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Risk of ischaemic stroke among new users of glucosamine and chondroitin sulphate: a nested case–control study

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Abstract

Background: Several studies have reported that the use of chondroitin sulphate (CS) and glucosamine may reduce the risk of acute myocardial infarction. Although it is thought that this potential benefit could be extended to ischaemic stroke (IS), the evidence is scarce.

Objective: To test the hypothesis that the use of prescription glucosamine or CS reduces the risk of IS.

Design: Case–control study nested in an open cohort.

Methods: Patients aged 40–99 years registered in a Spanish primary healthcare database (BIFAP) during the 2002–2015 study period. From this cohort, we identified incident cases of IS, applying a case-finding algorithm and specific validation procedures, and randomly sampled five controls per case, individually matched with cases by exact age, gender and index date. Adjusted odds ratios (AORs) and 95% confidence interval (CI) were computed through a conditional logistic regression. Only new users of glucosamine or CS were considered.

Results: A total of 13,952 incident cases of IS and 69,199 controls were included. Of them, 106 cases (0.76%) and 803 controls (1.16%) were current users of glucosamine or CS at index date, yielding an AOR of 0.66 (95% CI: 0.54–0.82) (for glucosamine, AOR: 0.55; 95% CI: 0.39–0.77; and for CS, AOR: 0.77; 95% CI: 0.60–0.99). The reduced risk among current users was observed in both sexes (men, AOR: 0.69; 95% CI: 0.49–0.98; women, AOR: 0.65; 95% CI: 0.50–0.85), in individuals above and below 70 years of age (AOR: 0.69; 95% CI: 0.53–0.89 and AOR: 0.59; 95% CI: 0.41–0.85, respectively), in individuals with vascular risk factors (AOR: 0.53; 95% CI: 0.39–0.74) and among current/recent users of nonsteroidal anti-inflammatory drugs (NSAIDs) (AOR: 0.71; 95% CI: 0.55–0.92). Regarding duration, the reduced risk was observed in short-term users (<365 days, AOR: 0.61; 95% CI: 0.48–0.78) while faded and became nonsignificant in long-term users (>364 days AOR: 0.86; 95% CI: 0.57–1.31).

Conclusions: Our results support a protective effect of prescription CS and glucosamine in IS, which was observed even in patients at vascular risk.

Mini abstract

Our aim was to analyse whether the use of glucosamine or chondroitin sulphate (CS) reduces the risk of ischaemic stroke (IS). We detected a significant decrease.

Keywords: chondroitin sulphate, glucosamine, stroke, SYSADOAs

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Introduction

SYSADOAs (symptomatic slow-acting drugs for osteoarthritis) are a heterogeneous group of drugs that have the ability to modify the symptoms of osteoarthritis (OA) slowly and independently of nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics or any other therapeutic option.[1,2] The main drugs included in this group are glucosamine (sulphate or hydrochloride) and chondroitin sulphate (CS), widely prescribed for the treatment of OA in some countries. Glucosamine and CS are chemically different natural compounds [glucosamine is an amino sugar and CS is a glycosaminoglycan (GAG)] involved in the biosynthesis of proteoglycans (PGs).[3,4] Although the efficacy of glucosamine and CS for the treatment of OA remains controversial,[1,3,5] several human, animal and laboratory studies have suggested that both drugs show anti-inflammatory properties[6–10] that could reduce the risk of several diseases.[11–13] In this context, several recent epidemiological investigations indicate that the use of glucosamine and CS could play a role in cardiovascular disease prevention,[14–17] as well as reduction of mortality,[16–18] and prevention of colorectal cancer[16,19–21] and other diseases.[14,16,22,23] In a recent study, our group found a reduced risk of acute myocardial infarction (AMI) associated with CS in both short- and long-term users, in both men and women, in individuals above and below 70 years of age and in patients at intermediate and high vascular risk, while no protection was found in individuals at low vascular risk. By contrast, no such effect was observed with glucosamine.[24]

In the present case–control study nested in a study cohort of nearly 3.8 million adult patients, we examined the association between the use of glucosamine and CS, as prescription medicines and risk of ischaemic stroke (IS) using a Spanish primary healthcare database (BIFAP; Base de datos para la Investigación Farmacoepidemiológica en Atención Primaria). We also analysed the potential effect modification by sex, age, background vascular risk and current/recent use of NSAIDs. Studies to date have included prevalent users which may have overestimated a protection by selection of less susceptible patients. To avoid this bias, we only included patients who initiated treatment with CS or glucosamine (the new-user design).[25]

Patients and methods

Data source

The 2016 version of BIFAP was used as the source of information for the present study. This database contains anonymised electronic medical records on clinical events, prescriptions and laboratory tests, prospectively recorded by primary care physicians (PCPs) as part of their routine clinical practice. BIFAP has been validated through multiple pharmacoepidemiological studies[15,26] providing results comparable with other well-known European databases (see online supplemental file 1 – supplementary methods).[27,28]

Study design and selection of cases and controls

The study follows a nested case–control design. We constructed a study cohort composed by all patients registered in the database during the study period (1 January 2002–31 December 2015), who were aged 40–99 years, had at least 1-year registry with their PCPs, and had no history of cancer or IS. The follow-up started when they fulfilled all these criteria and continued until the occurrence of incident IS diagnosis, cancer diagnosis, death, 100 years of age or the end of the study period, whichever occurred first. All potential IS cases were identified using specific diagnostic codes and free text associated with the diagnosis, from which a sample of potential cases were selected for manual review by two of the investigators to check whether they were valid cases of IS (with drug exposure information withheld) (see online supplemental file 1 – supplementary methods). In addition, a second validation was carried out to distinguish the most probable pathogenic subtype: cardioembolic and non-cardioembolic (including atherothrombotic, small cerebral vessel disease and unspecified/undetermined ischemic stroke), as described in online supplemental file 1 – supplementary methods.

Finally, we selected five controls per each stroke case following a risk set sampling (density-based): first, we identified all patients in the underlying cohort who, at the index date of cases, were of the same sex and age (exact) and were active in the database (to assure that at index date they were at risk) and, second, from this risk set, we randomly sampled five subjects.

