Glucosamine, a naturally occurring amino monosaccharide, has been used to treat or prevent osteoarthritis in humans. Recently, we have revealed that glucosamine inhibits platelet activation in vitro. However, the effect of in vivo administration of glucosamine has not yet been clear. Recently, we have evaluated the effect of oral glucosamine administration on platelet activation in guinea pigs. Guinea pigs were orally administered with glucosamine (an average of 400 mg glucosamine/animal/day) for 22 days and thereafter platelet functions were examined.

Glucosamine-administration suppressed platelet aggregation in response to ADP, but not platelet aggregation induced by collagen. Furthermore, glucosamine-administration inhibited the ADP-induced extracellular release of ATP and production of thromboxane A2. In contrast, glucosamine did not affect the body-weights, platelet counts and bleeding time in guinea pigs after the administration.

These observations suggest that glucosamine is likely to exert an inhibitory action on platelets in vivo by suppressing platelet aggregation, ATP release, and thromboxane A2 production. Thus, glucosamine could be expected as a novel and safe anti-platelet agent.


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can synthesis. Moreover, glucosamine has been suggested to exhibit protective actions on carrageenin-induced inflammation and inflammatory bowel disorders\textsuperscript{11,12}. More recently, glucosamine is reported to exhibit anti-inflammatory action by inhibiting neutrophil function such as superoxide generation, phagocytosis, granule enzyme release and chemotaxis\textsuperscript{13}.

In addition to the chondroprotective and anti-inflammatory actions, glucosamine was demonstrated to prolong the allergenic cardiac allograft survival by suppressing the activation of T-lymphoblasts and dendritic cells\textsuperscript{14}. Furthermore, glucosamine has been shown to abrogate the acute phase of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis by blockade of T helper-1 response (IFN-$\gamma$ and IL-17) and an up-regulation of T helper-2 cytokines (IL-5 and IL-10)\textsuperscript{15}. Above observations indicate that glucosamine functions not only as an immunosuppressant but also as an immunomodulator for treatment of autoimmune disorders.

Furthermore, we preliminarily found that glucosamine improves the fluidity of blood (hemorheology) analyzed by a microchannel array assay\textsuperscript{16}. Thereafter, we examined the effects of glucosamine on the platelet functions in vitro using human peripheral blood platelets. The results indicated that glucosamine can suppress platelet aggregation, release of granule contents, thromboxane A$_2$ production\textsuperscript{17}. Thus, glucosamine could be expected as a novel anti-platelet agent for treatment of thrombotic disorders.

However, the effect of in vivo administration of glucosamine has not yet been clear. Thus, we orally administered glucosamine to guinea pigs and examined its effects on platelet functions. In this review, we present the data that glucosamine suppressed ADP-induced platelet aggregation, secretion of granule contents and thromboxane A$_2$ (TXA$_2$) production, but did not affect the body-weights, platelet counts and bleeding time after the administration, suggesting that glucosamine could be a novel and safe anti-platelet agent\textsuperscript{18}.

**Effect of glucosamine administration on ADP-induced platelet aggregation**

Recently, we have reported that glucosamine can suppress the ADP-induced platelet aggregation, secretion of granule contents (ATP and platelet factor 4) and TXA$_2$: production in vitro using human platelets\textsuperscript{17}. To determine whether glucosamine could modulate platelet functions in vivo, glucosamine solution (an average of 400 mg glucosamine) was orally administered to guinea pigs ad libitum for 22 days. Thereafter, blood was collected, and platelet rich plasma (PRP) was obtained. Aliquots of PRP were stimulated with 2 $\mu$M ADP or 2 $\mu$g/ml collagen, and platelet aggregation was monitored by light transmission with an aggregometer. As shown in Fig.1A and B, ADP-induced aggregation was reduced, when platelets from glucosamine-administered animals were used. In contrast, collagen-induced platelet aggregation was not affected by the glucosamine administration (Fig.1C).

![Fig.1 Effect of oral administration of glucosamine to guinea pigs on ADP-induced platelet aggregation](image-url)
Effect of oral administration of glucosamine to guinea pigs on ADP-induced release of ATP from platelets

PRP from control or glucosamine-administered animals was incubated without (Resting) or with 2 μM ADP (ADP-stimulation) at 37°C for 15 min, and the supernatants were recovered for measuring ATP. Extracellular release of ATP was determined by using an ATP bioluminescent assay. Data represent the mean ± SD of 14 separate experiments in each group. Values were compared with Resting of control animals. Values were also compared between control and glucosamine-administered animals in ADP-stimulation. ***p < 0.001.

