of actions against various disease conditions remain uninvestigated.

Sialic acid, also called N-acetyl neuraminic acid (NeuAc), is a negatively charged, nine-carbon monosaccharide that is mostly located at the non-reducing end of glycoconjugates. Cell surface sialic acids have been reported to be involved in a variety of physiological and pathological functions, including cell-cell adhesion, recognition, migration, cancer progression and metastasis, as well as pathogen binding and infection. In addition, cell surface sialic acids are also ligands for selectins and participate in the rolling and recruitment of leukocytes during inflammation. Therefore, compounds that block protein-sialic acid binding can prevent infection by pathogens; the tethering, rolling, and adhesion of leukocyte to the extravascular sites; and the subsequent chemokine releasing and inflammation [21–25].

Using chromatography, we purified polyphenol-rich, alcohol-soluble compounds from pulverized cinnamon (Cinnamomum zeylanicum) bark. After HPLC separation and NMR characterization, the major component of the purified polyphenol is composed of a pentameric procyanidin type A polyphenol polymer called IND02 [15]. We first examined the effects of IND02 on the adhesion of THP-1 cells to HUVECs and the attachment of neutrophils to E-selectin/ICAM-1. The glycan binding preferences of recombinant E-, L- and P-selectins were then characterized. We also analyzed the inhibition abilities of IND02 in selectin-glycan interactions. Finally, we tried to determine the possible anti-inflammatory mechanisms of IND02. Based on our findings, IND02 can interact with sialosides and reduce the interactions of leukocytes with cells.

Materials and Methods

Purification and characterization of IND02

The cinnamon-derived compound, IND02, was provided by Indus Biotech Private Limited, Pune, India and characterized as described (PCT Publication No: 011/018793A1, WO2011018793 A1) [15, 16]. IND02 is a standardized alcohol extract of Cinnamomum zeylanicum bark with pentameric type-A procyanidine flavonoid (S1 Fig). The solutions of IND02 were freshly prepared by dissolving in methanol or double distilled water.

Cells

Human leukemia monocytic THP-1 cells were purchased from Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan) and cultured in RPMI-1640 medium (GIBCO BRL, Grand Island, USA). Human umbilical vein endothelial cells (HUVECs) were purchased from Invitrogen (NY, USA) and cultured in Medium 199 (GIBCO BRL, Grand Island, USA). Cells were cultured by the recommended condition. HUVECs from passage 2 to 4 were used in the experiments.

Monocytic cells adhesion assay

HUVECs were grown to confluence on the flow chamber slide (μ-Slide I4.8, ibidi, Martinsried, Germany) and activated by tumor necrosis factor (TNF)-α (10 ng/mL) for 16–18 h. Before the assay, THP-1 monocytic cells (5x10⁷/mL) were preincubated with or without IND02 (200 μg/mL, final concentration) for 30 minutes at 37°C. After washing HUVECs by serum-free medium, THP-1 cells were perfused in the flow chamber at a constant flow rate of 0.5 dynes/cm². The number of adherent THP-1 cells was determined after 20 minutes of perfusion. In these experiments, the shear flow was generated using the perfusion loop system and an air pressure pump (ibidi, Martinsried, Germany), and images were recorded every 10 seconds.
The number of adherent cells was calculated in 10–15 random fields in a single experiment and, the statistical test were calculated from 3 independent experiments.

Isolation of human neutrophils
As previously described [26], whole blood was collected into tubes containing heparin. After RBC sedimentation by 3% dextran T-100 (Sigma-Aldrich, USA), leukocyte-rich plasma was centrifuged at 1000 rpm for 10 minutes to pellet cells. Cells were then resuspended by saline, and Ficoll-Hypaque (Pharmacia, USA) solution was layered beneath the cell suspension. After centrifuging at 1400 rpm for 40 minutes, supernatant was removed to leave pellet consisting of neutrophils and residual RBC. After removing RBC by hypotonic lysis, neutrophils were resuspended in ice-cold PBS/glucose. Cells were used within 2 hours. The obtaining of human cells was approved by the institutional review board of National Cheng Kung University Hospital, Tainan, Taiwan.