Exposure definition

Drugs of interest were SYSADOAs, including glucosamine (sulphate or hydrochloride) and CS, available in Spain as prescription medicines. Although hyaluronic acid and diacerein are usually considered among SYSADOAs, they are different types of drugs and were not considered in this study. Nutraceuticals containing SYSADOAs were not considered either. Exposure was characterised as (a) 'current users' of SYSADOAs when the last prescription ended within 30 days before the index date; (b) 'recent users' when it ended between 31 and 365 days before the index date; (c) 'past users' when it ended more than 365 days before the index date and (d) 'non-users' when there was no recorded prescription of SYSADOAs before the index date.

Among current users, treatment duration was calculated by summing up consecutive prescriptions (defined as such when the interval between the end of one prescription and the start of the next was no more than 90 days). Duration was categorised as less than 365 days and 365 days or more. Daily dose was only studied for CS, as glucosamine showed no variability.

To comply with a new-user design, we restricted the analysis to SYSADOAs initiating patients. To this end, we excluded patients with recorded prescriptions for SYSADOAs before the start of follow-up.[25]

Potential confounding factors

The selection of potential confounding variables was based on expert knowledge avoiding data-driven methods. The following variables, recorded before the index date, were considered: number of visits to the PCP in the year prior to the index date (as a general indicator of comorbidity), body mass index (BMI), smoking status, alcohol abuse (recorded as such by the PCP), any recorded diagnosis of transient ischaemic attack (TIA), ischaemic heart disease (including history of myocardial infarction, angina pectoris and use of nitrates as an indicator of angina), atrial fibrillation, thromboembolic disease, heart failure, peripheral artery disease (PAD), hypertension, diabetes (recorded as such and/or use of glucose-lowering drugs), dyslipidaemia (recorded as such and/or use of lipid-lowering drugs), chronic obstructive pulmonary disease (COPD), knee or hip OA, rheumatoid arthritis, chronic renal failure, gout and asymptomatic hyperuricaemia. Also, we included the use of the following drugs in the 30 days prior to the index date: antiplatelet drugs, oral anticoagulants, paracetamol, metamizole, NSAIDs, opioids, corticosteroids, calcium supplements (with or without vitamin D), angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, beta-blockers, alpha-blockers, diuretics, proton pump inhibitors and H2-receptor antagonists.

Statistical analysis

Conditional logistic regression models were constructed to assess the association between SYSADOAs use and incident IS. We calculated adjusted odds ratios (AORs) and their corresponding 95% confidence intervals (CIs) including the main exposure plus all potential confounders described above.

Subsequently, the interaction with the following factors was evaluated: sex, age (stratified as below 70 years and above 70 years), concomitant use of NSAIDs and background vascular risk. The latter was categorised as follows: *high risk*, patients with an established vascular disease: history of PAD, ischaemic heart disease, atrial fibrillation, TIA or diabetes; *intermediate risk*, patients with vascular risk factors (hypertension, dyslipidaemia, chronic renal failure, current smoking or obesity, defined as BMI $\geqslant 30\,kg/m^2$), but none of the aforementioned established diseases; and *low risk* for the rest. Diabetes was considered among high-risk factors as it has been reported to be equivalent to ischaemic heart disease.[29] Statistical evaluation of the interaction was performed by running fully adjusted models within the different categories of the potentially interacting variable. The AORs associated with SYSADOAs across different strata of the interacting variable were compared using the interaction test described by Altman and Bland.[30] All results were considered statistically significant when the p value was below 0.05. For the stratified analysis by concomitant use of NSAIDs and background vascular risk, as well as for the analysis restricted to patients with knee/hip OA, we performed an unconditional logistic regression including the matching variables in the model because conditional logistic regression provided unstable estimates.

Missing values for smoking (50.7%) and BMI (35.3%) were included in specific categories after

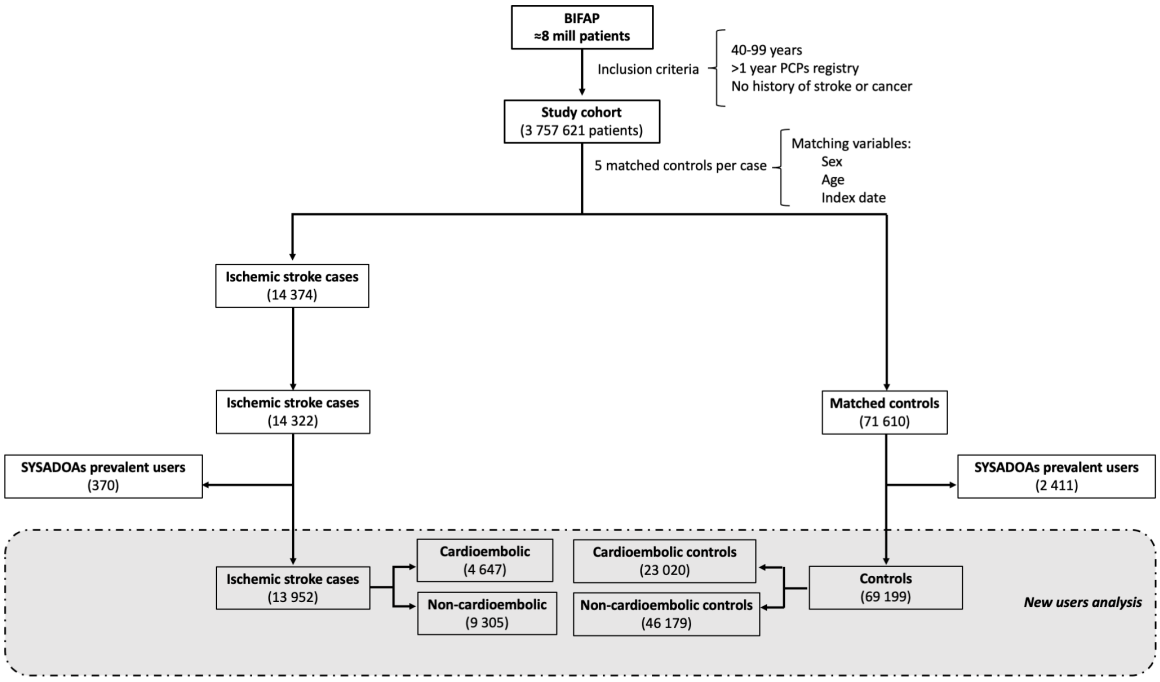

Figure 1. Flow chart of patient selection.
BIFAP, Base de datos para la Investigación Farmacoepidemiológica en Atención Primaria; SYSADOAs, symptomatic slow-acting drugs for osteoarthritis.

checking that the distribution of missing values did not vary across main exposure categories.[31] Notwithstanding, as sensitivity analyses, we also run multiple imputation with chained equations (MICEs) models[32] (see online supplemental file 1 – supplementary methods).

