Mini Review

Inhibitory effect of oral glucosamine administration on platelet activation in guinea pigs

Jian Hua[1)], Shiori Suguro[1)], Koji Sakamoto[2)] and Isao Nagaoka[1,*)]

[1)]Department of Host Defense and Biochemical Research, Juntendo University, School of Medicine, Tokyo, Japan

[2)]Koyo Chemical Co., Ltd., Tokyo, Japan

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 A_2. In contrast, glucosamine did not affect the bodyweights, 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 A_2 production. Thus, glucosamine could be expected as a novel and safe anti-platelet agent.

Rec.5/11/2006, Acc.7/25/2006, pp446-452

*Correspondence should be addressed to:
Isao Nagaoka, Department of Host Defense and Biochemical Research, Juntendo University, School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Phone: +81-3-5802-1032, Fax: +81-3-3813-3157, e-mail: nagaokai@med.juntendo.ac.jp

Key words glucosamine, platelet, aggregation, ADP, guinea pig

Introduction

Glucosamine, a naturally occurring amino monosaccharide, has been used for a treatment of osteoarthritis for more than two decades in Europe[1-4)]. Several short-time and long-time of clinical trials in osteoarthritis have shown the significant symptom-modifying effect of glucosamine with no side effects[5-7)]. Glucosamine, as a constituent of glycosaminoglycans, can increase proteoglycan synthesis, thereby exhibiting a therapeutic potential for arthritis[8)]. Recently, glucosamine has been shown to inhibit the expression of inducible nitric oxide (NO) synthase in rats, thereby suppressing the excess production of NO that is implicated in the pathogenesis of arthritis[9)]. In addition, glucosamine has been shown to act on chondrocytes to interfere with the IL-1β-mediated cellular responses[10)], such as production of NO and prostaglandin E_2, and suppression of galactose-β-1,3-glucuronosyltransferase, a key enzyme for glycosaminogly-

can synthesis. Moreover, glucosamine has been suggested to exhibit protective actions on carrageenin-induced inflammation and inflammatory bowel disorders[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[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[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-γ and IL-17) and an up-regulation of T helper-2 cytokines (IL-5 and IL-10)[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[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[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[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[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 μM ADP or 2 μg/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).

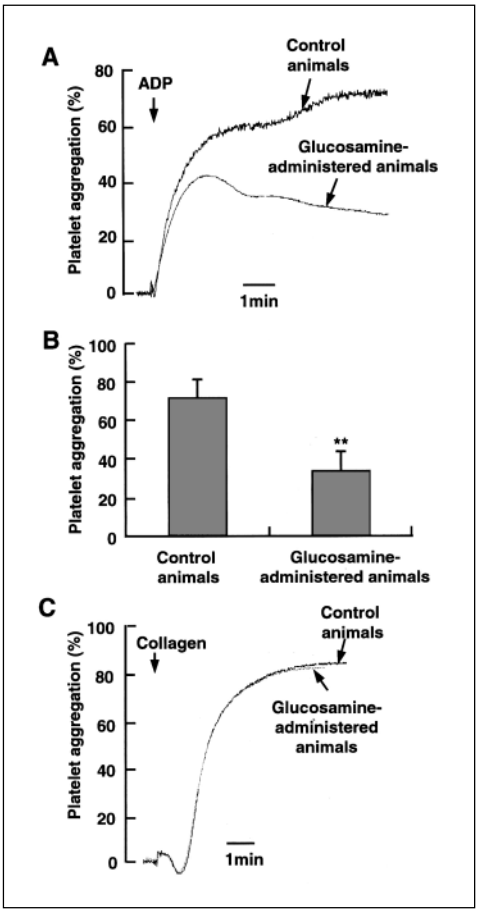

Fig.1 Effect of oral administration of glucosamine to guinea pigs on ADP-induced platelet aggregation

(A) PRP from control or glucosamine-administered animals was preincubated for 1 min, and then stimulated with 2 μM ADP at 37℃ for 15 min for monitoring platelet aggregation with an aggregometer.

(B) The extent of aggregation was estimated quantitatively by measuring the maximum curve height above the baseline level. Data represent the mean ± SD of 14 separate experiments in each group. $^{**}p < 0.01$.

(C) PRP from control or glucosamine-administered animals was preincubated for 1 min, and then stimulated with 2 μg/ml collagen for monitoring platelet aggregation.

