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Original Article

Efficacy and Safety of Aspirin for Prevention of Hepatocellular Carcinoma: An Updated Meta-analysis

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Abstract

Background and Aims: Previous meta-analyses have shown that aspirin use may reduce the risk of hepatocellular carcinoma (HCC). However, the optimal dose, frequency, and duration of aspirin use or the safety and efficacy of aspirin in target populations for HCC prevention remain unclear. The study aim was to investigate the efficacy and safety of aspirin for prevention of HCC. ***Methods:*** Publications were retrieved by a comprehensive literature research of several databases. Based on a random-effects model, hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) were used to assess the pooled risk. The dose-response relationship between aspirin use and HCC risk was assessed with a restricted cubic spline model. ***Results:*** Twenty-two studies were included in the meta-analysis. Aspirin use was associated with a reduced risk of HCC (HR=0.64, 95% CI: 0.56–0.75). The effect was robust across sex and age; however, women and the non-elderly had the greatest benefit from aspirin use. The preventive effect was well reproduced in those with comorbidities. Daily use and long-term use of aspirin appeared to offer greater benefits. Aspirin 100 mg/d was associated with maximum reduction of HCC risk. Aspirin use did slightly increase the risk of bleeding (HR=1.14, 95% CI: 1.02–1.27). ***Conclusions:*** Our meta-analysis confirmed that use of aspirin significantly reduced the incident risk of HCC. Regular and long-term aspirin use offers a greater advantage. Aspirin use was associated with an increased risk of bleeding. We recommend 100 mg/d aspirin as a feasible dose for further research on primary prevention of HCC in a broad at-risk population.

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Keywords: Aspirin; Hepatocellular carcinoma; Prevention; Meta-analysis; Dose-response analysis.

Abbreviations: CI, confidence interval; COX, cyclo-oxygenase; HCC, hepatocellular carcinoma; HR, hazard ratio; IFN-α, interferon alpha; NOS, Newcastle-Ottawa scale; NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio; RR, relative risk; TXA_2, thromboxane A2.

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer in adults. It is the sixth most commonly diagnosed cancer and the third leading cause of cancer-related deaths in the world.[1] Early-stage HCC tends to cause no or minimal symptoms, but grows aggressively with a high rate of metastasis; therefore, a large proportion of patients are diagnosed at an advanced stage with limited treatment options.[2] Owing to poor treatment outcomes and unfavorable prognosis, identification of chemopreventive agents against HCC is a key research imperative.

Chronic inflammation is believed to be a key enabling characteristic of cancer and is known to be involved in all stages of malignant progression, from the initial transformation phase to invasion and metastasis. HCC is a prototypical inflammation-associated cancer, with approximately 90% of HCC being associated with chronic hepatitis.[2] Besides, platelets play an important part in facilitating chronic inflammation in the liver and have been implicated in the genesis and progression of HCC.[3,4] Aspirin is a widely used nonsteroidal anti-inflammatory drug (NSAID). It irreversibly inhibits cyclo-oxygenase enzyme 1 (COX1), thus inhibiting platelet aggregation because of a reduction of thromboxane A2 (TXA_2) synthesis. In addition, aspirin also has an anti-inflammatory effect by inhibiting the activity of COX2 that catalyzes the production of prostaglandins. Experimental studies have suggested aspirin as a promising chemoprotective agent for the prevention of HCC, which might be attributable to its anti-inflammatory effect, anti-platelet effect, and modulation of bioactive lipids.[5–8]

Previous systematic reviews have investigated the potential effect of aspirin in the prevention of HCC.[9–13] However, there was insufficient evidence to arrive at a convincing conclusion because of inconsistency and intractable heterogeneity among the studies. Previous studies did not focus on the efficacy of aspirin use in specific populations. The optimal duration, frequency, or dosage of aspirin for HCC prevention remains unclear. The associated risk of bleeding has also not been systematically analyzed. Thus, there is a need to perform an updated meta-analysis to account for the effect of potential confounding factors and to explore a feasible regimen for further research.

Methods

Data sources and searches

We searched PubMed, Embase, Web of Science, and the Cochrane Library databases for studies published through 20 March 2021. The key words included subject terms (non-steroidal anti-inflammatory agents OR aspirin) AND (hepatocellular carcinoma) AND (risk OR mortality OR cohort) and their related entry terms. The reference lists of the included publications were manually searched to identify additional relevant studies.