We conducted all analyses using STATA version 15/SE (StataCorp. College Station, Texas. 77845, USA).

Sensitivity analyses

Three sensitivity analyses were performed: (1) including prevalent users of SYSADOAs in the analysis; (2) using MICE models to assess the impact of missing values for BMI and smoking and (3) restricting the analysis to patients with a recorded diagnosis of knee/hip OA.

Ethical aspects

The BIFAP Scientific Committee granted access to fully anonymised electronic medical records (project #04/2016; approval date: 26 May 2016). Afterwards, on 1 July 2020, this committee approved specifically the analysis proposed for this study. In addition, the Research Ethics Committee of the Hospital Fundación Alcorcon (Ref 20/76) approved the study on 4 May 2020.

The present study was carried out following the Strengthening the Reporting of Observational studies in Epidemiology (STROBE)[33] guideline (online supplemental file 1 – supplementary guideline).

Patient and public involvement

We engaged patients through the Osteoarthritis International Foundation (OAFI; a nonprofit association of OA patients) in the development of the research question which was based on the patients' priorities identified by OAFI. Also, they will be involved in the reporting and dissemination of our research. OAFI did not take part in the design and conduct of the study.

Results

A total of 13,952 incident cases of IS and 69,199 matched controls were included (Figure 1). Characteristics are shown in Table 1. The mean age of patients was 74.6 years, and both men and women were equally represented. As expected, the prevalence of vascular risk factors and the use

Table 1. Cases and controls characteristics.

	Cases (%) *N* = 13,952	**Controls (%) *N* = 69,199**	**Unadjusted OR[a] (95% CI)**
Age; mean (*SD*)	74.6 (12.4)	74.6 (12.4)	–
Men	7059 (50.59)	35,083 (50.70)	–
Visits (last 12 months)			
Up to 5	2457 (17.61)	19,502 (28.18)	1 (ref.)
6–15	5153 (36.93)	25,991 (37.56)	1.67 (1.58–1.76)
16–24	3230 (23.15)	12,750 (18.43)	2.26 (2.13–2.40)
25+	3112 (22.31)	10,956 (15.83)	2.66 (2.50–2.83)
BMI, kg/m^2			
Up to 24.9	1989 (14.26)	9566 (13.82)	1 (ref.)
25–29	4125 (29.57)	20,164 (29.14)	0.98 (0.93–1.04)
30–34	2473 (17.73)	11,336 (16.38)	1.05 (0.98–1.12)
35–39	718 (5.15)	2960 (4.28)	1.18 (1.07–1.29)
40+	230 (1.65)	763 (1.10)	1.45 (1.24–1.69)
Unknown	4417 (31.66)	24,410 (35.28)	0.87 (0.82–0.92)
Smoking			
Never smoking	4599 (32.96)	22,650 (32.73)	1 (ref.)
Current smoker	2219 (15.90)	7777 (11.24)	1.49 (1.40–1.58)
Past smoker	942 (6.75)	3683 (5.32)	1.32 (1.22–1.43)
Unknown	6192 (44.38)	35,089 (50.71)	0.88 (0.84–0.91)
Alcohol abuse	408 (2.92)	1074 (1.55)	1.93 (1.72–2.17)
TIA	764 (5.48)	1591 (2.30)	2.50 (2.29–2.74)
Ischaemic heart disease			
Acute myocardial infarction	850 (6.09)	2437 (3.52)	1.86 (1.72–2.02)
Angina pectoris[b]	1251 (8.97)	4373 (6.32)	1.52 (1.43–1.63)
Thromboembolic disease	313 (2.24)	1155 (1.67)	1.35 (1.19–1.54)
Heart failure	1052 (7.54)	3177 (4.59)	1.74 (1.61–1.87)
Atrial fibrillation	2064 (14.79)	5271 (7.62)	2.18 (2.06–2.30)
PAD	692 (4.96)	1702 (2.46)	2.10 (1.91–2.30)
Hypertension	8734 (62.60)	36,767 (53.13)	1.55 (1.49–1.61)

(Continued)

Table 1. (Continued)

	Cases (%) *N* = 13,952	Controls (%) *N* = 69,199	Unadjusted OR[a] (95% CI)
Diabetes[c]	3999 (28.66)	13,163 (19.02)	1.73 (1.65–1.80)
Dyslipidaemia[d]	6151 (44.09)	27,157 (39.24)	1.23 (1.19–1.28)
COPD	1202 (8.62)	5064 (7.32)	1.21 (1.13–1.29)
Knee/hip osteoarthritis	1684 (12.07)	7835 (11.32)	1.08 (1.02–1.14)
Rheumatoid arthritis	120 (0.86)	606 (0.88)	0.99 (0.81–1.20)
Hyperuricaemia			
Asymptomatic	1089 (7.81)	4905 (7.09)	1.13 (1.06–1.21)
Gout	692 (4.96)	2665 (3.85)	1.32 (1.21–1.44)
Chronic renal failure	796 (5.71)	2592 (3.75)	1.57 (1.44–1.70)
Current use of			
Antiplatelet drugs	3733 (26.76)	11,360 (16.42)	2.14 (2.04–2.24)
Oral anticoagulants	1097 (7.86)	3835 (5.54)	1.53 (1.43–1.64)
Paracetamol	2378 (27.52)	11,714 (16.93)	1.15 (1.08–1.22)
Metamizole	693 (4.97)	2749 (3.97)	1.37 (1.26–1.50)
NSAIDs	1275 (9.14)	6728 (9.72)	1.00 (0.94–1.07)
Opioids	740 (5.30)	3061 (4.42)	1.28 (1.17–1.39)
Corticosteroids	327 (2.34)	1253 (1.81)	1.32 (1.17–1.49)
Calcium/vitamin D supplements	771 (5.53)	3819 (5.52)	1.02 (0.93–1.10)
ACEIs	2952 (21.16)	12,111 (17.50)	1.39 (1.33–1.46)
ARBs	2474 (17.13)	10,613 (15.34)	1.26 (1.20–1.33)
CCBs	2169 (15.55)	8566 (12.38)	1.41 (1.34–1.49)
Beta-blockers	2050 (14.69)	6124 (8.85)	1.87 (1.77–1.98)
Alpha-blockers	353 (2.53)	1497 (2.16)	1.19 (1.06–1.34)
Diuretics	2551 (18.28)	9437 (13.64)	1.58 (1.50–1.66)
PPIs	4592 (32.91)	18,848 (27.24)	1.43 (1.37–1.50)
H_2 receptor blockers	323 (2.32)	1174 (1.70)	1.39 (1.23–1.58)

