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REGULAR ARTICLE

Effects of higenamine and its 1-naphthyl analogs, YS-49 and YS-51, on platelet TXA_2 synthesis and aggregation

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Abstract The effects of higenamine and its 1-naphthyl analogs, YS-49 and YS-51, on thromboxane A_2 (TXA_2) formation from arachidonic acid (AA) and aggregation in platelets, were investigated. YS-49 and YS-51 (IC_{50}; 32.8 and 39.4 μM respectively) exhibited much stronger inhibitory effects on TXA_2 formation than higenamine (IC_{50}; 2.99 mM). The higher inhibitory potencies of YS-49 and YS-51 (IC_{50}: 3.3 and 5.7 μM respectively) than higenamine (IC_{50}: 140 μM) on AA induced rat platelet aggregation was presumed to be the result of low inhibitory effect of higenamine than YS-49 and YS-51 on TXA_2 production from AA. Among the present three compounds, the more hydrophobic naphthylmethyl groups were supposed to be more favorable than *p*-hydroxybenzyl moiety, at 1-position of the tetrahydroisoquinoline ring, to display the inhibitory effects on TXA_2 production and AA induced aggregation of platelets. In addition, higenamine, YS-49 and YS-51 were observed directly antagonistic on TXA_2 receptor (TP receptors) by displaying inhibitory effects to U46619 (TXA_2 mimetic) induced platelet aggregation, however all of the three compounds showed similar order of inhibitory potencies. The present results are suggestive that YS-49 and YS-51 exert their inhibitory effects on AA-induced platelet aggregation partly by inhibiting the production of TXA_2 from AA and partly by directly blocking the TP receptor, in addition to the previously reported effects on α_2-adrenergic receptor. On the other hand, higenamine is supposed to antagonize AA-induced platelet aggregation by mostly directly blocking the TP receptor.

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Higenamine (CAS 5843-65-2), a 1-benzyl-1,2,3,4-tetrahydroisoquinoline alkaloid, was isolated as optically inert racemic mixtures from several plant materials, including Aconite root, Asiasaari radix, *Annona squamosa* and *Gnetum parvifolium* [1–5]. Higenamine possesses a catecholamine moiety as a part of its structure, although it belongs to isoquinoline alkaloid that is chemically completely distinct from catecholamines. With the catecholamine moiety, higenamine was expected to have effects on adrenergic receptors and was reported to possess positive inotropic and chronotropic effects through β-adrenoceptor stimulation. Higenamine was also observed to behave as an α-adrenoceptor antagonist on rat vascular smooth muscle and blood platelets. This compound relaxed endothelium-denuded aorta pre-contracted with phenylephrine and showed selective inhibitory effects on epinephrine induced platelet aggregation. The above mentioned effects, as a whole, were suggested to be beneficial for congestive heart failure by reducing cardiac afterload, increasing inotropic potential and increasing blood fluidity [6–9]. In addition, higenamine was observed to inhibit inducible nitric oxide synthase (iNOS) mRNA expression and accompanying NO production in lipopolysaccharide (LPS) with or without other cytokine treated macrophage and other cells. Higenamine pre-treatment reduced the drastic increase in blood NO concentration and the resulting decrease in heart rate and blood pressure in LPS bolus-injected rats [10–12]. Two of the 1-naphthylmethyl analogs of higenamine, 1-(α-naphthylmethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (YS-49, CAS 213179-96-5) and 1-(β-naphthylmethyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (YS-51, CAS 213179-96-5), were also reported to possess various biological activities either equivalent or superior to higenamine [8,13–17]. Moreover, the above three tetrahydroisoquinolines, higenamine, YS-49 and YS-51, were reported to ameliorate various symptomatic blood factors of disseminated intravascular coagulation (DIC) and/or accompanying multiple organ failure (MOF) in LPS induced experimental animal DIC model [18,19].

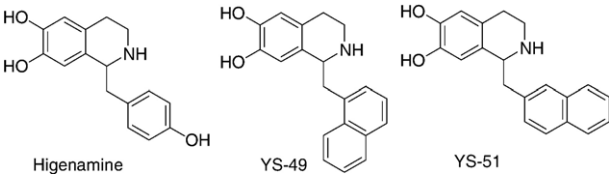

Figure 1 Chemical structure of higenamine, YS-49 and YS-51.

The purpose of the present study was to investigate the effects of higenamine, YS-49 and YS-51 on platelet thromboxane A_2 receptor (TP receptor) and on thromboxane A_2 (TXA_2) production induced by arachidonic acid (AA) in platelets.

Materials and methods

Materials and animals

Higenamine [1-(4′-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline], YS-49 and YS-51 (Fig. 1) were synthesized as hydrobromide salts according to the previously described method [13,20,21]. Collagen was purchased from Chrono-Log Corp. (USA). Sodium arachidonate (AA), U46619 (9,11-dideoxy-11α, 9α-epoxymethanoprostaglandin $F_{2\alpha}$), yohimbine hydrochloride, pentolamine hydrochloride, and acetylsalicylic acid (ASA) were obtained from Sigma Chem. Co. (USA). Thromboxane B_2 Biotrack Assay (ELISA) Kit was purchased from Amersham Bioscience. The rats (Sprague–Dawley; 250±20 g) were bred at the Animal Station of Natural Products Research Institute, Seoul National University. They were fed with a diet of animal chow and trap water and were housed at 23±0.5 °C and 10% humidity in a 12 h light–dark cycle in accordance with the Guide for the Care and Use of Laboratory Animals by Seoul National University.

Aggregation assay with rat platelets

Blood, collected from rat heart after surgery using syringe containing 0.1 volume of 2.2% sodium citrate, was centrifuged at 200×*g* for 10 min to obtain the supernatant platelet rich plasma (PRP). Platelet poor plasma (PPP) was prepared from the residue by centrifugation at 900×*g* for 30 more minutes. PRP was diluted with saline to adjust the number of platelets (400–450×10^6/ml) with the aid of platelet counter (PLT-4, HEMA-1, Texas International Laboratories, Inc., USA). The percentage of platelet aggregation was measured on a platelet aggregometer (500VS, Chrono-Log Corp., USA) at 37 °C with stirring at 1000 rpm, assuming that PRP representing 0% aggregation and PPP representing 100% aggregation [22]. After 3 min pre-incubation, sample or vehicle was added to the adjusted PRP followed by the addition of threshold concentration of collagen (0.5–1.0 μg/ml) at 30 s. And then, platelet aggregation was induced with the addition of an aggregating agent [AA (10–40 μM) or U46619 (1–5 μM)] at 1 min.