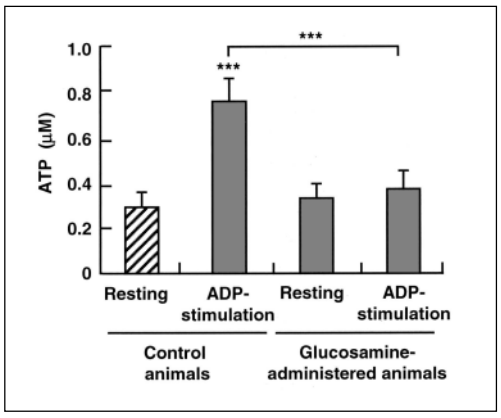

Fig.2 Effect of oral administration of glucosamine to guinea pigs on ADP-induced release of ATP from platelets

PRP from control or glucosamine-administered animals was incubated without (Resting) or with 2 μM ADP (ADP-stimulation) at 37℃ 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. ***$p < 0.001$.

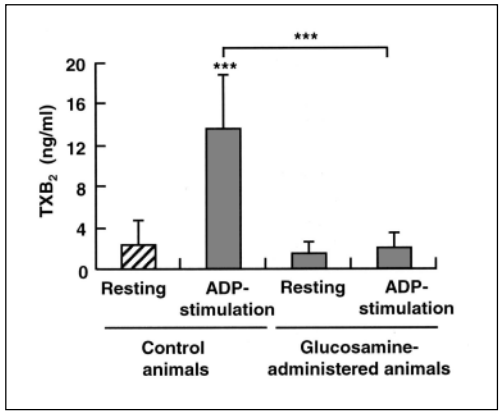

Fig.3 Effect of oral administration of glucosamine to guinea pigs on TXB_2 production by platelets

PRP from control or glucosamine-administered animals was incubated without (Resting) or with 2 μM ADP (ADP-stimulation) at 37℃ for 15 min, and the supernatants were recovered for measuring TXB_2, a stable metabolite of TXA_2 by a TXB_2 ELISA kit. 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[19]. 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 TXA_2 production from ADP-stimulated platelets

It is known that upon stimulation of platelets, arachidonic acid is liberated from membrane phospholipids by phospholipase A_2, and metabolized to TXA_2[20]. To determine whether glucosamine administration can inhibit TXA_2 production, we measured the levels of TXB_2, a stable metabolite of TXA_2 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 TXB_2 in response to ADP; however, TXB_2 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

Table 1 Effects of glucosamine-administration on body-weights, platelet counts and bleeding

	Control animals	Glucosamine-administered animals
Body-weights (g)	369.14 ± 25.50	361.57 ± 32.80
Platelet counts (× 10^3 cells/μl)	556 ± 182	653 ± 80
Bleeding time (s)	289 ± 19	279 ± 37

These parameters were measured on day 22 after glucosamine-administration, and body weights of guinea pigs were 250 ± 31 g on 0 day.

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 *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 μM)-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 *in vitro*, as observed with platelets from control and glucosamine-administered animals (data not shown).

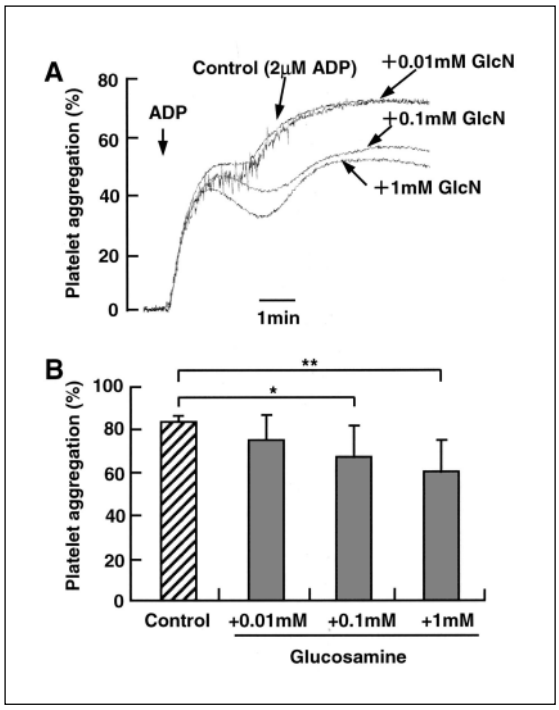

Fig.4 *In vitro* effect of glucosamine on ADP-induced platelet aggregation

(A) PRP from control animals was preincubated in the absence (Control) or presence of 0.01 ～ 1mM glucosamine (+GlcN) for 10 min, and then stimulated with 2 μM ADP for evaluating the effect of glucosamine on platelet aggregation with an aggregometer.