ACEIs, angiotensin-converting enzyme inhibitors; AMI, acute myocardial infarction; ARBs, angiotensin II receptor blockers; BMI, body mass index; CCBs, calcium channel blockers; CI, confidence interval; COPD, chronic obstructive pulmonary disease; NSAIDs, nonsteroidal anti-inflammatory drugs; OR, odds ratio; PAD, peripheral artery disease; PPIs, proton pump inhibitors; SD, standard deviation, TIA, transient ischaemic attack.
[a]Adjusted only for matching factors (age, sex and calendar year).
[b]Recorded as such, and/or when patients were using nitrates.
[c]Recorded as such, and/or when patients were using glucose-lowering drugs.
[d]Recorded as such, and/or when patients were using lipid-lowering drugs.

of cardiovascular drugs were higher for cases when compared with controls.

SYSADOAs (glucosamine and CS) use and IS risk

In total, 106 cases (0.76%) and 803 controls (1.16%) were current users of SYSADOAs, leading to an unadjusted OR of 0.65 (95% CI: 0.53–0.79), which hardly changed after full adjustment: AOR: 0.66 (95% CI: 0.54–0.82). Such decreased risk disappeared upon discontinuation (partially in recent users and completely in past users) (Table 2). When glucosamine and CS were analysed separately, we observed that the association with a reduced risk was driven by glucosamine (AOR: 0.55; 95% CI: 0.39–0.77), while a lower but still statistically significant risk reduction was observed with CS (AOR: 0.77; 95% CI: 0.60–0.99) (Table 2). Due to the very small number of patients currently using the combination of CS and glucosamine (seven cases and 45 controls), the result associated with such combination was too imprecise (AOR: 0.86; 95% CI: 0.38–1.95). By pathogenic subtype of IS, we did not find a difference: cardioembolic IS, AOR: 0.61 (95% CI: 0.41–0.92); non-cardioembolic IS, AOR: 0.69 (95% CI: 0.53–0.89).

SYSADOAs (glucosamine and CS) use and IS risk: duration of treatment and daily dose

Current use of SYSADOAs in accordance with treatment duration (less than 365 days, 365 days or more) was lower among cases (0.57% and 0.19%, respectively) than among controls (0.92% and 0.24%, respectively), leading to unadjusted ORs of 0.61 (95% CI: 0.48–0.77) and 0.80 (95% CI: 0.53–1.21), respectively, as compared with non-users. After full adjustment, results were barely modified: AOR: 0.61 (95% CI: 0.48–0.78) and AOR: 0.86 (95% CI: 0.57–1.31), respectively. Table 3 shows the results by pharmacological group and by each individual drug. Regarding dose, a significant decreased risk was only observed with CS daily doses of 800 mg or higher (AOR: 0.70; 95% CI: 0.52–0.95), though few patients used the lower dose (online supplemental file 1 – supplementary Table 2).

SYSADOA (glucosamine and CS) use and IS risk in different subgroups

No evidence of statistical interaction with age and sex was found (Figure 2).

As for the background vascular risk, a significant reduced risk of IS associated with SYSADOAs was observed in the group of patients with vascular risk factors (intermediate-risk group; AOR: 0.53; 95% CI: 0.39–0.74), while in the low- and high-risk groups, a reduced risk was suggested, but no statistical significance was reached (AOR: 0.77; 95% CI: 0.42–1.41 and AOR: 0.90; 95% CI: 0.65–1.22, respectively). Interestingly, the reduced risk of IS associated with SYSADOAs was observed in the stratum of current/recent users of NSAIDs (AOR: 0.71; 95% CI: 0.55–0.92), as well as in the stratum of non-users/past users of NSAIDs (AOR: 0.64; 95% CI: 0.45–0.93) (see online supplemental file 1 – supplementary Table 3 for more details and results by each individual drug).

Sensitivity analyses

(1) The analysis including prevalent users of SYSADOAs yielded the same results but with greater precision (AOR: 0.66; 95% CI: 0.56–0.78); in this analysis, there was no difference between glucosamine (AOR: 0.68; 95% CI: 0.54–0.86) and CS (AOR: 0.68; 95% CI: 0.55–0.84) (Table 4); (2) the use of MICE models to address missing values for BMI and smoking did not materially change the results of the main analysis (AOR for SYSADOAs: 0.66; 95% CI: 0.54–0.81) (online supplemental file 1 – supplementary Table 4) and (3) the analysis carried out in patients with knee/hip OA yielded a similar result for SYSADOA (AOR: 0.64; 95% CI: 0.45–0.92) and for glucosamine (AOR: 0.40; 95% CI: 0.21–0.74), albeit a non-significant AOR was obtained for CS (AOR: 0.89; 95% CI: 0.60–1.34) (Table 5) (the main characteristics of cases and controls with knee/hip OA are shown in online supplemental file 1 – supplementary Table 5).