Effect of glucosamine administration on ATP release from ADP-stimulated platelets

To examine the effect of glucosamine administration on the extracellular release of granule contents, we measured the amounts of ATP (a content of dense granules) in the supernatants of ADP-stimulated platelets by using an ATP bioluminescent assay. As shown in Fig. 2, in response to ADP, platelets from control animals released approximately 0.8 μM ATP. Of note, ATP release was significantly suppressed in platelets from glucosamine-administered animals, compared with those from control animals.

Effect of glucosamine administration on TXA₂ production from ADP-stimulated platelets

It is known that upon stimulation of platelets, arachidonic acid is liberated from membrane phospholipids by phospholipase A₂, and metabolized to TXA₂. To determine whether glucosamine administration can inhibit TXA₂ production, we measured the levels of TXB₂, a stable metabolite of TXA₂ in the supernatants of ADP-stimulated platelets by using a double antibody sandwich enzyme linked immunosorbent assay (ELISA). As shown in Fig. 3, platelets from control animals produced approximately 13 ng/ml TXB₂ in response to ADP; however, TXB₂ production was significantly suppressed in platelets from glucosamine-administered animals, compared with those from control animals.

Effects on glucosamine administration on body-weights, platelet counts and bleeding time

Furthermore, we evaluated body-weights, platelet counts and bleeding time after glucosamine administration. As shown in Table 1, there were no substantial differences between control and glucosamine-administered animals in body-weights, platelet counts and bleeding time.

In vitro effect of glucosamine on ADP-induced platelet aggregation

Based on the intake amounts of glucosamine, 400 mg glucosamine was administered to each animal by gastric gavage, and
plasma glucosamine levels were measured by a high performance liquid chromatography method using phenylisothiocyanate-derivatized glucosamine\(^{21}\). The glucosamine level was found to reach 1.39 ± 2.56 mM after glucosamine administration, although glucosamine was hardly detected in the plasma from control animals. Thus, we evaluated the effects of 0.01 ~ 1 mM glucosamine on the ADP-induced platelet aggregation \textit{in vitro}. As shown in Fig. 4A and B, 0.1 mM and 1 mM glucosamine significantly inhibited the ADP-induced platelet aggregation. Interestingly, 1 mM glucosamine suppressed both the initial and secondary phases of ADP (2 \(\mu\)M)-induced platelet aggregation by 17 and 28\%, respectively (Fig. 4A). Thus, glucosamine could inhibit both the initial and secondary phases of ADP-induced platelet aggregation; however, the suppressive effect seemed to be more prominent on the secondary phase than the first phase of aggregation. In contrast, collagen-induced platelet aggregation was not affected by glucosamine \textit{in vitro}, as observed with platelets from control and glucosamine-administered animals (data not shown).

Effects of glucosamine on ADP-binding to the receptors

ADP activates platelets via the binding of ADP to its receptors\(^{22}\). To determine whether the inhibitory action of glucosamine is mediated by the suppression of ADP-binding to the receptors, we analyzed the ADP-binding using \(^{3}H\)-labeled ADP as a ligand. As shown in Fig. 5A, glucosamine modestly inhibited the binding of ADP to its receptors. To further clarify whether glucosamine affects the binding of ADP to high and/or low affinity binding sites on platelets\(^{23}\), we performed Scatchard analysis. As shown in Fig. 5B, 1 mM glucosamine reduced the maximum number of both high and low affinity binding sites without significant effect on the apparent dissociation constants (Kd) for ADP, suggesting that glucosamine suppresses the ADP-binding to the receptor in a noncompetitive manner, in which an inhibitor possibly binds to either the receptor or the agonist-receptor complex\(^{24}\). Moreover, we previously demonstrated that glucosamine is unable to bind with a ligand (ADP) itself to form a glucosamine-ADP complex, using Sephadex G-10 gel filtration chromatography\(^{27}\).