Neutrophils adhesion assay
For adhesion of neutrophils to immobilized E-selectin in combine with ICAM-1 under flow, a laminar flow chamber slide (μ-Slide I.0.8 Luer, ibidi, Martinsried, Germany) was filled with protein G (25 μg/mL; Invitrogen, USA) overnight. After washing with phosphate-buffered saline, the slide was filled with E-selectin (5 μg/mL; R&D system, USA) and ICAM-1 (5 μg/mL; R&D system, USA) for 2 hours and blocked with 1% bovine serum albumin (Sigma-Aldrich, USA) for 1 hour. Freshly isolated human neutrophils (5x10^5 cells) were preincubated without or with INDO2 at RT for 1 hour, followed by perfusion at a constant flow rate of 1 dynes/cm^2. The number of adherent cells (stationary over 5 seconds) was determined after 3 minutes of perfusion. The shear flow was generated by using the perfusion loop system and an air pressure pump (ibidi, Martinsried, Germany), and the images were captured by the microscope. The number of adherent cells was calculated in 10–15 random fields in a single experiment and the statistical test was calculated from 3 experiments.

Carbohydrate binding specificities of selectins (AlphaScreen assay)
AlphaScreen assays were performed on a PerkinElmer Envision instrument. Streptavidin-coated donor beads, protein A-conjugated acceptor beads and ProxiPlate-384 assay plates were purchased from PerkinElmer Life Sciences, Inc. (Boston, MA, U.S.A.). Biotinylated polyacrylamide-based sugar polymers were purchased from GlycoTech (Gaithersburg, MD, U.S.A.). Anti-human IgG antibody was purchased from Acris (BM478, Herford, Germany). Rabbit anti-mouse IgG was purchased from Zymed, Inc. (South San Francisco, CA, U.S.A.). Recombinant human selectin Fc chimera proteins (including E-, L- and P-selectins) were purchased from R&D Systems (Minneapolis, MN, U.S.A.). All of the procedures and incubations were performed in the dark. The buffer used in selectin assay was 25 mM Tris buffer, pH = 7.0, 1.0 mM CaCl_2, 0.1% BSA. Donor beads (20 ng/well) and biotin-PAA-sugars (20 ng/well) mixed with selectins (5 ng/well) were incubated at ambient temperature for 1 h (total 15 μL). The mixture of acceptor beads (20 ng/well), mouse anti-human IgG antibody (50 ng/well) and rabbit anti-mouse IgG antibody (25 ng/well) was added to the reaction to a final volume of 25 μL. After 2 h of incubation, the binding signals were obtained using a PerkinElmer Envision instrument and the AlphaScreen program. The optimized concentrations of selectins were measured and used for binding assays. The results were depicted using units of relative intensities.
Amplification and purification of influenza A virus

The human influenza A virus (seasonal H1N1 strains, A/Taiwan/211/2009) used in this study was collected from the Virology Laboratory of National Cheng Kung University Medical Center. The virus was amplified in MDCK cells and purified using a sucrose gradient. The purified virus was inactivated by treating with 0.01% Merthiolate (final concentration). The protein concentrations of the purified virus were determined by a protein assay (Bio-Rad Laboratories, CA, USA).

RBC agglutination and inhibition assays

Two-fold serially diluted influenza A virus in row A and B (column 1 to 11, 50 μg/mL to 50 ng/mL) and two-fold serially diluted IND02 in row C and D (column 1 to 11, 50 μg/mL to 50 ng/mL) were prepared in the microtiter plate. Washed human RBCs from blood bank of National Cheng Kung University Hospital (5% v/v) were added to the wells and incubated at 37°C for 1 h. The agglutination titers of influenza A virus and IND02 were 1:16 (3.125 μg/mL) and 1:64 (0.78125 μg/mL), respectively.

Two-fold serially diluted fetuin (column 1 to 11, 50 μg/mL to 50 ng/mL) were prepared. Influenza A virus (row A and B, 10 μg/mL), IND02 (row C and D, 10 μg/mL), or PBS (row E and F) were added and incubated with fetuin at 37°C for 0.5 h. Washed RBCs (5%) were then added to the reaction and incubated at 37°C for 1 h.

Statistical analysis

All of the results were obtained from at least three independent experiments. The data were analyzed using Student’s t test (two-tailed) or one-way ANOVA followed by post hoc test (Figs 1d and 2d) on GraphPad Prism 5 software. P values of less than 0.05 were considered to be statistically significant. (*: P < 0.05; **: P < 0.01; ***: P < 0.001)