(B) The extent of aggregation was estimated quantitatively by measuring the maximum curve height above the baseline level. Date represent the mean ± SD of six separate experiments. Values were compared with Control (without addition of glucosamine). $*p < 0.05$, $**p < 0.01$.

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[17].

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-

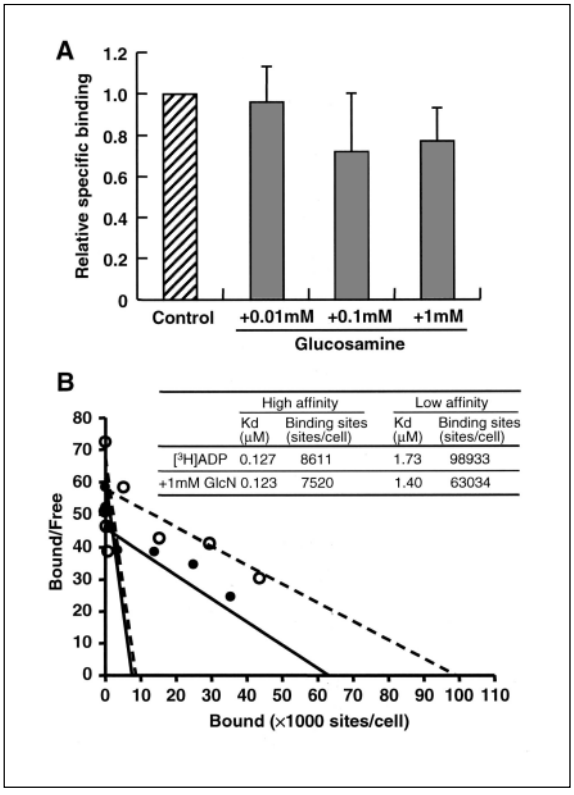

Fig.5 Effect of glucosamine on the binding of [^{3}H] ADP to platelets

(A) Paraformaldehyde-fixed platelets were incubated with 10^{-8} M of [^{3}H] ADP in the absence or presence of 0.01～1 mM glucosamine for 30 min. After washing over a glass-fiber filter, the radioactivities of platelets were measured by scintillation counting, and specific binding was determined. The binding of [^{3}H] ADP was expressed as a percentage of that obtained with platelets (Control) incubated in the absence of glucosamine. Data represent the mean ± SD of three separate experiments.

(B) For Scatchard analysis of [^{3}H] ADP-binding to platelets, paraformaldehyde-fixed platelets were incubated with various concentrations (10^{-9} ～ 10^{-6} M) of [^{3}H] ADP in the absence (○) or presence (●) of 1 mM glucosamine (GlcN). Binding parameters for binding sites and dissociation constants (Kd) were calculated by non-linear regression (Prism) using a two-site binding equation. Broken lines and solid lines were predicted by binding sites and Kd values in the absence and presence of glucosamine, respectively. Inserted table shows the results from Scatchard analysis.

nent 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[1-7,25]. As mechanisms for the chondroprotective action, glucosamine is assumed to increase proteoglycan synthesis, and to inhibit the degradation of proteoglycan[26], thereby exhibiting the therapeutic efficacy in arthritis[8]. 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[13].

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[17]. 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[17], the suppressive actions of glucosamine were observed using platelets from glucosamine-administered animals; ADP- but not collagen-induced aggregation, ATP release and TXA_2 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[21]. 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 (K_d) 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[22,23,27-29]. 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[30]. In recent years, a number of anti-platelet drugs such as aspirin, ADP receptor inhibitors (clopidogrel and ticlopidine), and glycoprotein IIb/IIIa

