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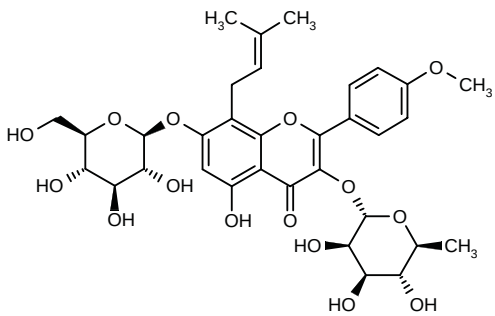
Icariin

Immunostimulant
Neuronal Injury Inhibitor
Antineoplastic
Calcium Regulator

3-(6-Deoxy-α-L-mannopyranosyloxy)-7-(β-D-glucopyranosyloxy)-5-hydroxy-2-(4-methoxyphenyl)-8-(3-methyl-2-butenyl)-4*H*-1-benzopyran-4-one



$C_{33}H_{40}O_{15}$ Mol wt: 676.67

CAS: 000489-32-7

EN: 260563

Introduction

Icariin is a flavanol glycoside isolated from the aerial parts of *Epimedium grandiflorum* Morr, *E. sagittatum* (Sieb. et Zucc) Maxim (1), *E. koreanum* Nakai (2), and the stems and leaves of *E. brevicornum* Maxim (3). This paper reviews the extensive pharmacological studies on icariin that have been carried out in recent years.

Immunomodulatory Effects

The synergistic effects of icariin on inducing IL-2, IL-3, IL-6 and enhancing natural killer (NK) and lymphokine activated killer (LAK) cell activity were studied by using dependent cell lines and assaying lactate dehydrogenase release. The results showed that in combination with phytohemagglutinin, icariin induces IL-2, IL-3 and IL-6 production in tonsil mononuclear cells in a dose-dependent manner. IL-2 induction peaked after 48 h, whereas IL-6 induction peaked after 72 h. The enhanced NK and LAK cell activity induced by icariin was dose-dependent, with the LAK activity peaking after 72 h. The results indicate that icariin may be an effective biological response modifier (4, 5).

The immunological effects of *Epimedium polysaccharide* (EPS) and icariin on mouse thymus have been reported (6). When the compounds were given subcutaneously, EPS enhanced IL-2 production and proliferation of thymocyte but decreased L3T4 and Lyt2 cell number and response to Con A stimulation of the thymus, whereas icariin increased the response of mouse thymus cells to Con A stimulation. The results suggest that both EPS and icariin have an immunostimulatory effect on the thymus, and that EPS possibly promotes the migration of thymocytes from the thymus to the peripheral tissues.

Li *et al.* (7) investigated the influence of icariin on murine spleen lymphocyte production and the colony stimulating factor (CSF)-like activity induced by priming such lymphocytes with Con A or lipopolysaccharides, as determined by the degree of [^{3}H]-TdR incorporation and by the uptake of [^{3}H]-TdR by the bone marrow cells. The results showed that *in vitro*, icariin significantly enhanced Con A-induced mice lymphocyte proliferation. Increased CSF-like activity was also detected in the supernatant of Con A-primed mouse lymphocyte culture after icariin was added to the culture medium, suggesting that icariin increases immunologic function and enhances hemopoiesis.

The effects of icariin on murine peritoneal macrophage function have also been studied (8). Icariin in concentrations of 0.01-1 μg/ml significantly increased the macrophage phagocytic activity as measured by the amount of phagocytic neutral red. Furthermore, icariin in concentrations of 0.001-10 μg/ml dose-dependently increased the production of IL-1 and tumor necrosis factor from lipopolysaccharide-treated macrophages. These results indicate that icariin activates macrophages and may regulate murine immune function.