Results

IND02 reduced leukocyte attachment

Cinnamon extracts have been shown to exhibit anti-inflammatory activity in several experimental models [12–14]. The primary response of the innate immune system to inflammation is the recruitment of leukocytes. To investigate whether IND02 affects this first step of inflammation (leukocyte attachment), we analyzed the adhesion of THP-1 cells with TNF-α activated HUVECs under shear flow with or without IND02 pretreatment. IND02 significantly reduced the adhesion of THP-1 cells to TNF-α-activated HUVECs when compared with untreated group. (Fig 1 and S1–S3 Movies). In addition, the effects of IND02 on neutrophil attachment to E-selectin and ICAM-1 coated on slides were also evaluated. We observed that neutrophils were able to spread in the absence of IND02 (Fig 2a); however, the shape of cell retained round up in the pretreatment of IND02 (Fig 2b). Moreover, the number of adherent neutrophils to E-selectin/ICAM-1-coated surface was significantly reduced by the treatment of IND02 (Fig 2c). To confirm the viability of the attached neutrophils, the slides were stained with trypan blue. The viability of the attached neutrophils was higher than 95% (data not shown). These results suggested that IND02 may inhibit inflammatory responses by blocking leukocyte adhesion.

IND02 blocked the binding of selectins with sialosides

Because the recruitment of neutrophils/leukocytes to inflamed tissue is mediated by selectin-sialoside interactions, human selectins were subjected to carbohydrate binding and IND02 inhibition assays to explore the molecular mechanism of IND02 in leukocyte recruitment. The
substrate specificities of recombinant human E-, L- and P-selectins (human Fc chimera, purchased from R&D) were profiled primarily by solution glycan microarray [27]. The PAA-glycans used in this binding assay are listed in S1 Table. E-selectin preferentially bound to Leb, sialyl-Lea, sialyl Lex, and 3'Sulfated Lea/Lex (Fig 3a, relative intensity >30%). L-selectin interacted with sulfated glycans, NeuGc, 6'Sialyl lactose, NeuAcα2-3Galβ1-4GlcNAc, NeuAcα2-3Galβ1-3GalNAc, Leb, sialyl-Lea and sialyl Lea (Fig 3b, relative intensity >30%). P-selectin recognized several glycans including sulfated glycans, NeuGc, NeuAcα2-6GalNAc, NeuGcα2-6GalNAc, NeuAcα2-3Gal, NeuAcα2-3GalNAc, 6'Sialyl lactose, 3’6’disialyl-GalNAc, Galβ1-4Galβ1-4Glc, NeuAcα2-3Galβ1-4GlcNAc, NeuAcα2-3Galβ1-3GalNAc, Leb, sialyl-Lea and sialyl Lea (Fig 3c, relative intensity >30%). Among the common ligands for the three recombinant human selectins, the common ligand of selectins, sialyl Lex, was used to analyze the IC50 of IND02 [28]. Serially diluted IND02 (from 0.5 μg/mL to 1000 μg/mL) were preincubated with biotin-PAA-sialyl Lea and then interacted with selectins. The results showed that the IC50 values of IND02 for E-, L- and P-selectin were 31.6, 5.0 and 28.2 μg/mL, respectively (Fig 4).

IND02 agglutinated human red blood cells by interacting with sialosides

Influenza virus can agglutinate animal RBCs through the binding of hemagglutinin, one of the major proteins on influenza viral envelop, with cell surface α2–3 or α2–6 linked sialic acids [29]. The levels of influenza virus in sample can be quantified by hemagglutination test (HA
The ability of compounds or proteins that prevent hemagglutination can be determined by hemagglutination inhibition test (HI test) [30]. Since we found that IND02 may interact with sialosides and reduce the binding of selectins to carbohydrate ligands, we tried to assess the inhibition capacity of IND02 using HI test. Unexpectedly, IND02 agglutinated human RBC independently of influenza virus addition, and the HA titer of influenza A virus and IND02 were 1:16 (3.125 μg/mL) and 1:64 (0.78125 μg/mL), respectively (Fig 5a). To investigate whether sialosides were involved in IND02-induced human RBC agglutination, fetuin, a highly sialylated glycoprotein, was used to disrupt these interactions [31]. As shown in Fig 5b, the addition of fetuin significantly reduced the influenza A virus and IND02-induced human RBC agglutination. These findings suggested that IND02-induced human RBC agglutination was due to interactions of IND02 with cell surface sialosides.