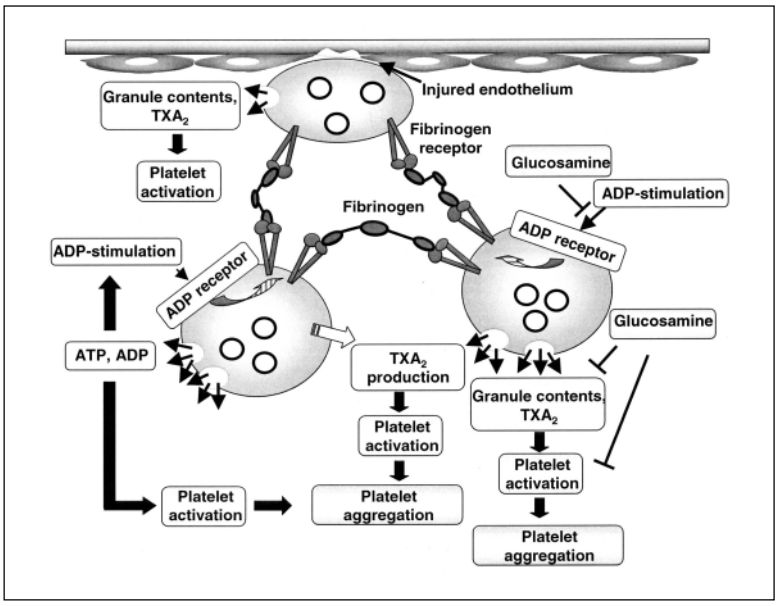

Fig.6 Schematic representation of the effect of glucosamine on platelet functions

Left half: When activated by ADP and injured endothelium, platelets extracellularly release granule contents (ADP, ATP) and produce TXA_2, which subsequently activates platelets to induce aggregation.

Right half: glucosamine can suppress (⊥) release of granule contents, TXA_2 production and platelet aggregation via the inhibition of ADP binding to its receptors.

receptor antagonists (e.g., abciximab) have been clinically used to treat or prevent the thrombotic disorders[27,30-33]. However, most of these agents have a significantly higher rate of complication with bleeding upon treatment of thrombotic disorders[27,30-34]. In addition, aspirin causes gastrointestinal damage[35], and ticlopidine and abciximab show adverse effects such as neutropenia and thrombocytopenia, respectively[27,33]. 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[7]. 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 TXA_2 production) possibly via the inhibition of ADP-induced activation (Fig.6).

Acknowledgments

This work was supported in part by grants from The Institute for Environmental and Gender-specific Medicine, Juntendo University School of Medicine, and the Foundation for Presymptomatic Medicine and Anti-aging, Institute of Gerontology, Hakujikai, Japan.

References

1) Crolle G, D'Este E: Glucosamine sulphate for the management of arthrosis: a controlled clinical investigation. Curr Med Res Opin, 7: 104-109, 1980.
2) Drovanti A, Bignamini AA, Rovati AL: Therapeutic activity of oral glucosamine sulfate in osteoarthrosis: a placebo-controlled double-blind investigation. Clin Ther, 3: 260-272, 1980.
3) Tapadinhas MJ, Rivera IC, Bignamini AA: Oral glucosamine sulphate in the management of arthrosis: report on a multi-centre open investigation in Portugal. Pharmatherapeutica, 3: 157-168, 1982.
4) Lopes Vaz A: Double-blind clinical evaluation of the relative efficacy of ibuprofen and glucosamine sulphate in the management of osteoarthrosis of the knee in out-patients. Curr Med Res Opin, 8: 145-149, 1982.
5) Houpt JB, McMillan R, Wein C, Paget-Dellio SD: Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. J Rheumatol, 26: 2423-2430, 1999.
6) Noack W, Fischer M, Forster KK, Rovati LC, Setnikar I: Glucosamine sulfate in osteoarthritis of the knee. Osteoarthritis Cartilage, 2: 51-59, 1994.
7) Reginster JY, Deroisy R, Rovati LC, Lee RL, Lejeune E, Bruyere O, Giacovelli G, Henrotin Y, Dacre JE, Gossett C: Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. Lancet, 357: 251-256, 2001.
8) Towheed TE, Anastassiades TP: Glucosamine therapy for