Inclusion and exclusion criteria

The abstracts and titles of the articles retrieved on database search were independently screened by two reviewers (L-JY and S-YY) to identify articles that were eligible for further review. Studies were included in the meta-analysis if they satisfied the following criteria: (1) the study design was a randomized control trial, retrospective or prospective cohort, or case-control study; (2) the study population included adult patients with HCC and ≥ 18 years of age; (3) HCC was confirmed histologically or the diagnosis was made by physicians based on clinical manifestations, radiographic results, laboratory results, and other clinical data; (4) the reported outcomes included hazard ratios (HRs), relative risk (RR), or odds ratios (ORs) estimating the risk of HCC in aspirin users or studies for which adequate raw data was available for calculation of the effect size. Patients with recurrent HCC or liver metastases or HCC accompanied with cholangiocarcinoma were excluded. If the sources of the recruited participants overlapped in different studies, the study with the smaller number of HCC patients was excluded.

Data extraction and quality assessment

Data were independently extracted from the full-text articles by two authors (L-JY and S-YY). Any disagreements were identified and resolved with the participation of a third author (H-CL). Data pertaining to the following variables were extracted: geographical area, study design, study period, number of participants, follow-up period, data source, drug assessment, definition of drug use, and the crude or adjusted HR/RR/OR and their 95% confidence intervals (CIs). For quality assessment, each study was assessed and scored using the Newcastle-Ottawa Scale (NOS) with a maximum of 9 points.

Statistical analysis

We extracted the RR, HR, OR, or incidence density ratio from the included studies evaluating the risk of HCC or adverse events in aspirin users. If unadjusted and adjusted effect sizes were both available, we extracted the latter. The HRs were treated as the common measure of association across studies. RRs were directly considered as HRs. The heterogeneity of RRs among the included studies was assessed using the Cochrane Q statistic and I^2 statistic. I^2>50% accompanied by a p-value of <0.10 for the Q statistic was considered indicative of substantial heterogeneity. Univariate random-effects model meta-regressions of the HRs from the included studies were performed. Publication bias was assessed using the Egger's test and funnel plots.

Dose-response analysis was performed for studies that reported sufficient data in terms of the number of subjects, incidence of HCC during follow-up, and the dosage of aspirin. We examined the potential nonlinear association between aspirin intake and risk of HCC using study-specific restricted cubic spline models with three knots at fixed 10%, 50%, and 90% percentiles of the exposure distribution. Statistical analysis was performed with the Stata 16.0 (Stata Corporation, College Station, TX, USA).

Results

Literature search

The search retrieved 2,006 citations, 56 from PubMed, 1,048 from Embase, 29 from the Cochrane Library, and 873 from Web of Science. After elimination of duplicate records, the titles and abstracts of 1,371 citations were screened. After preliminary exclusion of manuscripts that did not meet the inclusion criteria, 74 were further assessed by full-text review. After screening and reviewing, 22 studies met the inclusion criteria and were included in the meta-analysis (Fig. 1).

Baseline characteristics

The characteristics of the 22 studies included in the meta-analysis are listed in Table 1.[14–35] The combined study population was 2,531,742. Of the 22, five were case-control studies and 17 were cohort studies (14 retrospective studies and three prospective studies). Fourteen studies were conducted in Asian countries and eight in Western countries (three in Europe and five in America). Except for the study by Oh *et al*.,[36] which did not report the method of data collection, most gathered data from electronic databases and two obtained data through questionnaires. Sixteen studies assessed aspiring exposure by counting prescriptions dispensed by hospitals, four collected participant medication history through questionnaires, and two did not report the method used to assess aspirin use. The detailed NOS items and study scores are shown in Supplementary Tables 1 and 2. The median NOS score was 7, and 17 of the 22 studies were considered to be of high quality for achieving an NOS score ≥7.

Risk of HCC in aspirin users

We collected data from 23 cohorts in the 22 studies. Ho *et al*. 2018[37] reported data from two cohorts that included patients infected with either HBC or HCV. The pooled HR for the incident risk of HCC in aspirin users (Supplementary Fig. 1) was 0.64 (95% CI: 0.56–0.75). We detected substantial heterogeneity in the analysis (I^2=89.9%, p<0.001). To explore the source of heterogeneity and examine the protective effect in subgroups, we conducted meta-regression analysis by study design, geographical area, drug assessment, and comorbidity and further performed subgroup analysis based on the four covariates (Supplementary Table 3, and Supplementary Fig. 2). Among the covariates, comorbidities of the participants were found to be a potential source of heterogeneity, and explained 54.33% of between-study variance (p=0.03). When stratified by geographical region, HRs for the risk of HCC in aspirin users in Western countries and Asian countries were 0.64 (95% CI: 0.51–0.80) and 0.64 (95% CI: 0.56–0.75), respectively. We also performed sensitivity analysis to investigate the influence of each individual study on the pooled estimate. The study by Tsoi *et al*. 2018[38] was suspected of excessive influence (Supplementary Fig. 3). After exclusion of Tsoi 2018 from the meta-analysis, there was a 37.36% reduction in the Q-