Discussion

In this population-based case–control study, nested in a cohort of 3,757,621 adult patients, from which around 14,000 incident IS cases emerged, we obtained the following main findings: (1) current use of SYSADOAs as prescription medicines was associated with a reduced risk of IS after full adjustment for potential confounding factors; (2) the decreased risk is observed for both cardioembolic and non-cardioembolic IS; (3) it also was observed in both sexes and in subjects above and below 70 years of age; (4) likewise,

Table 2. Risk of ischaemic stroke associated with the use of SYSADOAs (chondroitin sulphate and glucosamine).

	Cases (%) *N* = 13,952	Controls (%) *N* = 69,199	Unadjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)
SYSADOAs (all)				
Non-users	13,271 (95.12)	65,569 (94.75)	1 (ref.)	1 (ref.)
Current	106 (0.76)	803 (1.16)	0.65 (0.53–0.79)	0.66 (0.54–0.82)
Recent	155 (1.11)	878 (1.27)	0.88 (0.74–1.04)	0.85 (0.71–1.01)
Past	420 (3.01)	1949 (2.82)	1.06 (0.96–1.19)	0.99 (0.89–1.11)
Glucosamine				
Non-users	13,557 (97.17)	67,157 (97.05)	1 (ref.)	1 (ref.)
Current	38 (0.27)	362 (0.52)	0.52 (0.37–0.72)	0.55 (0.39–0.77)
Recent	80 (0.57)	435 (0.63)	0.91 (0.72–1.16)	0.87 (0.67–1.11)
Past	277 (1.99)	1245 (1.80)	1.10 (0.96–1.25)	1.05 (0.91–1.21)
Chondroitin sulphate				
Non-users	13,580 (97.33)	67,143 (97.03)	1 (ref.)	1 (ref.)
Current	75 (0.54)	487 (0.70)	0.76 (0.59–0.97)	0.77 (0.60–0.99)
Recent	89 (0.64)	527 (0.76)	0.85 (0.68–1.07)	0.82 (0.65–1.04)
Past	208 (1.49)	1042 (1.51)	0.99 (0.85–1.15)	0.90 (0.77–1.05)
Chondroitin sulphate + glucosamine				
Non-users	13,866 (99.38)	68,731 (99.32)	1 (ref.)	1 (ref.)
Current	7 (0.05)	45 (0.07)	0.78 (0.35–1.74)	0.86 (0.38–1.95)
Recent	28 (0.20)	162 (0.23)	0.87 (0.58–1.31)	0.81 (0.53–1.23)
Past	51 (0.37)	261 (0.38)	0.95 (0.70–1.28)	0.88 (0.64–1.20)

CI, confidence interval; OR, odds ratio.
[a]Adjusted only for matching factors (age, sex and calendar year).
[b]Adjusted for matching factors (age, sex and calendar year) plus the history of transient ischaemic attack, ischaemic heart disease (acute myocardial infarction or angina pectoris–recorded as such, and/or when patients were using nitrates), thromboembolic disease, heart failure, atrial fibrillation, peripheral artery disease, hypertension, diabetes (recorded as such, and/or use of glucose-lowering medications), dyslipidaemia (recorded as such, and/or use of lipid-lowering medications), chronic obstructive pulmonary disease, knee/hip osteoarthritis, rheumatoid arthritis, asymptomatic hyperuricaemia, gout, chronic renal failure, the number of visits to the primary care practitioner in the year prior to the index date (as an indicator of comorbidities), body mass index, smoking, alcohol abuse, and use of the following drugs: antiplatelet drugs, oral anticoagulants, paracetamol, metamizole, non-steroidal anti-inflammatory drugs, opioids, corticosteroids, calcium supplements, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, beta-blockers, alpha-blockers, diuretics, proton pump inhibitors and H_2-receptor antagonists.

no interaction was observed with the background vascular risk, though the reduced risk only reached statistical significance in the group with vascular risk factors but no established vascular disease; and (5) it was equally observed in the subgroup of current/recent users of NSAIDs and in the subgroup of past-users/non-users of NSAIDs.

The fact that the reduced risk of IS associated with the current use of SYSADOAs (either

Table 3. Risk of ischaemic stroke associated with the use of SYSADOAs (chondroitin sulphate and glucosamine) according to duration of treatment among current users.

	Cases (%) *N* = 13,952	Controls (%) *N* = 69,199	Unadjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)
SYSADOAs				
Non-users	13,271 (95.12)	65,569 (94.75)	1 (ref.)	1 (ref.)
Current users				
< 365 days	79 (0.57)	638 (0.92)	0.61 (0.48–0.77)	0.61 (0.48–0.78)
365+ days	27 (0.19)	165 (0.24)	0.80 (0.53–1.21)	0.86 (0.57–1.31)
Glucosamine				
Non-users	13,557 (97.17)	67,157 (97.05)	1 (ref.)	1 (ref.)
Current users				
< 365 days	29 (0.21)	291 (0.42)	0.50 (0.34–0.73)	0.52 (0.35–0.77)
365+ days	9 (0.06)	71 (0.10)	0.61 (0.30–1.21)	0.66 (0.33–1.34)
Chondroitin sulphate				
Non-users	13,580 (97.33)	67,143 (97.03)	1 (ref.)	1 (ref.)
Current users				
< 365 days	52 (0.37)	359 (0.52)	0.71 (0.53–0.95)	0.70 (0.52–0.95)
365+ days	23 (0.16)	128 (0.18)	0.90 (0.58–1.40)	0.97 (0.61–1.53)

CI, confidence interval; OR, odds ratio.
[a]Adjusted only for matching factors (age, sex and calendar year).
[b]Adjusted for matching factors (age, sex and calendar year) plus the history of transient ischaemic attack, ischaemic heart disease (acute myocardial infarction or angina pectoris – recorded as such, and/or when patients were using nitrates), thromboembolic disease, heart failure, atrial fibrillation, peripheral artery disease, hypertension, diabetes (recorded as such, and/or use of glucose-lowering medications), dyslipidaemia (recorded as such, and/or use of lipid-lowering medications), chronic obstructive pulmonary disease, knee/hip osteoarthritis, rheumatoid arthritis, asymptomatic hyperuricaemia, gout, chronic renal failure, number of visits to the primary care practitioner in the year prior to the index date (as an indicator of comorbidities), body mass index, smoking, alcohol abuse, and use of the following drugs: antiplatelet drugs, oral anticoagulants, paracetamol, metamizole, non-steroidal anti-inflammatory drugs, opioids, corticosteroids, calcium supplements, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, beta-blockers, alpha-blockers, diuretics, proton pump inhibitors and H_2-receptor antagonists.

glucosamine or CS) waned after discontinuation (a lesser association with recent users and null among past users) is in favour of a pharmacological effect of these drugs as current and recent/past users are highly comparable populations and such differential effect is unlikely to be attributed to different personal factors.

