The Isoflavone Genistein Inhibits Proliferation and Increases Histamine Content in Human Leukemic Mast Cells

Michael G. Alexandrakis, M.D.,* Despina S. Kyriakou, M.D.,* Duraisamy Kempuraj, Ph.D.,# Man Huang, M.D., Ph.D.,# William Boucher, B.Pharm.,# Dimitris Seretakis, M.D.,* and Theoharis C. Theoharides, Ph.D., M.D.#

ABSTRACT

Mast cells are involved in allergic inflammation and some rare disorders such as systemic mastocytosis and mast cell leukemia. Certain naturally occurring flavonoids have been shown to inhibit mast cell activation and promote maturation of secretory granules. Here, we report that the isoflavone genistein inhibited the growth of human leukemic mast cells (HMC-1) by 68.8, 51.6, and 30.2% at 10^{-4}, 10^{-5}, and 10^{-6} M, respectively, at day 3 ($p < 0.001$). Genistein at 10^{-4} M increased the histamine content per 2×10^5 cells at day 3 from 5.9 ± 1.2 μg/mL to 11.1 ± 1.3 μg/mL ($n = 6$; $p < 0.0001$). These results indicate that genistein can inhibit proliferation and induce maturation of HMC-1 cells. (Allergy and Asthma Proc 24:373–377, 2003)

Mast cells are primary effector cells in immediate hypersensitivity responses and their numbers are increased in a wide spectrum of pathological conditions.[1] Mast cells exposed to allergens or other stimuli[2] can release many potent and rapidly acting mediators of inflammation, including histamine, proteases, prostaglandins, leukotrienes, and cytokines.[1,3–5] The mediator most readily associated with the mast cell is histamine.[6] Histamine plays an important role in the pathogenesis of various allergic disorders, including vasodilatation and increased vasopermeability. In addition, mast cells and histamine are involved in the pathophysiology of irritable bowel syndrome[7] and interstitial cystitis,[8] sterile inflammatory conditions associated with pain, and worsened by stress.[9]

Flavonoids are low molecular weight benzo-γ-pyrone derivatives ubiquitous in plants with a wide spectrum of biologic activities, such as inflammatory and immune responses, including inhibition of mast cells.[10] Genistein is a natural isoflavonoid phytoestrogen present in various plant foods, especially soy. This isoflavone has a heterocyclic, diphenolic structure similar to estrogen;[11] at micromolar concentrations, genistein was found to have an inhibitory effect on protein tyrosine kinases (PTK), presumably at the level of the adenosine triphosphate binding site.[12] Although the biologic effects of genistein have not been fully clarified, genistein could induce cell cycle arrest and apoptosis in leukemic cells[13,14] and also affect the growth and cell cycle progression of human leukemic MOLT-4 and HL-60 cells.[15] A possible interpretation of these observations is the ability of genistein to inhibit the activities of topoisomerases and tyrosine kinases and to induce differentiation in the human leukemia cells HL-60 and K-562.[16] In addition, genistein at 150 μM was shown to be an inhibitor of

*From the *Department of Hematology, University Hospital of Heraklion, School of Medicine University of Crete, Greece, and #Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston Massachusetts*

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Address correspondenceand reprint requests to T.C. Theoharides, Ph.D., M.D., Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111

angiogenesis,[17] *in vivo*.[10] Several studies have shown that genistein can induce apoptosis in breast, stomach, lung, head, and neck, as well as prostate cancer cell lines by modulation of cell cycle and apoptosis of regulatory molecules.[18–20]

Genistein has been observed to induce the differentiation of human myelogenous leukemia K562 cells.[21] Other flavones have been reported to induce maturation of rat basophilic leukemia cells.[22] This effect, as well as the inhibition of mast cell activation has been associated with particular structural requirements, especially of the B ring.[23]

In this study, we investigated the effect of genistein on the growth of human leukemic mast cells (HMC-1) and cellular concentrations of histamine. These results suggest that genistein may be an effective agent in the prevention and/or for the treatment of human mast cell disorders.

