Ability of Different Flavonoids To Inhibit the Procoagulant Activity of Adherent Human Monocytes

A. Lale and J. M. Herbert*

Sanofi Recherche, 195 Route d'Espagne, 31036 Toulouse Cedex, France

J. M. Augereau, M. Billon, and M. Leconte

Sanofi Recherche, Labege Innopole, 31328 Castanet Tolosan Cedex, France

J. Gleye

Faculté des Sciences Pharmaceutiques, 35 Chemin des maraichers, 31062 Toulouse, France

Received February 22, 1995⊗

Sixty-five natural flavonoids of various chemical classes were screened for their ability to inhibit the procoagulant activity of adherent human monocytes stimulated by endotoxin and interleukin-1β *in vitro*. Eighteen of these compounds inhibited the interleukin-1β-induced expression of tissue factor on human monocytes, but the most active compound was a biflavonoid: hinokiflavone.

Tissue factor is a ubiquitous membrane-anchored glycoprotein that initiates blood coagulation by forming a complex with circulating factors VII and VIIa.[1] Under normal circumstances, endothelial cells and monocytes do not express tissue factor activity although they constitutively express thrombomodulin, which accelerates the thrombin-catalyzed activation of protein C, thus contributing to the anticoagulant properties of the endothelium. In some pathological situations, when the endothelium or the monocytes are exposed to inflammatory mediators, they can acquire procoagulant properties.[2–4] Indeed, stimulation of these cells by such inflammatory compounds as endotoxin or interleukin-1β may alter the antithrombotic properties of the endothelium by inducing the expression of tissue factor and the down-regulation of thrombomodulin, thereby promoting coagulation and thrombosis.[2–5]

Flavonoids have been shown to affect a large variety of enzymes, to possess important enzyme-inducing activities, to possess free-radical scavenging activity, to chelate certain metal cations, to have antioxidant properties, and, especially, to affect cellular protein phosphorylation,[6] and the inhibitory effects of these compounds on platelet and leukocyte functions as well as their protective effect on endothelial cells suggest that they are of potential interest in the development of inhibitors of the interactions between blood and the vessel wall.[7]

Because flavonoids have been shown to inhibit several individual aspects of blood-vessel–wall interactions involved in the initiation of thrombosis,[7] this work was aimed at determining the inhibitory effect of several flavonoids on the expression of tissue factor on the surface of human monocytes induced by endotoxin or interleukin-1β.

Unstimulated human monocytes were devoid of tissue factor activity; that is, they demonstrated the same insignificant hydrolysis of S2222 in the test, but the addition of endotoxin or interleukin-1β to adherent cells resulted in a dose-dependent expression of tissue factor on the cell surface (Figure 1). These results confirm previous observations.[5,8–10]

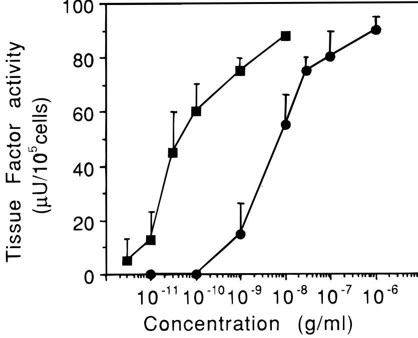


Figure 1. Effect of endotoxin and interleukin-1β on tissue factor expression. Human monocytes were incubated with increasing concentrations of endotoxin (●) or interleukin-1β (■). Tissue factor expression was quantified as described in "**Materials and Methods**". Results are expressed as mean ± SD ($n = 6$).