Perspective

Glycosaminoglycans are large complexes of negatively-charged carbohydrate chains that are present in mucous secretions, connective tissue, skin, tendons, ligaments and cartilage. Glucosamine, an amino monosaccharide is an essential compo-
ment of glycosaminoglycans. Because of its high concentration in joint cartilage, it was hypothesized that glucosamine supplements would provide symptomatic relief for osteoarthritis, and many clinical trials have shown the significant symptom-modifying effects of glucosamine for osteoarthritis. As mechanisms for the chondroprotective action, glucosamine is assumed to increase proteoglycan synthesis, and to inhibit the degradation of proteoglycan, thereby exhibiting the therapeutic efficacy in arthritis. Moreover, it has been suggested that glucosamine may have an anti-inflammatory action by suppressing the neutrophil function such as superoxide anion generation, phagocytosis, granule enzyme release and chemotaxis.

In addition to its chondroprotective and anti-inflammatory actions, glucosamine is also reported to exert the inhibitory actions on human platelet functions in vitro using human platelets. Recently, we have evaluated the effects of oral glucosamine administration on platelet functions in guinea pigs. Consistent with our in vitro study using human platelets, the suppressive actions of glucosamine were observed using platelets from glucosamine-administered animals; ADP- but not collagen-induced aggregation, ATP release and TXA2 production were inhibited in platelets from glucosamine-administered animals, compared with those from control animals. Interestingly, ADP-induced platelet aggregation was decreased after oral administration of glucosamine, and the serum levels of glucosamine reached 1.39 ± 2.56 mM after glucosamine administration, as measured by a high performance liquid chromatography method. Supporting this, glucosamine similarly inhibited platelet aggregation in vitro at 0.1–1 mM. Of note, glucosamine modestly inhibited the binding of ADP to its receptors on platelets (Fig. 5A). Moreover, Scatchard analysis revealed that glucosamine reduced the maximum number of both high and low affinity binding sites for ADP without significant effect on the apparent dissociation constants (Kd) for ADP; however, the suppressive effect was more intense on the low affinity binding sites than the high affinity binding sites. A high affinity ADP-binding site (P2Y1) induces an initial phase of small and rapid reversible platelet aggregation, while a low affinity ADP-binding site (P2Y12) participates in a full and stabilized secondary aggregation in response to ADP. Thus, it is tempting to speculate that glucosamine administration inhibits the low-affinity binding site-mediated secondary phase of aggregation more strongly than the high-affinity binding site-mediated initial phase aggregation in vivo by possibly suppressing the ADP binding to the receptors.

Platelets have a critical role in normal hemostasis, whereas they also contribute to thrombotic disorders such as myocardial infarction and peripheral vascular diseases. In recent years, a number of anti-platelet drugs such as aspirin, ADP receptor inhibitors (clopidogrel and ticlopidine), and glycoprotein IIb/IIIa...
receptor antagonists (e.g., abciximab) have been clinically used to treat or prevent the thrombotic disorders. However, most of these agents have a significantly higher rate of complication with bleeding upon treatment of thrombotic disorders. In addition, aspirin causes gastrointestinal damage, and ticlopidine and abciximab show adverse effects such as neutropenia and thrombocytopenia, respectively. The present study clearly indicated that there were no substantial differences between control and glucosamine-administered animals in body weight changes, platelet counts and bleeding time. Furthermore, long-term clinical trials with oral administration of glucosamine for 3 years to humans indicated that no apparent side effects were recorded and routine laboratory tests did not show any abnormalities during treatment of osteoarthritis. Together these observations suggest that glucosamine could be expected as a novel and safe anti-platelet agent.

In conclusion, we revealed that in vivo administration of glucosamine is able to moderately suppress platelet functions (aggregation, release of granule content and TXA2 production) possibly via the inhibition of ADP-induced activation (Fig.6).

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Fig.6 Schematic representation of the effect of glucosamine on platelet functions

Left half: When activated by ADP and injured endothelium, platelets extracellularly release granule contents (ADP, ATP) and produce TXA2, which subsequently activates platelets to induce aggregation.

Right half: Glucosamine can suppress (↓) release of granule contents, TXA2 production and platelet aggregation via the inhibition of ADP binding to its receptors.

References


8) Towheed TE, Anastassiades TP: Glucosamine therapy for


23) Jefferson JR, Harmon JT, Jamieson GA: Identification of high-affinity (Ka 0.35 μmol/L) and low-affinity (Ka 7.9 μmol/L) platelet binding sites for ADP and competition by ADP analogues. Blood, 71: 110-116, 1988.