MATERIALS AND METHODS

Genistein (4′,5,7-Trihydroxyisoflavone)

This compound was purchased from Sigma (St. Louis, MO) and was dissolved in propylene glycol, to make a 0.1 M solution; then, it was filter-sterilized and diluted with culture medium to get the final concentrations 10^{-4}–10^{-6} M.

Cell Culture

HMC-1[24] was cultured in Iscove's modified Dulbecco's medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum and 1.2 mM of monothioglycerol (Sigma) in 25-cm^2 tissue culture flasks (Becton Dickinson Labware, Franklin Lakes, NJ). The cells were incubated at 37°C in a humidified incubator with 5% CO_2 and 95% air. After 3 days of culture, cells were collected and centrifuged for 5 minutes at 1000 rpm. Cells then were resuspended in culture medium and plated in 12-well flat-bottom culture plates (Costar, Cambridge, MA) at a density of 2×10^5 cell/mL. Genistein was added in concentrations (10^{-4}–10^{-5} M); cells cultured in medium alone served as control.

Determination of Cell Viability

Cells with or without genistein were harvested, washed in phosphate-buffered saline and pelleted by centrifugation for 5 minutes at $400 \times g$ at room temperature. Next, cells were resuspended in Iscove's modified Dulbecco's medium supplemented as aforementioned. Trypan blue (0.1%) exclusion tests were performed at the end of culture for cell viability.

Determination of Inhibition of Cell Proliferation

The inhibition is expressed as the percentage of the number of live cells counted from each treatment group subtracted from the total number of the nontreatment group and then divided by the total number of cells from the nontreatment group, according to the formula:

$$\text{Inhibition (\%)} = \frac{1 - \text{number of live cells in treatment group}}{\text{number of live cells in nontreatment group}} \times 100.$$

Staining of HMC-1 Cells

Cells were grown on Lab-Tek culture chamber slides (NUNK, Naperville, IL). After washing with phosphate-buffered saline, cells were fixed for 5 minutes with 10% formaldehyde in 10% acetic acid (pH 2.5). Cells were stained for 5 minutes with 0.5% toluidine blue (pH 2.5), washed with deionized water, and air dried. Cells were photographed using an Olympus light microscope (Olympus Optical Co., Tokyo, Japan).

Histamine Measurement

Histamine was assayed by a luminescence spectrometer (Perkin-Elmer, Norwalk, CT) with a lower detection level of 5 ng/mL. HMC-1 cells were washed and harvested by centrifugation at $400 \times g$ for 5 minutes in 1.5-mL Eppendorf tubes. Then, cells were resuspendend in water. This suspension was sonicated in a Branson 1200 ultrasound device (Sonifier, Model W185D, Heat Systems—Ultrasonics, Inc., Plainview, NY) for 10 minutes, vortexed for 5 minutes, and pelleted by 10-minute centrifugation at $6000 \times g$ speed in an Eppendorf Microfuge. The total amount of histamine in the cell pellet is expressed as micrograms/2×10^5 mast cells/mL.

Statistics

The results are expressed as mean ± SD of either number of cell or of the histamine present in the cell pellet. Comparisons were made and probability was calculated with the nonparametric Mann-Whitney U test using analysis of variance. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effects of Genistein on Cell Proliferation

HMC-1 cells were cultured for 3, 4, and 5 days in the presence or absence of genistein at 10^{-4}, 10^{-5}, and 10^{-6} M. A concentration-dependent inhibition of growth was observed on the 3rd, 4th, and 5th day of culture. Cell viability on day 3 remained high with lower than 10% trypan blue–positive cells in cultures treated with any genistein concentration. On day 3 the number of cells ($\times 10^3$) was decreased from 977.4 ± 68.9 in the untreated condition to 304.6 ± 53.9 at 10^{-4} M genistein, to 472.6 ± 79.8 at 10^{-5} M and 681.8 ± 96.2 at 10^{-6} M; the corresponding degree of inhibition on proliferation of HMC-1 cells (Tables I and II) was 68.8% ($p < 0.001$) at 10^{-4} M genistein, 51.6% ($p < 0.001$) at 10^{-5} M, and 30.2% ($p < 0.002$) at 10^{-6} M, respectively.