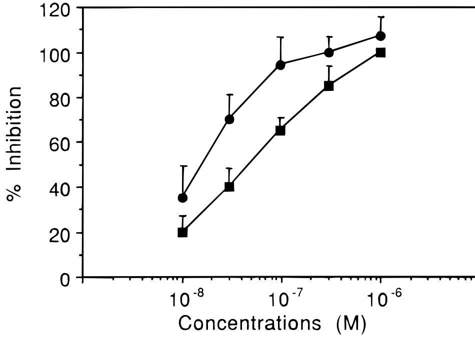


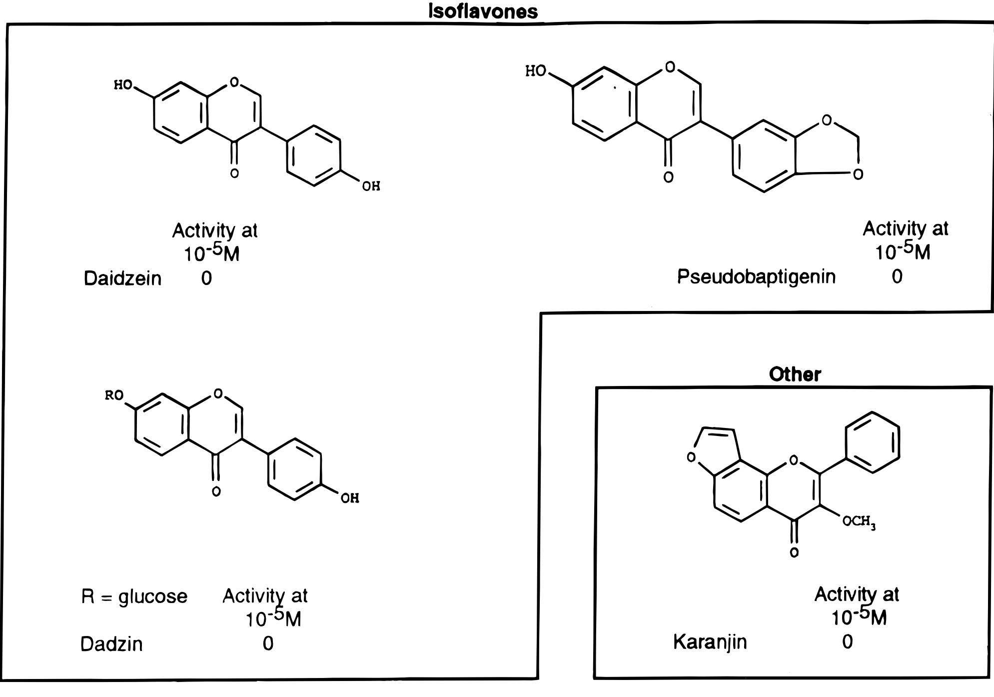
Figure 2. Effect of hinokiflavone on endotoxin- or interleukin-1β-induced expression of tissue factor in monocytes. Increasing concentrations of hinokiflavone were incubated with human monocytes in the presence of endotoxin (●) (10 ng/mL) or interleukin-1β (■) (0.5 ng/mL). Tissue factor expression was determined as described in "**Materials and Methods**". Results are expressed as percentage of inhibition ± SD of the control response ($n = 6$).

* To whom correspondence should be addressed. Tel: (33) 62 14 23 61. Fax: (33) 62 14 22 86.

⊗ Abstract published in *Advance ACS Abstracts,* February 1, 1996.

0163-3864/96/3259-0273$12.00/0 © 1996 American Chemical Society and American Society of Pharmacognosy

Chart 1



As shown in Table 1, several flavonoids counteracted interleukin-1-induced tissue factor induction in human monocytes. The IC_{50} values (concentrations that inhibited 50% of the interleukin-1-induced tissue factor expression) were between 1.2×10^{-6} and 6.8×10^{-8} M. The most active compound was the biflavonoid hinokiflavone. IC_{50} values with regard to endotoxin- and interleukin-1-induced tissue factor expression in human monocytes were 18 ± 3 and 48 ± 4 nM, respectively (means $\pm$ SD; $n = 6$) (Figure 2). This is the first demonstration of the activity of such a compound with regard to cytokine-induced cell activation. The etiology of such an effect is still unknown but cannot be attributed to an effect on the intracellular protein kinase C, which has already been shown to be inhibited in vitro by certain flavonoids[11-13] (data not shown).

The ability of hinokiflavone to inhibit endotoxin- and interleukin-1-induced tissue factor expression within the same range of concentration suggests that interleukin-1 may be a major participant in the response of monocytes to endotoxin, but definite evidence for the importance of interleukin-1 in this phenomenon will await the determination of the effect of hinokiflavone in a more global model of inflammation.

Table 1. Effect of Various Flavonoids on Interleukin-1-Induced Tissue Factor Expression on Human Monococytes