Discussion

Cell migration is important in innate and adaptive immune responses and the recruitment of leukocytes to the sites of acute or chronic inflammation [21, 32, 33]. Inadequate congregation of leukocytes may reduce inflammatory responses and initiate pathological processes of inflammation [32, 33]. Cell surface proteins, especially members of the integrin family and E-selectins of endothelial cells and L-selectins of leukocytes, are known to participate in cell-cell adhesion and trafficking [32, 34–36]. In addition, P-selectin expressed on platelets is also involved in these processes [37–39]. Selectins are single-chain transmembrane glycoproteins bearing C-
Fig 3. Carbohydrate binding specificities of E-, L- and P-selectin. Donor beads and biotin-PAA-sugars mixed with selectins were incubated at ambient temperature for 1 h (total 15 μL). The mixture of acceptor beads, mouse anti-human IgG antibody and rabbit anti-mouse IgG antibody was added to the reaction to a final volume of 25 μL. After 2 h of incubation, the binding signals were obtained using a PerkinElmer Envision instrument and the AlphaScreen program. The results were indicated by relative intensities of the highest fluorescence signal (y-axis). The sugar identities are designated by numbers (x-axis) and shown in detail in S1 Table. Glycans which showed more than 30% relative intensity are indicated as number on each figure.

doi:10.1371/journal.pone.0130389.g003
type lectins that contain calcium-dependent, carbohydrate recognition domains (CRDs). The CRDs of selectin recognize sialylated glycans and control the interactions between leukocytes and endothelial cells. The recruitment of leukocytes is always accompanied by the activation and expression of E-, P- and/or L-selectin ligands. The majority of the ligands for selectins are highly glycosylated, especially with sialyl Lex glycans [40]. Although the affinity of selectins to sialyl Lex is weak (with a dissociation constant in μM range), multivalent effects can compensate for the weak interactions [41–43].

Several selectin-directed anti-inflammatory compounds/strategies have been developed and evaluated in preclinical trials, including carbohydrate-based inhibitors, non-carbohydrate molecules, peptides, antibodies and nucleotide inhibitors [21, 44]. For example, P-selectin inhibitors reduce platelet aggregation, prolong heart allograft survival, decrease vein injury from thrombosis, and reduce acute pancreatitis in murine model [45–48]. Non-anticoagulant (NAC) heparin, a well-known selectin inhibitor, reduces tumor metastasis in selectin knockout mice [49, 50]. The pan-selectin antagonist Bimosiamose, a mannose-based dimer, could reduce airway inflammation [51]. Cinnamon extracts have also been reported to show anti-inflammatory responses [12–17]. However, the real roles of cinnamon extracts in reducing inflammatory responses are still unclear. In this study, we found that IND02 reduced the attachment of THP-1 to TNF-α activated HUVECs under shear flow. IND02 also attenuated the attachment of human neutrophils to E-selectin/ICAM-1-coated surface. E-selectin plays a critical role in mediating leukocyte rolling by interacting with sialic acids expressed on leukocyte surface. Therefore, E-selectin is a necessary adhesion molecule involved in the transition of leukocyte rolling to adhesion. Based on these findings, it is suggested that cinnamon extracts can interfere with selectin-sialosides interaction directly and resulted in a reduced leukocytes attachment.

While leukocytes stay in resting, the adhesion molecules are homogeneously expressed on the leukocyte surface. When leukocytes are rolling over the activated endothelium, the adhesion molecules on the surface of leukocytes are going to be redistributed via signals induced by
endothelial adhesion molecules, such as E-selectin. Redistribution of adhesion molecules on leukocytes can increase the binding avidity to their counter-act receptors and enhance leukocyte adhesion. In this study, neutrophils can adhere and spread out under the flow condition; however, under the treatment of IND02, the adherent neutrophils were easily detached. The morphology of neutrophils was also very different between control and IND02 treated cells (Fig 2a–2c). It is suggested that the distribution of adhesion molecules or arrangement of cytoskeleton is affected by IND02. Although THP-1 and neutrophils both belong to myeloid cell lineage, the characteristics of these two types of cells are different. After neutrophils were arrested, the cells spread to become firm adhesion. The transit from round up to spread out was apparent. However, we did not observe such morphology differences before and after the treatment of IND02 in THP-1 cells. It is possible that cells would have different behavior when adhesion to E-selectin/ICAM-1-coated surface or activated endothelial cells. Further studies are needed to dissect the detailed mechanisms, to investigate whether IND02 interfere with E-selectin-induced signaling, and to study whether IND02 interfere with the expression level of adhesion molecules.

To investigate the inhibitory mechanism of IND02, we analyzed the carbohydrate binding specificities of recombinant selectins. The recombinant selectins not only showed interactions with sialosides, especially to sialyl Lex, but also bound to some glycan without sialic acid modification [52]. Hence, PAA-sialyl Leα was subjected for the subsequent assays. It is meaningful
to use sialyl Le\(^\alpha\) in the inhibition assays because sialyl Le\(^\alpha\) is the natural ligand for selectins. The results showed that the binding of selectins with sialyl Le\(^\alpha\) dropped significantly after IND02 treatments. These findings suggest that selectin-sialic acid interactions may be one of the targets for IND02.

