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Ex vivo Platelet Anti-aggregating Activities of 3, 4-Dihydroxybenzoic Acid and It's Ethyl Ester

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Abstract—3, 4-Dihydroxybenzoic acid and it's artefact ethyl ester obtained from *Acanthopanax senticosus* Max. were tested *ex vivo* and found to show mild inhibitory activities against rat platelet aggregation.

Keywords—3, 4-Dihydroxybenzoic acid • ethyl 3, 4-dihydroxybenzoate • *ex vivo* platelet anti-aggregating activities

In the previous papers,[1~3] the authors reported the isolation of 3, 4-dihydroxybenzoic acid (DBA) from *Acanthopanax senticosus* Max. as an anti-platelet aggregating (*in vitro* results) component. The ethyl ester of 3, 4-dihydroxybenzoic acid (EDB) was also obtained as an artefact with the inhibitory activities during the activity-guided fractionations of the MeOH extract.

Since *in vitro* screening results are not necessarily coincide with the results obtained with the whole animals, DBA and EDB were tested for their ability to inhibit platelet aggregations *ex vivo*. DBA or EDB was administered to rats for three consecutive days at the dose of 100 mg/kg, i.p. and on the last day of the experiment blood samples were collected to measure the degrees of aggregations of the platelets induced by ADP, arachidonic acid (AA), or collagen. The degrees of platelet aggregations were measured with platelet rich plasma with the modified smear method[1] and were graded as ++; full aggregation, +; intermediate aggregation, ±; slight aggregation, and −; no aggregation.

Table I shows comparison of the degrees of platelet aggregation of the DBA- or EDB-treated rats with those of the control rats which received vehicle (physiological saline) alone and also with those of aspirin (100 mg/kg, i.p.) treated rats. Platelets from control rats responded to ADP, AA and collagen sho-

Table I. *Ex vivo* effects of ASA, DBA, and EDB on rat platelet aggregation induced by ADP, AA, or collagen.

	Control (Saline)	ASA[a]	DBA[b]	EDB[c]
Saline	−	−	−	−
ADP[d]	++	+	++	++~+
AA[e]	++	±~−	++~+	+~±
Collagen[f]	++	±~−	+~±	±~−
No of rats	10	8	18	12

a) 100 mg/kg, i.p. of aspirin, b) 100 mg/kg, i.p. of 3, 4-dihydroxybenzoic acid, c) 100 mg/kg, i.p. of ethyl 3, 4-dihydroxybenzoate, d) 7.5×10^{-7} g/ml of adenosine 5'-diphosphate, e) 6×10^{-5} g/ml of arachidonic acid, f) 1×10^{-5} g/ml of collagen.

The degree of platelet aggregation were measured with the platelet rich plasma with the modified smear method and were graded as ++; full aggregation, +; intermediate aggregation, ±; slight aggregation, −; no aggregation.

wing full aggregations (卄) while platelets from aspirin-treated rats showed intermediate aggregation (+) responding to ADP and almost no aggregation (±~−) to AA or collagen showing that aspirin was more inhibitory to AA or collagen induced aggregations than to ADP induced aggregation as has been described.[4~6] DBA was not inhibitory to ADP induced platelet aggregation giving full aggregation (卄) and showed mild inhibitions to either AA-induced or collagen-induced aggregations (卄~+ and +~±) however less inhibitory than aspirin. EDB was more inhibitory than DBA to either ADP-(卄~+ vs 卄), AA-(+~± vs 卄~+), and collagen-(±~− vs +~±) induced platelet aggregations. On the other hand, EDB was less active than aspirin to either ADP- and AA-induced aggregations, however showed similar inhibitory potencies as aspirin to collagen-induced platelet aggregations with the degrees of aggregation of ±~−.

Disseminated intravascular coagulation (DIC) is a syndrome in which a number of thrombi are produced in small vessels resulting in the reduction of coagulation factors and blood platelets.[7~9] At the dose of 100 mg/kg, i.p., neither DBA nor EDB exerted significiant effects on endotoxin-induced DIC symptoms. Aspirin was reported to give maximal preventive effects against DIC in rats at the dose of 30 ng/kg,[10] however large doses (3 mg/kg or higher) of aspirin could not prevent the aggravation of DIC[11] showing an opposite dose-effect relationships. Although DBA and EDB appeared to show no effects to the progress of DIC at the dose where platelet aggregabilities were reduced, the possibilities of biphasic effects can not be excluded with a maximum effect at low dose level as has been observed with aspirin.

Experimental

Materials

ADP (adenosine 5′-diphosphate dicyclohexylammonium salt), AA (arachidonic acid sodium salt), collagen (acid soluble, from calf skin) and endotoxin (lipopolysaccharide from *E. coli*) were purchased from Sigma Chem. Comp., U.S.A. DBA (3, 4-dihydroxybenzoic acid) and EDB (ethyl 3, 4-dihydroxybenzoate) were either obtained from *Acanthopanax senticosus* or purchased from Aldrich Chem. Co., U.S.A. and Tokyo Kasei, Japan respetively.

Ex vivo platelet aggregation testing

Aspirin (ASA), DBA or EDB was administered to Sprague-Dawley rats (males, 200±30 g) at the dose of 100 mg/kg, i.p. for 3 consecutive days. One hour after the last injection blood samples were drawn from hearts of $CHCl_3$-anesthetized rats into a plastic syringe containing 1/10 volume of 2.2% trisodium citrate solution. The preparation of platelet rich plasma (PRP) from the citrated blood and the measurements of the degrees of platelet aggregation were performed by the modified smear method of Yun-Choi et. al.[1] The following final concentrations of aggregation inducing agents were employed. ADP; 7.5×10^{-7} g/ml, AA; 6×10^{-5} g/ml and collage; 1×10^{-5} g/ml.

Effects on experimental disseminated intravascular coagulation (DIC)

The induction of the experimental DIC and the determination of the severity of DIC were performed by the method of Yoshikawa et. al.[8,9] Sprague-Dawley rats (males, 200±20 g) were injected with DBA or EDB at the dose of 100 mg/kg, i.p. A sustained infusion of endotoxin (100 mg/kg, 4 hr period) into the femoral vein was performed starting 30 min after the DBA or EDB injection. And various parameters including platelet count, prothrombin

time, partial thromboplastin time etc. were determined as usual.[9]

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