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	activity 10^{-5} M	IC_{50} (M)
Flavones											
flavone	H	H	H	H	H	H	H	H	H	6.8	
bacailein	H	OH	OH	OH	H	H	H	H	H	55.2	
chrysin	H	OH	H	OH	H	H	H	H	H		2.4×10^{-6}
apigenin	H	OH	H	OH	H	H	H	OH	H		1.2×10^{-6}
luteolin	H	OH	H	OH	H	H	H	OH	OH		1.5×10^{-6}
4-methoxyflavone	H	H	H	H	H	H	H	OCH_3	H	0	
acacetin	H	OH	H	H	H	H	OH	OCH_3	H	26	
chrysoeriol	H	OH	H	OH	H	H	H	OH	OCH_3		2.6×10^{-6}
diosmetin	H	OH	H	OH	H	H	H	OCH_3	OH		3×10^{-6}
eupatorin	H	OH	OCH_3	OCH_3	H	H	OH	OCH_3	H	0	
5,7-dihydroxy-3′,4′,5′-trimethoxyflavone	H	OH	H	OH	H	H	OCH_3	OCH_3	OCH_3	0	
eupatorin-5-methylether	H	OCH_3	OCH_3	OCH_3	H	H	OH	OCH_3	H	0	
sinensetin	H	OCH_3	OCH_3	OCH_3	H	H	H	OCH_3	OCH_3		10^{-5}
gardenin A	H	OH	OCH_3	OCH_3	H	H	OCH_3	OCH_3	OCH_3	0	
3,4′-dimethoxy-3′,5,7-trihydroxyflavone	OCH_3	OH	H	OH	H	H	H	OH	OCH_3		3.9×10^{-6}
tangeretin	OCH_3	OCH_3	OCH_3	OCH_3	H	H	H	OCH_3	H	0	
luteolin-3′,7-diglucoside	H	H	H	glucose	H	H	glucose	H	H		1.55×10^{-6}
vitexin-2-0-rhamnoside	H	OH	H	OH	gluco-rhamnose	H	H	OH	H	0	
Flavonols											
galanjin	OH	OH	H	OH	H	H	H	H	H	0	
kaempferol	OH	OH	H	OH	H	H	H	OH	H		2.7×10^{-6}
datiscetin	OH	OH	H	OH	H	OH	H	H	H	28.4	
fisetin	OH	H	H	OH	H	H	OH	OH	H		3.7×10^{-6}
robinetin	OH	H	H	OH	H	H	OH	OH	OH	38.5	
quercetin	OH	OH	H	OH	H	H	OH	OH	H	0	
morin	OH	OH	H	OH	H	OH	H	OH	H	0	
quercetagetin	OH	OH	OH	OH	H	H	OH	OH	H	0	
myricetin	OH	OH	H	OH	H	H	OH	OH	OH	1.2	
geraldol	OH	H	H	OH	H	H	OCH_3	OH	H		2.3×10^{-6}
kaempferide	OH	OH	H	OH	H	H	H	OCH_3	H		5.7×10^{-6}
tamarixetin	OH	OH	H	OH	H	H	H	OCH_3	OH		4.6×10^{-6}
rhamnetin	OH	OH	H	OCH_3	H	H	OH	OH	H	0	
isorhamnetin	OH	OH	H	OH	H	H	OCH_3	OH	H		3.8×10^{-6}
syringetin	OH	OH	H	OH	H	H	OCH_3	OH	OCH_3		2.1×10^{-6}
kaempferol-7,4′-dimethoxy-8-butyryl ester	OH	OH	H	OCH_3	$OC(O)CH_2C_2H_5$	H	H	H	H	0	
datiscetin-3-rutinose	rutinose	OH	H	OH	H	OH	H	H	H	28.4	
kaempferol 3-glucoside	O-glucose	OH	H	OH	H	H	H	OH	H	0	
avicularin	glucose	OH	H	OH	H	H	OH	OH	H	0	
myricitrin	rhamnose	OH	H	OH	H	H	OH	OH	OH	37.7	
isorhamnetin 3-glucoside	glucose	OH	H	OH	H	H	OCH_3	OH	H		3.8×10^{-6}
rutin	rutinose	OH	H	OH	H	H	OH	OH	H	9.1	
tiliroside	*p*-coumaryl glucose	OH	H	OH	H	H	OH	H	H		5.2×10^{-6}
quercetin-3-glucose 6″ acetate	glucosyl acetate	OH	H	OH	H	H	OH	OH	H	12.4	

Table 1 (Continued)

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	activity 10^{-5} M	IC_{50} (M)
				Flavanones							
flavanone	H	H	H	H	H	H	H	H	H	0	
naringenin	H	OH	H	OH	H	H	OH	H	H	10.6	
eriodictyol	H	OH	H	OH	H	H	H	OH	OH	39	
7-methoxypinocembrin	H	OH	H	OCH_3	H	H	H	H	H	0	
isosakuranetin	H	OH	H	OH	H	H	H	OCH_3	H	0	
hesperetin	H	OH	H	OH	H	H	OH	OCH_3	H		2.1×10^{-6}
homoeriodictyol	H	OH	H	OH	H	H	H	OH	OCH_3	0	
5,7-dihydroxyl-3′,4′,5′-trimethoxyflavone	H	OH	H	OH	H	H	OCH_3	OCH_3	OCH_3	41.3	
bavachinin	H	H	$CH_2CH{=}C(CH_3)CH_3$	OCH_3	H	H	H	OH	H		3.2×10^{-6}
neohesperidin	H	OH	H	*O*-neohesperidose	H	H	OH	OCH_3	H	0	
narirutin	H	OH	H	*O*-rutinose	H	H	OH	OCH_3	H	0	
				Dihydroflavonols							
taxifolin	OH	OH	H	OH	H	H	OH	OH	H	41	
dihydrorobinetin	OH	OH	H	OH	H	H	OH	OH	OH	0	
fustin	OH	H	H	OH	H	H	OH	OH	H	0	