TABLE I

Inhibitory Effect of Genistein on HMC-1 Cell Proliferation

Genistein	Day 3		Day 4		Day 5	
	Cells × 10^3	Inhibition (% Total)	Cells × 10^3	Inhibition (% Total)	Cells × 10^3	Inhibition (% Total)
Control	977.4 ± 68.90	—	1012.8 ± 97.3	—	723.3 ± 37.7	—
10^{-6} M	681.8 ± 96.2#	30.2	763.0 ± 92.4#	24.7	600.5 ± 72.6§	16.9
10^{-5} M	472.6 ± 79.7*	51.6	580.0 ± 69.5*	42.7	470.0 ± 63.9*	35.0
10^{-4} M	304.6 ± 53.9*	68.8	377.5 ± 33.3*	62.6	382.0 ± 39.7*	47.1

n = 6

**p < 0.0001; #p < 0.002; §p < 0.005 using Mann-Whitney U test.*

TABLE II

Effect of Genistein on Total Histamine Content of HMC-1 Cells

Genistein	Total Histamine (μg/2 × 10^5 Mast Cells/mL)		
	Day 3 (n = 6)	Day 4 (n = 8)	Day 5 (n = 8)
Control	5.9 ± 1.2	5.8 ± 2.4	6.1 ± 1.7
10^{-5} M	9.5 ± 2.3	9.7 ± 1.8	8.3 ± 2.0*
	$p < 0.002$*	$p < 0.02$*	$p < 0.05$*
10^{-4} M	11.1 ± 1.3	10.1 ± 4.5	10.1 ± 4.5
	$p < 0.0001$*	$p < 0.01$*	$p < 0.02$*

**Using Mann-Whitney U test.*

On day 4, cell viability was also high at 91.4%. The number of cells (×10^3) at the respective genistein concentrations of 10^{-4}, 10^{-5}, and 10^{-6} M was decreased from 1012.8 ± 97.3 to 377.3 ± 33.3, 580.0 ± 69.5 ($p < 0.001$; $n = 6$), and 763.0 ± 92.4 ($p < 0.002$), respectively. The corresponding degree of inhibition was 62.6, 42.7, and 24.7%. Finally, on day 5, the viability dropped to ~80%. The number of cells (×10^3) at the respective genistein concentrations of 10^{-4} and 10^{-5} was 382.0 ± 39.7 ($p < 0.001$), 470.1 ± 63.9 ($p < 0.001$), and 600.5 ± 72.6 ($p < 0.005$), respectively; the corresponding degree of inhibition was 47.1, 35.0, and 16.9% (Table I).

Effects of Genistein on Histamine Content

Mast cells were cultured without or with 10^{-4} and 10^{-5} M of genistein. Genistein increased (Table II) histamine content on day 3 of culture from 5.9 ± 1.2 mg/2 × 10^5 cells/mL in untreated cells to 9.5 ± 2.3 μg/2 × 10^5 cells/mL at 10^{-5} M and to 11.1 ± 1.3 μg/2 × 10^5 cells/mL at 10^{-4} M. On day 4, histamine increased from 5.8 ± 2.4 μg/mL to 9.7 ± 1.8 μg/2 × 10^5 cells/mL at 10^{-5} M and to 10.1 ± 4.5 μg/2 × 10^5 cells/mL at 10^{-4} M, respectively. On day 5, genistein increased the histamine content from 6.1 ± 1.7 μg/2 × 10^5 cells/mL to 8.3 ± 2.0 μg/2 × 10^5 cells/mL at 10^{-5} M and to 10.1 ± 4.5 μg/mL at 10^{-4} M ($p < 0.02$).