To further elucidate the possible inhibitory mechanisms for IND02, we performed HA test, HI test, and glycoprotein blocking assay for IND02 [53]. Surprisingly, IND02 agglutinated human RBC in a dose dependent manner. The ability of RBC agglutination for IND02 may lead to some possible side effects. IND02 may cause aggregation and obstruct small vessel in some special condition. However, it is known that influenza virus can agglutinate human RBC in 96-well assays, but will not agglutinate RBC in blood vessel. To verify whether the IND02-induced agglutination was sialic acid dependent, a well-known inhibitor for RBC agglutination, fetuin, was subjected to a blocking assay. We found that IND02-induced human RBC agglutination was significantly reduced by fetuin treatment. Taken together, this evidence suggested that IND02 exhibits anti-inflammatory activity by interacting with cell surface sialosides to reduce leukocyte attachment. Injection of IND02 into the vein or the site of inflammation may reduce leukocyte recruitment and decrease the subsequent leukocyte infiltration.

In conclusion, we show for the first time that IND02, a cinnamon-derived type A procyandrin molecule, reduces inflammatory responses by interaction with sialosides and blocking leukocyte adhesion to interacting sialosides. These findings not only confirm the reported anti-inflammatory abilities of IND02, but also dissect the inhibition mechanisms of IND02. Further studies are in progress to explore novel biological functions and anti-inflammatory/anti-infection mechanisms for IND02.

**Supporting Information**

**S1 Fig. HPLC analysis and structure of IND-02.** (a) HPLC absorbance chromatogram (at 280 nm) of alcohol-soluble compounds from pulverized cinnamon (*Cinnamomum zylanicum*) bark. 4% of the eluted material is procyanidin trimer and 92% of the eluted material are pentamer polyphenols (IND-02). Unknown material (peak 1, 2, 4 and 5) represents 4.1% of the total UV trace. (b) Structure of the pentameric procyanidin A (IND-02). (TIF)

**S1 Movie. Negative control.** THP-1 monocytic cells were preincubated with PBS for 0.5 h at 37°C followed by perfusion into a flow chamber. The rolling (attachment) of THP-1 with HUVECs were recorded. (MP4)

**S2 Movie. Positive control.** THP-1 monocytic cells were preincubated with PBS for 0.5 h at 37°C followed by perfusion into a flow chamber. The rolling (attachment) of THP-1 with TNF-\(\alpha\) activated HUVECs were recorded. (MP4)

**S3 Movie. IND02 treatment.** THP-1 monocytic cells were preincubated with IND-02 (200 \(\mu\)g/mL) for 0.5 h at 37°C followed by perfusion into a flow chamber. The rolling (attachment) of THP-1 with TNF-\(\alpha\) activated HUVECs were recorded. (MP4)

**S1 Table. Glycan list for selectin binding assay.** (DOCX)
Author Contributions
Conceived and designed the experiments: CFC CCT HBC. Performed the experiments: WLL SYG. Analyzed the data: WLL SYG. Contributed reagents/materials/analysis tools: EP MV. Wrote the paper: CFC.