Wang and He (9) have reported on the marked inhibitory effects of a methanol extract of *Epimedium* on the *in vitro* proliferation response of mouse lymphocytes to mitogens (Con A and LPS) and on the mixed lymphocyte reaction. The inhibitory effect was found to be proportional to the concentration of the mitogen. In addition, *in vivo* studies indicated that intraperitoneal injection of the methanol extract prolonged the survival time of the

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half-heart allograft in newborn mice. In another study icariin was found to significantly enhance antibody production (10).

Vascular Effects

The effects of icariin on cerebral blood flow in animals have been studied (11). In anesthetized rabbits and dogs, intravenous injection of icariin (1 mg/kg) brought about a sustained increase in cerebral blood flow and a decrease in cerebrovascular resistance, accompanied by a slight fall in blood pressure. In another study, the effects of icariin and flavenol on cerebral blood flow in rabbits were investigated (12). In anesthetized rabbits intravenous injection of icariin (1 mg/kg) or flavenol (3 mg/kg) resulted in a sustained increase in cerebral blood flow, a decrease in cerebrovascular resistance and a similar slight fall in blood pressure. Both compounds were found to delay the ECG flattening time, and offered some protection against anoxemia.

The effects of icariin on the vascular smooth muscle of rabbits and dogs have been studied by Wang *et al.* (13), who found that the drug decreased the resting tension of blood vessels. They also studied the effects of icariin, verapamil, papaverine and phentolamine on the dose-response curves to norepinephrine in rabbit thoracic aorta. Phentolamine competitively antagonized norepinephrine, whereas icariin, verapamil and papaverine acted noncompetitively. In a calcium-free Krebs-Henselheit solution, norepinephrine caused an initial fast response that could not be induced by icariin; when calcium was added to the solution, the calcium-dependent response was markedly reduced by icariin. In addition, icariin inhibited the potassium-evoked contraction in thoracic aorta, mesenteric aorta and the coronary artery, and also inhibited the 5-HT-induced contraction of the cerebral basialis artery. These findings suggest that icariin-induced vasodilatation results from its inhibitory effects on receptor-operated and potential-dependent calcium influx.

Guan *et al.* (14) studied the vasodilatory mechanism of icariin using isolated rabbit aorta strips. They found that icariin (20 and 40 mg/l) antagonized the dose-response curves of norepinephrine, potassium chloride and calcium chloride in a noncompetitive manner. Icariin in a concentration of 30 mg/l significantly inhibited the extracellular calcium-dependent contraction of the aortic strips induced by norepinephrine, but did not inhibit the corresponding intracellular calcium-dependent contractions of the aortic strips. These results indicate that the vasodilatory mechanism of icariin may be linked with its calcium channel blocking activity.

The effects of icariin on murine fibrinolysis and hemorrhagic mortality have been investigated by Shan *et al.* (15). Plasminogen activator activity induced by icariin-stimulated macrophages was 0.731 IU/ml ($p < 0.01$), some 2.8 times that of the control, as determined by a spectrophotometric assay, and was associated with a rapid fibrinolysis of the macrophages.

Anticancer Effects

The inhibitory effects of icariin on a human promyelocytic leukemia cell line (HL-60) have been investigated (16). The results showed that icariin in concentrations of 62.5, 125 and 250 mg/l dose-dependently inhibited HL-60 cells after 12 h incubation. Icariin (100 mg/l) increased the reduction of nitroblue tetrozolium ($p < 0.01$) and the mean optical density after 48 h of incubation. After exposure to icariin, the nuclear morphology of HL-60 cells showed changes with the formation of rod and lobular forms and a reduction of nuclear volume, indicating that the compound may induce differentiation of HL-60 cells.

The effects of icariin on the differentiation of HL-60 cells were also studied by Zhao *et al.* (17), who used nitroblue tetrozolium reduction test, ^{125}I-cAMP and ^{125}I-cGMP double antibody radioimmunoassay and electron micro-scopy techniques. They found that after treating HL-60 cells with icariin at a concentration of 0.1 g/l, there was a reduction of nitroblue tetrozolium and the cAMP/cGMP ratio increased; fine wrinkles and ball-like images on the cell surface were observed. The results indicate that icariin influences the induction of HL-60 cell differentiation, possibly via a mechanism involving the elevation of the cAMP/cGMP ratio.