DISCUSSION

Our results clearly show that genistein inhibits HMC-1 cell proliferation and induces accumulation of histamine in a dose-dependent manner. HMC-1 was derived from a patient with mast cell leukemia.[24] HMC-1 cells have less histamine than human mast cells in general.[23] Previous studies have shown that HMC-1 expresses a phenotype and several markers, *i.e.*, tryptase, histamine, heparin, and chondroitin sulfate, similar to that of human mast cells, and releases or expresses the mRNA of a number of chemokines and cytokines that are known to be secreted by human mast cells.[25,26] Flavonoids are molecules rich in plants, fruits, and seeds with potent antioxidant effects.[10] Flavonoids are low molecular weight benzo-γ-pyrone derivatives with a wide spectrum of biologic activities including regulation of inflammatory and immune responses, especially inhibition of mast cell activation.[10,23]An inverse relationship has been reported between the consumption of flavonoids and the incidence of established coronary artery disease,[10,27] as well the incidence of lung cancer.[10,28,29]

In this study, we examined the effect of genistein, a natural isoflavone phytoestrogen present in various plant foods, especially soy beans, with a heterocyclic, diphenolic structure similar to estrogen,[11] on HMC-1 growth and histamine content. Genistein is known to inhibit both PTKs[12] and DNA topoisomerase II.[30,31] At micromolar concentrations, genistein was found to have an inhibitory effect on PTKs, presumably at the level of the adenosine triphosphate binding site.[12,13] Genistein could induce cell cycle arrest and apoptosis in leukemic cells,[14] affect the growth and cell cycle progression of human leukemic MOLT-4 and HL-60 cells[15] and induce differentiation in the human leukemia cells HL-60 and K-562.[16] Other studies showed that some of the naturally occurring flavonoids, including genistein at 10–100 μM, inhibit the human promyelocytic leukemia cell line (HL-60).[15,32] In addition, genistein at 150 μM has been shown to be an inhibitor of angiogenesis, which has a significant effect on tumor growth and metastasis.[17] Several

studies have shown that genistein can induce apoptosis in breast, stomach, lung, head, and neck, as well as prostate cancer cell lines, by modulation of cell cycle and apoptosis regulatory molecules.[18–20] Genistein has been shown previously to be an inhibitor of tumor growth by induction of cell cycle arrest at G2/M phase.[18–20] Moreover genistein induced thymocyte apoptosis through the inhibition of topoisomerase II[30] and may affect cell proliferation either *via* its ability to interact with phospholipase C and phosphatidyl-inositol-kinases or by inhibition of DNA-topoisomerase II.[31] Geneistein also has been reported to delay mammary tumorigenesis;[33] moreover, recently, it was shown to prevent HER-2 activation and induce apoptosis of human breast epithelial cells.[34]

Genistein has been observed to induce the differentiation of human myelogenous leukemia K562 cells,[21] The present findings confirm previous reports that genistein dose dependently inhibits stimulated histamine release from rat peritoneal mast cells.[10,22,35,36] Other studies have shown that genistein inhibits immunologically stimulated mast cell activation[37–39] although not by substance P.[40] Moreover, genistein was shown to inhibit angiogenesis factor-induced mast cell chemotaxis.[41] A role of flavonoids in immunoglobulin E-mediated secretion was suggested by our observation that a flavone PTK inhibitor inhibited rat basophilic leukemia cell proliferation and β-hexosaminidase secretion stimulated by anti-dinitrophenol (DNP) and DNP-bovine serum albumin; it also induced secretory granule accumulation as evidenced by light and electron microscopy.[22]

Mast cells are critical for immediate hypersensitivity responses and inflammatory processes; consequently, mast cells are increased in many pathological conditions.[1,6] Mast cells activated immunologically or by other nonimmune triggers[2] can release many potent and rapidly acting mediators of inflammation such as histamine, proteases, prostaglandins, leukotrienes, and cytokines.[3–5] The mediator most readily associated with the mast cell is histamine.[6] Histamine plays an important role in the pathogenesis of various allergic disorders, including vasodilatation and increased vasopermeability. Mast cells and histamine also are involved in the pathophysiology of irritable bowel syndrome and interstitial cystitis,[8] sterile inflammatory conditions associated with pain, and worsened by stress.[9]

Like genistein, the flavone quercetin also inhibits the proliferation of HMC-1 cells and induces accumulation of granule-stored mediators. However, in addition to inhibiting PTKs involved with mast cell activation, there is also inhibition of protein kinases involved with the final steps of mast cell secretion.[42] In conclusion, we show that genistein is effective in inhibiting HMC-1 cell proliferation and may have synergistic effects when combined with quercetin.