References
1. Kwon HK, Hwang JS, So JS, Lee CG, Sahoo A, Ryu JH, et al. Cinnamon extract induces tumor cell death through inhibition of NFkappaB and AP1. BMC Cancer. 2010; 10: 392. doi: 10.1186/1471-2407-10-392 PMID: 20653974
2. Koppikar SJ, Choudhari AS, Suryavanshi SA, Kumari S, Chattopadhyay S, Kaul-Ghanekar R. Aqueous cinnamon extract (ACE-c) from the bark of Cinnamomum cassia causes apoptosis in human cervical cancer cell line (SiHa) through loss of mitochondrial membrane potential. BMC Cancer. 2010; 10: 210. doi: 10.1186/1471-2407-10-210 PMID: 20482751
3. Lu J, Zhang K, Nam S, Anderson RA, Jove R, Wen W. Novel angiogenesis inhibitory activity in cinnamon extract blocks VEGFR2 kinase and downstream signaling. Carcinogenesis. 2010; 31: 461–468. doi:10.1039/carin/bgp292 PMID: 19969552
4. Kwon HK, Jeon WK, Hwang JS, Lee CG, So JS, Park JA, et al. Cinnamon extract suppresses tumor progression by modulating angiogenesis and the effector function of CD8+ T cells. Cancer Lett. 2009; 278: 174–182. doi: 10.1016/j.canlet.2009.01.015 PMID: 19203831
5. Shan B, Cai YZ, Brooks JD, Corke H. Antibacterial properties and major bioactive components of cinnamon stick (Cinnamomum burmanii): activity against foodborne pathogenic bacteria. J Agric Food Chem. 2007; 55: 5484–5490. PMID: 17967033
6. Mau J, Chen C, Hsieh P. Antimicrobial effect of extracts from Chinese chive, cinnamon, and corni fructus. J Agric Food Chem. 2001; 49: 189–188. PMID: 11707575
7. Sartorius T, Peter A, Schulz N, Drescher A, Bergheimer I, Machann J, et al. Cinnamon extract improves insulin sensitivity in the brain and lowers liver fat in mouse models of obesity. PLoS One. 2014; 9: e92358. doi: 10.1371/journal.pone.0092358 PMID: 24643026
8. Shen Y, Honma N, Kobayashi K, Jia LN, Hosono T, Shindo K, et al. Cinnamon Extract Enhances Glucose Uptake in 3T3-L1 Adipocytes and C2C12 Myocytes by Inducing LKB1-AMP-Activated Protein Kinase Signaling. PLoS One. 2014; 9: e87894. doi: 10.1371/journal.pone.0087894 PMID: 24551069
9. Jiao L, Zhang X, Huang L, Gong H, Cheng B, Sun Y, et al. Proanthocyanidins are the major anti-diabetic components of cinnamon water extract. Food Chem Toxicol. 2013; 56: 398–405. doi:10.1016/j.fct.2013.02.049 PMID: 23499750
10. Kim SH, Hyun SH, Choung SY. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. J Ethnopharmacol. 2006; 104: 119–123. PMID: 16213119
11. Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y. Cinnamon extract (traditional herb) potentiates in vivo insulin-regulated glucose utilization via enhancing insulin signaling in rats. Diabetes Res Clin Pract. 2003; 62: 139–148. PMID: 14625128
12. Hong JW, Yang GE, Kim YB, Eom SH, Lew JH, Kang H. Anti-inflammatory activity of cinnamon water extract in vivo and in vitro LPS-induced models. BMC Complement Altern Med. 2012; 12: 237. doi: 10.1186/1472-6882-12-237 PMID: 23190501
13. Hagenlocher Y, Bergher I, Zacheja S, Schaffer M, Bischoff SC, Lorenz A. Cinnamon extract inhibits degranulation and de novo synthesis of inflammatory mediators in mast cells. Allergy. 2013; 68: 490–497. doi: 10.1111/all.12122 PMID: 23409344
14. Lee BJ, Kim YJ, Cho DH, Sohn NW, Kang H. Immunomodulatory effect of water extract of cinnamon on anti-CD3-induced cytokine responses and p38, JNK, ERK1/2, and STAT4 activation. Immunopharmacol Immunotoxicol. 2011; 33: 714–722. doi: 10.3109/08923973.2011.564185 PMID: 22053946
15. Rath B, Bodhanker S, Mohan V, Thakurdesai P. Ameliorative Effects of a Polyphenolic Fraction of Cinnamomum zeylanicum L. Bark in Animal Models of Inflammation and Arthritis. Sci Pharm. 2013; 81: 567–589. doi: 10.7577/scipharm.1301-16 PMID: 23833722
16. Sachin V BS, Mohanb V, Thakurdesai PA. Anti-inflammatory and anti-arthritis activity of type-A procyandine polyphenols from bark of Cinnamomum zeylanicum in rats. Food Science and Human Wellness. 2013; 2: 59–67.
17. Kandhare AD BS, Singh V, Mohan V, Thakurdesai PA. Anti-asthmatic effects of type-A procyanidine polyphenols from cinnamon bark in ovalbumin-induced airway hyperresponsiveness in laboratory animals. Biomedicine & Aging Pathology. 2013; 3: 23–30.
18. Lu T, Sheng H, Wu J, Cheng Y, Zhu J, Chen Y. Cinnamon extract improves fasting blood glucose and glycosylated hemoglobin level in Chinese patients with type 2 diabetes. Nutr Res. 2012; 32: 408–412. doi:10.1016/j.nutres.2012.05.003 PMID: 22749176

19. Mohamed Sham Shihabudeen H, Hansi Priscilla D, Thirumurugan K. Cinnamon extract inhibits alpha-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. Nutr Metab (Lond). 2011; 8: 46. doi: 10.1186/1743-7075-8-46 PMID: 21711570