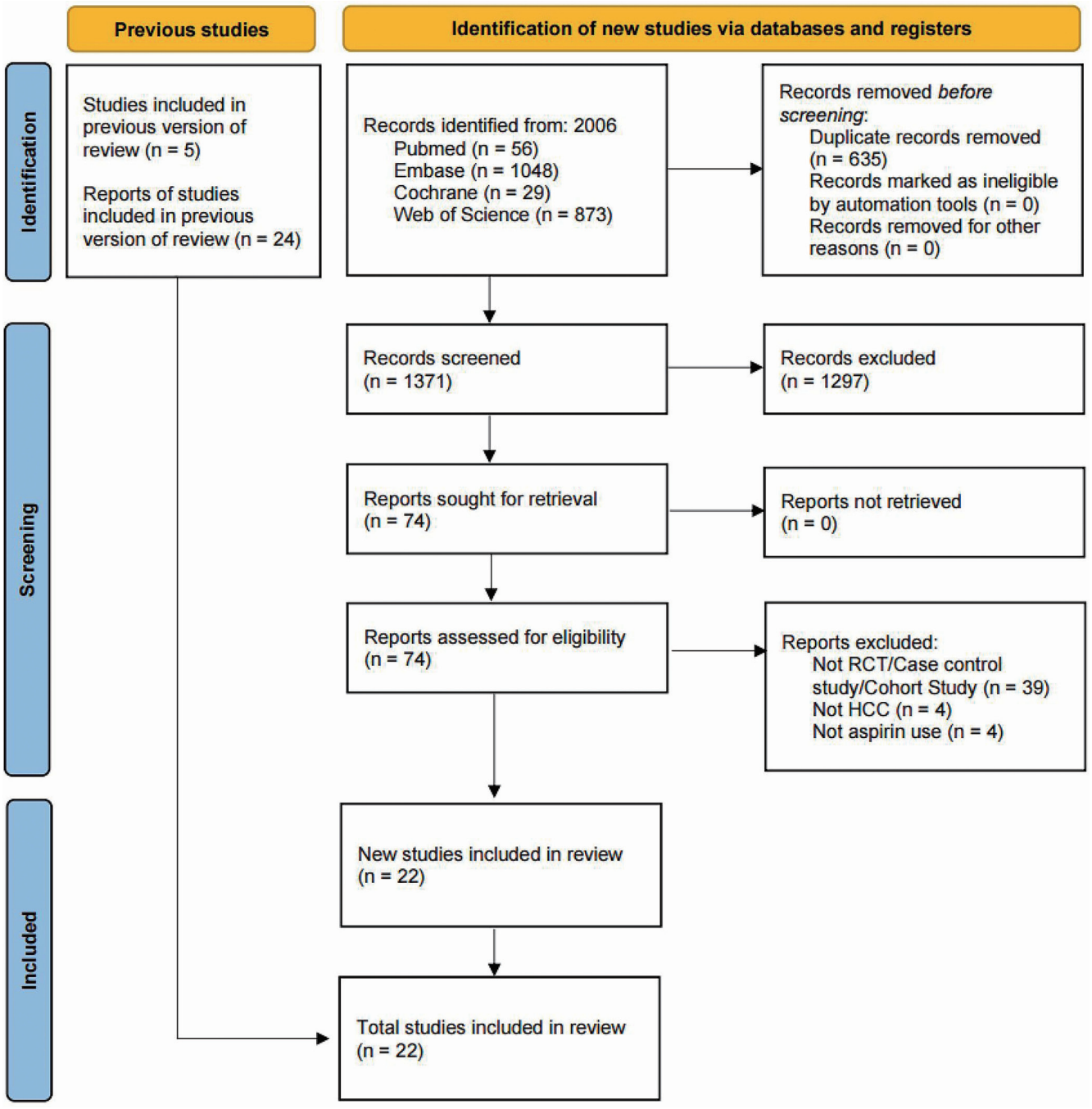

Fig. 1. Flowchart of meta-analysis following PRISMA guidelines.

value (decrease from 217.83 to 136.45). Publication bias was examined by Egger's regression asymmetry test and funnel plots (Supplementary Fig. 4), which indicated that there was no significant publication bias (p=0.589).