The potential protective effect of SYSADOA appeared to fade with long-term use (particularly evident for CS), a finding that is against a sustained biological effect of these drugs, though there may be other explanations. For instance, it is important to bear in mind that the number of patients on long-term treatment (365 days or greater) with both CS and glucosamine was rather small (23 and 9 cases, respectively), and, then, chance could have played a role, as shown by the wide CIs we found in long-term users. Also, a lower adherence at long run may account for this finding: the age of patients (mean of 74.6 years), the complex posology recommended for CS (3 months on treatment followed by a 2-month break) and, perhaps, the reduced efficacy of these drugs as OA progresses, are factors that may favour a lack of adherence to treatment at

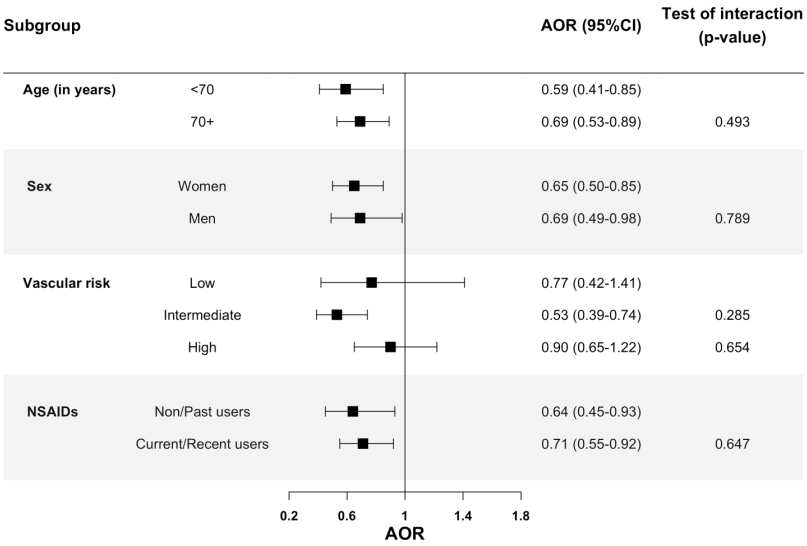

Figure 2. Risk of ischaemic stroke associated with current use of SYSADOAs by sex, age, background vascular risk and concomitant use of NSAIDs.
AOR, adjusted odds ratio; CI, confidence interval; NSAIDs, nonsteroidal anti-inflammatory drugs.

long-term and, consequently, contribute to the observed fading of the protective effect.

We observed a reduced risk of IS in patients with vascular risk factors but not among those with established vascular diseases (including diabetes). This finding is not consistent with the results we found in a previous study on AMI, where we detected the greatest effect of SYSADOAs in the group at the highest vascular risk.[24] We do not have a clear idea on how to explain this finding, but it is important to bear in mind that we are estimating measures of association, constructed in a multiplicative scale, and then highly influenced by the background vascular risk: the same risk reduction in an absolute scale translates into ORs of less magnitude as long as the background risk increases. In support of this explanation is the fact that the patients taking part in this study were on average 7 years older than those included in the AMI study (74.6 *versus* 67.0 years old). In this sense, it is interesting that the reduced risk associated with CS was of lower magnitude among patients with 70 years old or older (AOR: 0.85; 95% CI: 0.62–1.16) than among younger patients (AOR: 0.57; 95%: 0.62–0.95).

Our findings are in line with several previous studies that show inverse associations of SYSADOA use with vascular risk and mortality. In a cross-sectional study of 266,844 Australian participants, glucosamine use was found to be inversely associated with risks of AMI or angina (AOR: 0.79; 95% CI: 0.73–0.86) and other heart diseases (AOR: 0.82; 95% CI: 0.76–0.89).[34] In a prospective cohort study in the UK Biobank, glucosamine use was associated with a significantly lower risk of total cardiovascular events (AOR: 0.85; 95% CI: 0.80–0.90), cardiovascular death (AOR: 0.78; 95% CI: 0.70–0.87), coronary heart disease (AOR: 0.82; 95% CI: 0.76–0.88) and stroke (AOR: 0.91; 95% CI: 0.83–1.00).[14,16] The first evidence of a possible vascular protective effect of CS in humans was raised by Morrison and colleagues[35,36] in the 1970s, in an open-label clinical trial carried out with 120 patients with ischaemic heart disease, assigned in a 1:1 ratio to the experimental (CS) and control group. While 42 patients (70%) in the control group had one cardiac event per month and 14 (23%) died after a 6-year follow-up, only six (10%) patients receiving CS experienced an acute cardiac event, and only four (6.6%) died.[35,36] Nevertheless, no additional clinical trial using current quality standards has been carried out since then. In the Vitamins and Lifestyle (VITAL) cohort study, glucosamine and CS use was significantly associated with an 18% and 16%, respectively, lower risk of total mortality.[18,37] Finally, in a recent study of our group, we observed that current use of CS was associated with a reduced risk of AMI.[24]

Several potential mechanisms could explain the observed protective effect of SYSADOAs in vascular diseases. In the National Health and Nutrition Examination Survey (NHANES) study,

Table 4. Risk of ischaemic stroke associated with the use of SYSADOAs (glucosamine/chondroitin sulphate), including prevalent users.