20. Cao H, Graves DJ, Anderson RA. Cinnamon extract regulates glucose transporter and insulin-signaling gene expression in mouse adipocytes. Phytomedicine. 2010; 17: 1027–1032. doi: 10.1016/j.phymed.2010.03.023 PMID: 20554184

21. Bedard PW, Kaila N. Selectin inhibitors: a patent review. Expert Opin Ther Pat. 2010; 20: 781–793. doi: 10.1517/13543771003767468 PMID: 20408735

22. Endemann G, Abe Y, Bryant CM, Feng Y, Smith CW, Liu DY. Novel anti-inflammatory compounds induce shedding of L-selectin and block primary capture of neutrophils under flow conditions. J Immunol. 1997; 158: 4879–4888. PMID: 9144504

23. Martens CL, Cwirla SE, Lee RY, Whitehorn E, Chen EY, Bakker A, et al. Peptides which bind to E-selectin and block neutrophil adhesion. J Biol Chem. 1995; 270: 21129–21136. PMID: 7545665

24. Hatakeyama S, Sakai-Tagawa Y, Kiso M, Goto H, Kawakami C, Mitamura K, et al. Enhanced expression of an alpha2,6-linked sialic acid on MDCK cells improves isolation of human influenza viruses and evaluation of their sensitivity to a neuraminidase inhibitor. J Clin Microbiol. 2005; 43: 4139–4146. PMID: 16081961

25. Woodhall T, Williams G, Berry A, Nelson A. Synthesis of screening substrates for the directed evolution of sialic acid aldolase: towards tailored enzymes for the preparation of influenza A sialidase inhibitor analogues. Org Biomol Chem. 2005; 3: 1795–1800. PMID: 15858666

26. Kurosawa S, Esmon CT, Stearns-Kurosawa DJ. The soluble endothelial protein C receptor binds to activated neutrophils: involvement of proteinase-3 and CD11b/CD18. J Immunol. 2000; 165: 4697–4703. PMID: 11035113

27. Chang CF, Pan JF, Lin CN, Wu IL, Wong CH, Lin CH. Rapid characterization of sugar-binding specificity by in-solution proximity binding with photosensitizers. Glycobiology. 2011; 21: 895–902. doi: 10.1093/glycob/cwr021 PMID: 21325337

28. Pavlovic D, Leteux C, Ovchinnikova T, Tsvetkov Y, Nifant’ev N, Feizi T. Chemically synthesized solid phase oligosaccharide probes for carbohydrate-binding receptors. Interactions of the E-, L- and P-selectins with sialyl-Le(x) and O-sulphated forms linked to biotin or to polyacrylamide. J Immunol Meth. 2002; 264: 53–58. PMID: 12191509

29. Ito T, Suzuki Y, Mitnau L, Vines A, Kida H, Kawaoka Y. Receptor specificity of influenza A viruses correlates with the agglutination of erythrocytes from different animal species. Virology. 1997; 227: 493–499. PMID: 9018149

30. Morokutti A, Redlberger-Fritz M, Nakowitsch S, Krenn BM, Wressnigg N, Jungbauer A, et al. Validation of the modified hemagglutination inhibition assay (mHAI), a robust and sensitive serological test for analysis of influenza virus-specific immune response. J Clin Virol. 2013; 56: 323–330. doi: 10.1016/j.jcv.2012.12.002 PMID: 23375739

31. Cumming DA, Hellerqvist CG, Harris-Brandts M, Michnick SW, Carver JP, Bender B. Structures of asparagine-linked oligosaccharides of the glycoprotein fetus having sialic acid linked to N-acetylgalactosamine. Biochemistry. 1989; 28: 6500–6512. PMID: 2477057

32. Lowe JB. Glycosylation in the control of selectin counter-receptor structure and function. Immunol Rev. 2002; 186: 19–36. PMID: 12234359

33. Ley K, Kansas GS. Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. Nat Rev Immunol. 2004; 4: 325–335. PMID: 15122198

34. Kleinhaus H, Kafli JT, Mann O, Reinknecht F, Freitag M, Hansen B, et al. The role of vascular adhesion molecules PECAM-1 (CD31), VCAM-1 (CD 106), E-selectin (CD62E) and P-selectin (CD62P) in severe porcine pancreatitis. Histol Histopathol. 2009; 24: 551–557. PMID: 19283663

35. Chu A, Hong K, Berg EL, Ehrhardt RO. Tissue specificity of E- and P-selectin ligands in Th1-mediated chronic inflammation. J Immunol. 1999; 163: 5086–5093. PMID: 10528215