REFERENCES

1. Metcalfe DD, Baram D, and Mekori YA. Mast cells. Physiol Rev 77:1033–1079, 1997.
2. Theoharides TC. The mast cell: a neuroimmunoendocrine master player. Int J Tissue React 18:1–21, 1996.
3. Serafin WE, and Austen KF. Mediators of immediate hypersensitivity reactions. N Engl J Med 317:30–34, 1987.
4. Galli SJ. New concepts about the mast cell. N Engl J Med 328:257–265, 1993.
5. Grabbe J, Welker P, Möller A, et al. Comparative cytokine release from human monocytes, monocyte-derived immature mast cells and a human mast cell line (HMC-1). J Invest Dermatol 103:504–508, 1994.
6. Church MK, and Levi-Schaffer F. The human mast cell. J Allergy Clin Immunol 99:155–160, 1997.
7. Stead RH, Dixon MF, Bramwell NH, et al. Mast cells are closely apposed to nerves in the human gastrointestinal mucosa. Gastroenterology 97:575–585, 1989.
8. Theoharides TC, Kempuraj D, and Sant GR. Mast cell involvement in interstitial cystitis: a review of human and experimental evidence. Urology 57:47–55, 2001.
9. Theoharides TC. Mast cells and stress—a psychoneuroimmunological perspective. J Clin Psychopharmacol 22:103–108, 2002.
10. Middleton E Jr, Kandaswami C, and Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 52:673–751, 2000.
11. Miksicek RJ. Commonly occurring plant flavonoids have estrogenic activity. Mol Pharmacol 44:37–43, 1993.
12. Ferriola PC, Cody V, and Middleton E. Protein kinase inhibition by plant flavonoids, kinetic mechanisms, and structure-activity relationships. Biochem Pharmacol 38:1617–1624, 1989.
13. Spinozzi F, Pagliacci MC, Migliorati G, et al. The natural tyrosine kinase inhibitor genistein produces cell cycle arrest and apoptosis in Jurkat T-leukemia cells. Leuk Res 18:431–439, 1994.
14. Bergamaschi G, Rosti V, Danova M, et al. Inhibitors of tyrosine phosphorylation induce apoptosis in human leukemic cell lines. Leukemia 7:2012–2018, 1993.
15. Traganos F, Ardelt B, Halko N, et al. Effects of genistein on the growth and cell cycle progression of normal human lymphocytes and human leukemic MOLT-4 and HL-60 cells. Cancer Res 52:6200–6208, 1992.
16. Constantinou A, Kiguchi K, and Huberman E. Induction of differentiation and DNA strand breakage in human HL-60 and K-562 leukemia cells by genistein. Cancer Res 50:2618–2624, 1990.
17. Pepper MS, Aktas E, Breit S, et al. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. Cancer Res 57:2916–2921, 1997.
18. Li Y, Bhuiyan M, Mohammad RM, et al. Induction of apoptosis in breast cancer cells by TPA. Oncogene 17:2915–2920, 1998.
19. Alhasan SA, Ensley JF, and Sarkar FH. Genistein induced molecular changes in a squamous cell carcinoma of the head and neck cell line. Int J Oncol 16:333–338, 2000.
20. Davis JN, Singh B, Bhuiyan M, et al. Genistein-induced upregulation of p21WAF1, downregulation of cyclin B, and induction of apoptosis in prostate cancer cells. Nutr Cancer 32:123–131, 1998.
21. Honma Y, Okabe-Kado J, Kasukabe T, et al. Inhibition of abl oncogene tyrosine kinase induces erythroid differentiation of human myelogenous leukemia K562 cells. Jpn J Cancer Res 81:1132–1136, 1990.
22. Alexandrakis M, Singh L, Boucher W, et al. Differential effect of flavonoids on inhibition of secretion and accumulation of secretory granules in rat basophilic leukemia cells. Int J Immunopharmacol 21:379–390, 1999.
23. Theoharides TC, Alexandrakis M, Kempuraj D, et al. Anti-inflammatory actions of flavonoids and structural requirements for new design. Int J Immunopathol Pharmacol 14:119–127, 2001.
24. Butterfield JH, Weiler D, Dewald G, et al. Establishment of an immature mast cell line from a patient with mast cell leukemia. Leuk Res 12:345–355, 1988.