	Cases (%) *N* = 14,322	Controls (%) *N* = 71,610	Unadjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)
SYSADOAs (all)				
Nonusers	13,271 (92.66)	65,569 (91.56)	1 (ref.)	1 (ref.)
Current	172 (1.20)	1347 (1.88)	0.63 (0.53–0.74)	0.66 (0.56–0.78)
Recent	218 (1.52)	1354 (1.89)	0.79 (0.69–0.92)	0.78 (0.68–0.91)
Past	661 (4.62)	3340 (4.66)	0.97 (0.89–1.06)	0.92 (0.84–1.00)
Glucosamine				
Non-users	13,641 (95.25)	67,843 (94.74)	1 (ref.)	1 (ref.)
Current	83 (0.58)	660 (0.92)	0.62 (0.50–0.79)	0.68 (0.54–0.86)
Recent	118 (0.82)	720 (1.01)	0.81 (0.67–0.99)	0.81 (0.66–0.99)
Past	480 (3.35)	2387 (3.33)	1.00 (0.90–1.10)	0.95 (0.85–1.05)
Chondroitin sulphate				
Nonusers	13,805 (96.39)	68,515 (95.68)	1 (ref.)	1 (ref.)
Current	99 (0.69)	754 (1.05)	0.65 (0.53–0.80)	0.68 (0.55–0.84)
Recent	122 (0.85)	777 (1.09)	0.78 (0.64–0.94)	0.77 (0.63–0.94)
Past	296 (2.07)	1564 (2.18)	0.94 (0.82–1.06)	0.88 (0.77–1.00)
Chondroitin sulphate + glucosamine				
Nonusers	14,175 (98.97)	70,789 (98.85)	1 (ref.)	1 (ref.)
Current	10 (0.07)	65 (0.09)	0.77 (0.39–1.49)	0.85 (0.43–1.68)
Recent	47 (0.33)	276 (0.39)	0.85 (0.62–1.16)	0.83 (0.61–1.15)
Past	90 (0.63)	480 (0.67)	0.94 (0.75–1.17)	0.88 (0.69–1.11)

CI, confidence interval; OR, odds ratio.
[a]Adjusted only for matching factors (age, sex and calendar year).
[b]Adjusted for matching factors (age, sex and calendar year) plus the history of transient ischaemic attack, ischaemic heart disease (acute myocardial infarction or angina pectoris – recorded as such, and/or when patients were using nitrates), thromboembolic disease, heart failure, atrial fibrillation, peripheral artery disease, hypertension, diabetes (recorded as such, and/or use of glucose-lowering medications), dyslipidaemia (recorded as such, and/or use of lipid-lowering medications), chronic obstructive pulmonary disease, knee/hip osteoarthritis, rheumatoid arthritis, asymptomatic hyperuricaemia and gout, chronic renal failure, the number of visits to the primary care practitioner in the year prior to the index date (as an indicator of comorbidities), body mass index, smoking, alcohol abuse, and use of the following drugs: antiplatelet drugs, oral anticoagulants, paracetamol, metamizole, nonsteroidal anti-inflammatory drugs, opioids, corticosteroids, calcium supplements, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, beta-blockers, alpha-blockers, diuretics, proton pump inhibitors and H_2-receptor antagonists.

the regular use of SYSADOAs was associated with a statistically significant reduction in C-reactive protein levels, which is a marker of systemic inflammation.[10] In animal studies, SYSADOAs have been shown to inhibit nuclear factor kappa B (NF-kB), thereby reducing several inflammation markers,[6,38–42] thus, it is conceivable that such anti-inflammatory actions could have a preventive role in the pathophysiology of vascular diseases. In addition, glucosamine use has been shown to mimic a low-carbohydrate diet by decreasing glycolysis and increasing amino acid

Table 5. Risk of ischaemic stroke associated with the use of SYSADOAs (chondroitin sulphate and glucosamine) among patients with a record of knee/hip osteoarthritis.

	Cases (%) *N* = 1684	Controls (%) *N* = 7835	Unadjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)
SYSADOAs (all)				
Non-users	1446 (85.87)	6654 (84.93)	1 (ref.)	1 (ref.)
Current	37 (2.20)	285 (3.64)	0.59 (0.42–0.84)	0.64 (0.45–0.92)
Recent	58 (3.44)	263 (3.36)	1.01 (0.76–1.35)	1.10 (0.81–1.49)
Past	143 (8.49)	633 (8.08)	1.01 (0.83–1.23)	1.02 (0.83–1.25)
Glucosamine				
Nonusers	1545 (91.75)	7112 (90.77)	1 (ref.)	1 (ref.)
Current	11 (0.65)	140 (1.79)	0.36 (0.20–0.68)	0.40 (0.21–0.74)
Recent	26 (1.54)	152 (1.94)	0.80 (0.52–1.21)	0.90 (0.58–1.39)
Past	102 (6.06)	431 (5.50)	1.07 (0.86–1.34)	1.09 (0.86–1.38)
Chondroitin sulphate				
Non-users	1550 (92.04)	7210 (92.02)	1 (ref.)	1 (ref.)
Current	29 (1.72)	161 (2.05)	0.82 (0.55–1.23)	0.89 (0.60–1.34)
Recent	35 (2.08)	137 (1.75)	1.17 (0.80–1.71)	1.20 (0.81–1.77)
Past	70 (4.16)	327 (4.17)	0.96 (0.74–1.25)	0.97 (0.73–1.28)
Chondroitin sulphate + glucosamine				
Non-users	1649 (97.92)	7668 (97.87)	1 (ref.)	1 (ref.)
Current	3 (0.18)	16 (0.20)	0.85 (0.25–2.93)	0.95 (0.27–3.36)
Recent	12 (0.71)	52 (0.66)	1.07 (0.57–2.00)	1.01 (0.52–1.96)
Past	20 (1.19)	99 (1.26)	0.91 (0.56–1.48)	0.99 (0.60–1.64)