In another study, icariin was shown to have a marked inhibitory effect on oncocyte proliferation (18).

Skeletal Effects

The effects of an *E. sagittatum* Maxim (EES) extract on the prevention of bone loss was studied by Li *et al.* (19), who used orchidectomized (OCT) rats. EES was given in oral doses of 5 g/kg 6 days a week for 7 weeks, and the response was compared with that of ageing control animals. The proximal tibia of the OCT control rats were characterized by a significant increase in the erosion perimeter (+108%), in the label perimeter (+76%), in the osteoid perimeter (+89%) and in the bone formation rate (+65%), indicating a higher bone turnover rate. The OCT rats treated with EES showed an increase of 11% in the tibial trabecular area together with a significant decrease in perimeter erosion (–13%) and a comparable decrease of –14% in osteoid perimeter erosion, reflecting a partial inhibition of the high bone turnover and a reduction of bone loss. Bone formation and label perimeter rates were not inhibited, and were higher than in the control group. The results show that EES can prevent trabecular bone loss and maintain bone structure in OCT rats.

The effects of EES on the prevention of osteoporosis in rats have also been studied by Li *et al.* (20). They used 3-month old Sprague-Dawley rats divided randomly into 3 groups of 8 animals each: a control group, a hormone group and a hormone-traditional Chinese medicine group. The hormone group was treated with prednisone acetate at doses of 4.5 mg/kg twice weekly. The hormone-traditional Chinese medicine group received similar

doses of prednisone together with a fluid extract of EES in doses of 2 g/kg/day. After 90 days of treatment, the histomorphometric parameters of the three groups were measured. In the hormone-treated group, the percentage of trabecular area and trabecular number (density) was increased, and the trabecular separation increased as compared with the control group, indicating that bone resorption exceeded the formation of new bone. However, in the hormone-traditional Chinese medicine group, bone mass increased, as the bone resorption rate (70%) was less than that in the hormone only group.

In another study by Liu *et al.* (21), icariin was found to markedly increase [^{3}H]-TdR incorporation into the DNA of bone marrow cells *in vitro*.

Miscellaneous Effects

Xiong and Zhou (22) have reported the effects of icariin on the *in vivo* development of sex organs of immature male mice and the *in vitro* activity of rat Leydig cells. They found that icariin brought about a significant increase in the weights of the epididymides and seminal vesicles and that in cultures of adult rat Leydig cells icariin increased basal testosterone secretion and cAMP production. These results suggest that icariin possesses androgen-like properties, and may cause a cAMP-mediated increase in testosterone production.

The antihepatotoxic effects of icariin were studied by measuring the release of glutamic pyruvic transaminase (GPTase) and sorbitol dehydrogenase in cultures of CCl_4-treated hepatocytes (23). The results showed that icariin brought about a significant reduction in the levels of released GPTase and sorbitol dehydrogenase comparable to a 76% protection against toxicity at concentrations of 1-20 μM. The antihepatotoxic effects of icariin were also evaluated by determining the total cytochrome P450 and glutathione-S-transferase activity of the hepatocytes obtained from CCl_4-treated rat hepatocytes.

The quantitative distribution of icariin was investigated by Guo *et al.* (24) using [^{3}H]-icariin as the radiolabeled agent. It was injected into mice via the caudal vein, and after 48 h the content of [^{3}H]-icariin in 405 samples was measured. The samples included 15 types of organs or tissues, and were taken at 9 different time points. The work was designed to study the dynamic quantitative distribution and excretion of [^{3}H]-icariin from Chinese medicine epimedium. The results showed that the amounts of [^{3}H]-icariin in various organs and at various time points were significantly different ($p < 0.001$). Distribution was mainly in the liver, adrenal glands, small intestine, kidney and bronchus.

Source

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