25. Nilsson G, Blom T, Kusche-Gullberg M, et al. Phenotypic characterization of the human mast-cell line HMC-1. Scand J Immunol 39:489–498, 1994.
26. Selvan RS, Butterfield JH, and Krangel MS. Expression of multiple chemokine genes by a human mast cell leukemia. J Biol Chem 269:13893–13898, 1994.
27. Rimm EB, Katan MB, Ascherio A, et al. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. Ann Intern Med 125:384–389, 1996.
28. Lian F, Bhuiyan M, Li YW, et al. Genistein-induced G2-M arrest, p21WAF1 upregulation, and apoptosis in a non-small-cell lung cancer cell line. Nutr Cancer 31:184–191, 1998.
29. Le Marchand L, Murphy SP, Hankin JH, et al. Intake of flavonoids and lung cancer. J Natl Cancer Inst 92:154–160, 2000.
30. McCabe MJ, and Orrenius S. Genistein induces apoptosis in immature human thymocytes by inhibiting topoisomerase II. Biochem Biophys Res Commun 194:944–950, 1993.
31. Markovits J, Linassier C, Fosse P, et al. Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Res 49:5111–5117, 1989.
32. Hirano T, Gotoh M and Oka K. Natural flavonoids and lignans are potent cytostatic agents against human leukemic HL-60 cells. Life Sci 55:1061–1069, 1994.
33. Jin Z, and MacDonald RS. Soy isoflavones increase latency of spontaneous mammary tumors in mice. J Nutr 132:3186–3190, 2002.
34. Katdare M, Osborne M, and Telang NT. Soy isoflavone genistein modulates cell cycle progression and induces apoptosis in HER-2/neu oncogene expressing human breast epithelial cells. Int J Oncol 21: 809–815, 2002.
35. Pearce FL, Befus AD, and Bienenstock J. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells. J Allergy Clin Immunol 73:819–823, 1984.
36. Nagai S, Kitani S, Hirai K, et al. Pharmacological study of stem-cell-factor-induced mast cell histamine release with kinase inhibitors. Biochem Biophys Res Commun 208:576–581, 1995.
37. Wong WS, Tsang F, Li H, et al. Effects of inhibitors of the tyrosine kinase signaling cascade on an *in vitro* model of allergic airways. Asian Pac J Allergy Immunol 17:229–237, 1999.
38. Suzuki H, Takei M, Yanagida M, et al. Early and late events in FcεRI signal transduction in human cultured mast cells. J Immunol 159: 5881–5888, 1997.
39. Iwagoe H, Kohrogi H, Fujii K, et al. Inhibitory effect of tyrosine kinase inhibitors on antigen-induced tracheal contraction and histamine release in guinea pigs. Int Arch Allergy Immunol 111:291–298, 1996.
40. Columbo M, Horowitz EM, Kagey-Sobokta A, et al. Substance activates the release of histamine from human skin mast cells through a pertussis toxin-sensitive and protein kinase C-dependent mechanism. Clin Immunol Immunopathol 81:68–73, 1996.
41. Gruber BL, Marchese MJ, and Kew R. Angiogenic factors stimulate mast-cell migration. Blood 86:2488–2493, 1995.
42. Theoharides TC, Wang L, Pang X, et al. Cloning and cellular localization of the rat mast cell 78kD protein phosphorylated in response to the mast cell "stabilizer" cromolyn. J Pharmacol Exp Ther 294: 810–821, 2000. □