osteoarthritis. J Rheumatol, 26: 2294-2297, 1999.
9) Meininger CJ, Kelly KA, Li H, Haynes TE, Wu G: Glucosamine inhibits inducible nitric oxide synthesis. Biochem Biophys Res Commun, 279: 234-239, 2000.
10) Gouze JN, Bordji K, Gulberti S, Terlain B, Netter P, Magdalou J, Fournel-Gigleux S, Ouzzine M: Interleukin-1β down-regulates the expression of glucuronosyltransferase I, a key enzyme priming glycosaminoglycan biosynthesis: influence of glucosamine on interleukin-1β-mediated effects in rat chondrocytes. Arthritis Rheum, 44: 351-360, 2001.
11) Setnikar I, Pacini MA, Revel L: Antiarthritic effects of glucosamine sulfate studied in animal models. Arzneimittelforschung, 41: 542-545, 1991.
12) McCarty MF: Vascular heparan sulfates may limit the ability of leukocytes to penetrate the endothelial barrier--implications for use of glucosamine in inflammatory disorders. Med Hypotheses, 51: 11-15, 1998.
13) Hua J, Sakamoto K, Nagaoka I: Inhibitory actions of glucosamine, a therapeutic agent for osteoarthritis, on the functions of neutrophils. J Leukoc Biol, 71: 632-640, 2002.
14) Ma L, Rudert WA, Harnaha J, Wright M, Machen J, Lakomy R, Qian S, Lu L, Robbins PD, Trucco M, Giannoukakis N: Immunosuppressive effects of glucosamine. J Biol Chem, 277: 39343-39343, 2002.
15) Zhang GX, Yu S, Gran B, Rostami A: Glucosamine abrogates the acute phase of experimental autoimmune encephalomyelitis by induction of Th2 response. J Immunol, 175: 7202-7208, 2005.
16) Kikuchi Y, Sato K, Ohki H, Kaneko T: Optically accessible microchannels formed in a single-crystal silicon substrate for studies of blood rheology. Microvasc Res, 44: 226-240, 1992.
17) Hua J, Suguro S, Iwabuchi K, Tsutsumi-Ishii Y, Sakamoto K, Nagaoka I: Glucosamine, a naturally occurring amino monosaccharide, suppresses the ADP-mediated platelet activation in humans. Inflamm Res, 53: 680-688, 2004.
18) Lu-Suguro JF, Hua J, Sakamoto K, Nagaoka I: Inhibitory action of glucosamine on platelet activation in guinea pigs. Inflamm Res, 54: 493-499, 2005.
19) Crouch SP, Kozlowski R, Slater KJ, Fletcher J: The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. J Immunol Methods, 160: 81-88, 1993.
20) Bauch HJ, Ilsemann K, Beeck H, Voss B, Balleisen L: A double antibody sandwich microELISA for measuring human platelet factor 4. Thromb Res, 37: 573-582, 1985.
21) Liang Z, Leslie J, Adebowale A, Ashraf M, Eddington ND: Determination of the nutraceutical, glucosamine hydrochloride, in raw materials, dosage forms and plasma using precolumn derivatization with ultraviolet HPLC. J Pharm Biomed Anal, 20: 807-814, 1999.
22) Gachet C: ADP receptors of platelets and their inhibition. Thromb Haemost, 86: 222-232, 2001.
23) Jefferson JR, Harmon JT, Jamieson GA: Identification of high-affinity (Kd 0.35 μmol/L) and low-affinity (Kd 7.9 μmol/L) platelet binding sites for ADP and competition by ADP analogues. Blood, 71: 110-116, 1988.
24) Berg JM, Tymozko JL, Stryer L: Enzymes: Basic concepts and kinetics; Biochemistry, Vol 5. W.H. Freeman and Company, New York, pp189-225, 2002.
25) McAlindon TE, LaValley MP, Gulin JP, Felson DT: Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. JAMA, 283: 1469-1475, 2000.
26) Fenton JI, Chlebek-Brown KA, Peters TL, Caron JP, Orth MW: Glucosamine HCl reduces equine articular cartilage degradation in explant culture. Osteoarthritis Cartilage, 8: 258-265, 2000.
27) Sharis PJ, Cannon CP, Loscalzo J: The antiplatelet effects of ticlopidine and clopidogrel. Ann Intern Med, 129: 394-405, 1998.
28) Savi P, Beauverger P, Labouret C, Delfaud M, Salel V, Kaghad M, Herbert JM: Role of P2Y1 purinoceptor in ADP-induced platelet activation. FEBS Lett, 422: 291-295, 1998.
29) Savi P, Laplace MC, Herbert JM: Evidence for the existence of two different ADP-binding sites on rat platelets. Thromb Res, 76: 157-169, 1994.
30) George JN: Platelets. Lancet, 355: 1531-1539, 2000.
31) Quinn MJ, Fitzgerald DJ: Ticlopidine and clopidogrel. Circulation, 100: 1667-1672, 1999.
32) Kam PC, Nethery CM: The thienopyridine derivatives (platelet adenosine diphosphate receptor antagonists), pharmacology and clinical developments. Anaesthesia, 58: 28-35, 2003.
33) Topol EJ, Byzova TV, Plow EF: Platelet GPIIb-IIIa blockers. Lancet, 353: 227-231, 1999.
34) Fitzmaurice DA, Blann AD, Lip GY: Bleeding risks of antithrombotic therapy. BMJ, 325: 828-831, 2002.
35) Bjorkman DJ: The effect of aspirin and nonsteroidal anti-inflammatory drugs on prostaglandins. Am J Med, 105: 8S-12S, 1998.