Materials and Methods

Procoagulant activity was assayed according to Suprenant and Zuckerman.[14] Briefly, mononuclear cells were obtained from human heparinized blood, as described by Boyum.[15] Cells were plated for 30 min at 37 °C into 96-well microplates ($1-2 \times 10^5$ cells/well). Nonadherent cells were then removed, and adherent monocyte cells (5×10^3 cells/well) were used for the assay. In these conditions, interexperiment variations in adherent cell number never exceeded 15% (data not shown). Adherent cells were incubated for 18 h at 37 °C in M-199 (without phenol red), with endotoxin (lipopolysaccharide from *Escherichia coli* strain 055:B5) (Sigma, France) or interleukin-1β (Tebu, France) in the absence or presence of the indicated concentrations of the compounds to be tested. The medium was removed, and the wells were washed twice with 1 mL of phosphate buffered saline and incubated for 45 min at 37 °C with 250 μL of M-199 containing PPSB (a mixture of coagulation factors II, VII, IX, and X) (0.44 U/mL Factor VII) (Intertransfusion, France), and 100 μg/mL of substrate S2222 [Bz-Ile-Glu-(piperidyl)-Gly-Arg-pNA] (Kabi, Sweden). The optical density was measured at 405 nm. The tissue factor activity was obtained from a standard curve (log [ΔOD_{405}/min] vs. log [U/mL]) using serial dilutions of rabbit-brain thromboplastin in M-199 assayed as described above. Undiluted thromboplastin was arbitrarily assigned a value of 1 U/mL. The tissue factor activity was normalized to the cell counts from the same well and expressed as microunits of tissue factor/10^5 cells. The various flavonoids tested were purchased from Extrasynthèse (Genay, France).

References and Notes

(1) Nemerson, Y. *Blood* **1988**, *71*, 1.
(2) Bevilacqua, M. P.; Pober, J. S.; Majeau, G. R.; Cotran, R. S.; Gimbrone, M. A. *J. Exp. Med.* **1984**, *160*, 618.
(3) Bevilacqua, M. P.; Pober, J. S.; Majeau, G. R.; Fiers, W.; Cotran, R. S.; Gimbrone, M. A. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 4333.
(4) Colucci, M.; Balconi, G.; Lorenzet, R.; Pietra, A.; Locati, D.; Donati, M. B.; Semeraro, N. *J. Clin. Invest.* **1983**, *71*, 1893.
(5) Nawroth, P. P.; Handley, D. A.; Esmon, C. T.; Stern, D. M. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 3460.
(6) Middleton, E.; Kandaswami, C. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman & Hall: London, 1993; pp 619−651.
(7) Beretz, A.; Cazenave, J. P. In *Plant Flavonoids in Biology and Medicine*; Cody, V.; Middleton, E.; Harborne, J. B.; Beretz, A., Eds.; Alan R. Liss: New York, 1988; pp 187−200.
(8) Kapiotis, S.; Besemer, J.; Bevec, D.; Valent, P.; Bettelheim, P.; Lechner, .; Speiser, W. *Blood* **1991**, *78*, 410.
(9) Herbert, J. M.; Savi, P.; Laplace, M. C.; Lalé, A. *FEBS Lett.* **1992**, *310*, 31.
(10) Achipoff, G.; Beretz, A.; Freyssinet, J. M.; Klein-Soyer, C.; Brisson, C.; Cazenave, J. P. *Biochem. J.* **1991**, *273*, 679.
(11) Graziani, Y.; Chayoth, R.; Karny, N.; Feldman, B.; Levy, J. *Biochem. Biophys. Acta* **1982**, *714*, 415.
(12) Gcshwendt, M.; Horn, F.; Kittstein, W.; Marks, F. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 444.
(13) Ferriola, P. C.; Cody, V.; Middleton, E. *Biochem. Pharmacol.* **1989**, *38*, 1617.
(14) Surprenant, Y. M.; Zuckerman, S. H. *Thromb. Res.* **1989**, *53*, 339.
(15) Boyum, A. *Scand. J. Immunol.* **1976**, *5*, 9.

NP960057S