36. Sperandio M. Selectins and glycosyltransferases in leukocyte rolling in vivo. FEBS J. 2006; 273: 4377–4389. PMID: 16953722

37. Geng JG, Chen M, Chou KC. P-selectin cell adhesion molecule in inflammation, thrombosis, cancer growth and metastasis. Curr Med Chem. 2004; 11: 2153–2160. PMID: 15279555

38. Wynn TA, Hesse M, Sandler NG, Kaviratne MG, Hoffmann KA, Chiaradonna MG, et al. P-selectin suppresses hepatic inflammation and fibrosis in mice by regulating interferon gamma and the IL-13 decoy receptor. Hepatology. 2004; 39: 676–687. PMID: 14999686
39. Rosenkranz AR, Mendrick DL, Cotran RS, Mayadas TN. P-selectin deficiency exacerbates experimental glomerulonephritis: a protective role for endothelial P-selectin in inflammation. J Clin Invest. 1999; 103: 649–659. PMID:10074481

40. Foxall C, Watson SR, Dowbenko D, Fennie C, Lasky LA, Kiso M, et al. The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis(x) oligosaccharide. J Cell Biol. 1992; 117: 895–902. PMID:1374413

41. Beaucharnois ME, Lindquist KC, Marathe D, Vanderslice P, Xia J, Matta KL, et al. Affinity and kinetics of sialyl Lewis-X and core-2 based oligosaccharides binding to L- and P-selectin. Biochemistry. 2005; 44: 9507–9519. PMID:15996105

42. Shamay Y, Paulin D, Ashkenasy G, David A. Multivalent display of quinic acid based ligands for targeting E-selectin expressing cells. J Med Chem. 2009; 52: 5906–5915. doi:10.1021/jm900308r PMID:19746918

43. Papp I, Dernedde J, Enders S, Haag R. Modular synthesis of multivalent glycoarchitectures and their unique selectin binding behavior. Chem Commun (Camb). 2008: 5851–5853.

44. Schon MP. Inhibitors of selectin functions in the treatment of inflammatory skin disorders. Ther Clin Risk Manag. 2005; 1: 201–208. PMID:18360560

45. Carvalho AC, Sousa RB, Franco AX, Costa JV, Neves LM, Ribeiro RA, et al. Protective effects of fucoi-dan, a P- and L-selectin inhibitor, in murine acute pancreatitis. Pancreas. 2014; 43: 82–87. doi: 10.1097/MPA.0b013e3182a63b9d PMID:24177139

46. Stevenson JL, Varki A, Borsig L. Heparin attenuates metastasis mainly due to inhibition of P- and L-selectin, but non-anticoagulant heparins can have additional effects. Thromb Res. 2007; 120 Suppl 2: S107–111. PMID:18023703

47. Schon MP. Inhibitors of selectin functions in the treatment of inflammatory skin disorders. Ther Clin Risk Manag. 2005; 1: 201–208. PMID:18360560

48. Park JY, Lee DS, Song MH, Kim W, Won JM. Cylexin: a P-selectin inhibitor prolongs heart allograft survival in hypersensitized rat recipients. Transplant Proc. 1998; 30: 2927–2928. PMID:9838290

49. Bends G, Borsig L. Cancer cell adhesion and metastasis: selectins, integrins, and the inhibitory potential of heparins. Int J Cell Biol. 2012; 2012: 676731. doi:10.1155/2012/676731 PMID:22505933

50. Stevenson JL, Varki A, Borsig L. Heparin attenuates metastasis mainly due to inhibition of P- and L-selectin, but non-anticoagulant heparins can have additional effects. Thromb Res. 2007; 120 Suppl 2: S107–111. PMID:18023703

51. Watz H, Bock D, Meyer M, Schierhorn K, Vollhardt K, Woschwill C, et al. Inhaled pan-selectin antagonist Bimosiamose attenuates airway inflammation in COPD. Pulm Pharmacol Ther. 2013; 26: 265–270. doi:10.1016/j.pupt.2012.12.003 PMID:23257347

52. Etzioni A, Harlan JM, Pollack S, Phillips LM, Gershoni-Baruch R, Paulson JC. Leukocyte adhesion deficiency (LAD) II: a new adhesion defect due to absence of sialyl Lewis X, the ligand for selectins. Immunodeficiency. 1993; 4: 307–308. PMID:7513226

53. Aymard M, Quash GA, Million J. Determination of anti-neuraminidase antibody titres in human sera by inhibition of the agglutination of fetuin-latex by influenza viruses. J Biol Stand. 1982; 10: 125–133. PMID:7096361