CI, confidence interval; OR, odds ratio.
[a]Adjusted only for matching factors (age, sex and calendar year).
[b]Adjusted for matching factors (age, sex and calendar year) plus the history of transient ischaemic attack, ischaemic heart disease (acute myocardial infarction or angina pectoris – recorded as such, and/or when patients were using nitrates), thromboembolic disease, heart failure, atrial fibrillation, peripheral artery disease, hypertension, diabetes (recorded as such, and/or use of glucose-lowering medications), dyslipidaemia (recorded as such, and/or use of lipid-lowering medications), chronic obstructive pulmonary disease, rheumatoid arthritis, asymptomatic hyperuricaemia, gout, chronic renal failure, the number of visits to the primary care practitioner in the year prior to the index date (as an indicator of comorbidities), body mass index, smoking, alcohol abuse, and use of the following drugs: antiplatelet drugs, oral anticoagulants, paracetamol, metamizole, non-steroidal anti-inflammatory drugs, opioids, corticosteroids, calcium supplements, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, beta-blockers, alpha-blockers, diuretics, proton pump inhibitors and H_2-receptor antagonists.

catabolism in mice.[43] Other mechanisms could also be involved, such as the role of PGs and GAGs in atherosclerosis.[7] The luminal surface of the endothelium is covered by a gelatinous layer, the glycocalyx, composed of glycoproteins (GAGs and PGs), with CS being one of the most abundant GAGs.[44] Glycocalyx is involved in multiple physiological processes of the endothelium: filtration of fluids and macromolecules, regulation of vascular tone and haemostasis, as well as regulation of neutrophil migration across the endothelium.[44] In recent years, PGs and GAGs in the

glycocalyx have been postulated to play a role in the initiation and progression of atherosclerosis.[45] Interestingly, Melgar-Lesmes *et al.*[46] have observed in mice that CS directly latches onto the atheromatous plaque, drastically reducing its size, recedes tumour necrosis factor (TNF) effects, heals endothelial injury and decreases the monocyte/macrophage differentiation into foam cells. Furthermore, mice receiving CS showed 100% survival as compared with 85% of the control group.[46]

The strengths of the present study are the following: (1) although the access to the data for researchers was retrospective, primary clinicians collect the data prospectively, including all prescriptions filled (CS and glucosamine are both prescription drugs in Spain, reimbursed by the National Health System); (2) the sample size of the study was large and allowed us to estimate risks with reasonable precision; (3) researchers who conducted the validation of IS cases were fully blinded to drug exposure to avoid a differential misclassification of the event; (4) controls were randomly extracted from the person-time of the underlying cohort to make sure that they represent the population exposure (density sampling), this way, avoiding a selection bias and assuring that the ORs obtained are unbiased estimates of the incidence rate ratios;[46] (5) only 'new users' were considered in the main analysis thus avoiding a 'prevalent-user' bias;[25] and (6) all sensitivity analyses yielded similar results to the main analysis, including the one restricted to patients with knee or hip OA, albeit for CS we did not find a significant reduced risk, partly due to the reduction of the sample size.

Limitations of the study are the following: (1) despite our efforts to control for confounding factors, a residual confounding due to unknown or unmeasured factors may be present due to the observational nature of the study; (2) exposure misclassification is unlikely because all prescriptions are filled through the computer and then completely recorded, but treatment adherence by patients cannot be assured; (3) it was not possible to carry out a robust analysis with the combination of glucosamine and CS as the exposure was too low; (4) we had patients with missing values for smoking and BMI; however, applying multiple imputation the results were very similar as those given in the main analysis; and (5) regular use of SYSADOAs can be a marker of a healthy lifestyle, and then the reduced risk of IS observed among SYSADOAs users could be partly explained by a healthy-user effect; nonetheless, it is remarkable that the risk reduction was mainly observed in patients with vascular risk factors, which does not support this explanation; also, it is important to emphasize that SYSADOAs included in the present study are only those available as prescription medicines which may be less associated with a healthy lifestyle than nutraceuticals containing these substances, which were not considered in the present study. Actually, patients who used nutraceuticals were not recorded in the database and, then, classified as 'non-users', a potential misclassification that, if relevant, it would have diluted the magnitude of the effect of prescription SYSADOAs.

Conclusion

The results of the present study support the hypothesis that the use of SYSADOAs, as prescription medicines, is associated with a reduced risk of IS that was observed in both men and women and in individuals older and younger than 70 years. Importantly, such association with a risk reduction of IS was even observed in patients who were current/recent users of NSAIDs and in patients with vascular risk factors.

Declartions

Ethics approval and consent to participate

The BIFAP Scientific Committee granted access to fully anonymised electronic medical records (project #04/2016; approval date 26 May 2016). Afterwards, on 1 July 2020, this committee approved specifically the analysis proposed for this study. In addition, the Research Ethics Committee of the Hospital Fundación Alcorcon (Ref. 20/76) approved the study on 4 May 2020, which granted a waiver to obtain the informed consent, as all data were fully pseudonymised and investigators had no access to personal data.

Consent for publication

All authors read and approved the final version of the manuscript.

Author contributions

Ramón Mazzucchelli: Conceptualisation; Investigation; Supervision; Visualization; Writing – original draft; Writing – review & editing.

Sara Rodríguez-Martín: Conceptualisation; Data curation; Formal analysis; Investigation; Methodology; Software; Validation; Visualization; Writing – original draft; Writing – review & editing.

Natalia Crespí-Villarías: Investigation; Writing – review & editing.

Alberto García-Vadillo: Conceptualisation; Investigation; Writing – review & editing.

Miguel Gil: Data curation; Investigation; Software; Writing – review & editing.

Laura Izquierdo-Esteban: Investigation; Writing – review & editing.

Antonio Rodríguez-Miguel: Investigation; Writing – review & editing.

Diana Barreira-Hernández: Investigation; Writing – review & editing.

Encarnación Fernández-Anton: Investigation; Writing – review & editing.

Alberto García-Lledó: Investigation; Writing – review & editing.

Aina Pascual: Investigation; Writing – review & editing.

Marianna Vitaloni: Investigation; Writing – review & editing.

Josep Vergés: Investigation; Writing – review & editing.

Francisco J. De Abajo: Conceptualisation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Visualisation; Writing – original draft; Writing – review & editing

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Competing interests

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Availability of data and materials

Not applicable.

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Supplemental material

Supplemental material for this article is available online.

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