Sex and age

Three studies provided data on HCC incidence in aspirin users stratified by sex and age. The preventive effect was robust in both men and women. The HRs for the risk of HCC in men and women aspirin users were 0.75 (95% CI: 0.65–0.86, I^2=54.0%, p=0.114) and 0.67 (95% CI=0.57–0.79, I^2=0.0%, p=0.380), respectively (Fig. 2A). The association between HCC risk and age in aspirin users was assessed by dividing the participants into elderly and non-elderly groups following the criteria of the original studies. A reduced risk of HCC was observed (Fig. 2A) in both elderly (HR=0.65, 95% CI: 0.57–0.75, I^2=0.0%, p=0.531) and non-elderly groups (HR=0.72, 95% CI: 0.64–0.81, I^2=17.0%, p=0.300). We further calculated the incidence rate ratios (IRRs) to assess any sex and age differences in efficacy. The risk of devel-

Table 1. Baseline characteristics of studies included in the meta-analysis

Author, Year	Area	Study design	Study period	No. of subjects	Mean follow-up	Data source	Drug assessment	NOS score	Definition of drug use
Simon 2020[17]	Sweden	Retrospective cohort	2005–2015	50,275	7.9 years†	Database	Prescription	9	≥90 DDDs
Shin 2020[25]	Korea	Retrospective cohort	2003–2016	949	3.1 years†	Database	Prescription	8	100 mg/d for at least 6 months
Shen 2020[26]	US	Case-control Study	2011–2016	1,839	NA	Questionnaire	Self-reported	9	Regular use (at least once per week for 3 months or more)
Lee 2020[18]	Taiwan	Retrospective cohort	1997–2011	7,434	3.3 years	Database	Prescription	8	Continued daily therapy for 90 days or more
Tsoi 2019[16]	HongKong	Retrospective cohort	2000–2013	612,509	8.9 years	Database	Prescription	9	Prescribed for at least 6 months
Lee 2019[19]	Taiwan	Retrospective cohort	1997–2012	10,615	3.1 years†	Database	Prescription	8	Daily therapy ≥ 90 days
Du 2019[20]	China	Retrospective cohort	2000–2014	264	4.5 years†	Database	Prescription	9	100 mg/d
Tseng 2018[27]	Taiwan	Retrospective cohort	NA	85,787	5.3 years†	Database	Prescription	8	Any use at least 2 years
Simon 2018[23]	US	Prospective cohort	1980–2012	133,371	31.73 years	Questionnaire	Self-reported	8	≥ 2 standard-dose (325 mg) tablets per week
Lin 2018[28]	Taiwan	Retrospective cohort	2000–2011	18,243	1.27 years†	Database	Prescription	6	NA
Hwang 2018[21]	Korea	Retrospective cohort	2002–2013	460,755	6.44 years	Database	Prescription	9	≥30 DDDs
Ho 2018[15]	Taiwan	Retrospective cohort	2005–2014	15,597	4/4.6 years‡	Database	Prescription	7	Any use for antiplatelet therapy
Ramirez 2017[29]	US	Case-control Study	2012–2014	84	NA	Database	Na	5	NA
Oh 2017[14]	Korea	Retrospective cohort	NA	973	4.6 years†	Na	Na	5	NA
Lee 2017[30]	Taiwan	Retrospective cohort	1998–2012	18,080	6.32 years†	Database	Prescription	7	>1 day per month
Lee 2017[24]	Korea	Retrospective cohort	2002–2015	1,674	4.8 years†	Database	Prescription	7	100 mg/day for at least 6 months
Kim 2017[31]	Korea	Case-control Study	2002–2013	1,374	NA	Database	Prescription	7	At least one prescription between the cohort entry and the index date
Yang 2016[32]	UK	Case-control Study	1988–2011	5,835	NA	Database	Prescription	7	Two or more prescriptions
Petrick 2015[22]	US	Prospective cohort	1980–2010	803,248	11.9 years	Database	Self-reported	7	Any use
Sahasrabuddhe 2012[33]	US	Prospective cohort	1995–2008	300,504	9.15 years	Database	Self-reported	7	Any use
Chiu 2010[34]	Taiwan	Case-control Study	1996–2008	2,332	NA	Database	Prescription	6	At least one prescription over 1 year before the index date
Friis 2003[35]	Denmark	Retrospective cohort	1989–1997	NA	4.1 years	Database	Prescription	6	Low dose (75–150 mg) once a day

†median follow-up; ‡mean follow-up was 4.0 for the HBV and 4.6 years for the HCV cohorts. DDD, daily defined dose; NA, not applicable; NOS, Newcastle-Ottawa scale.

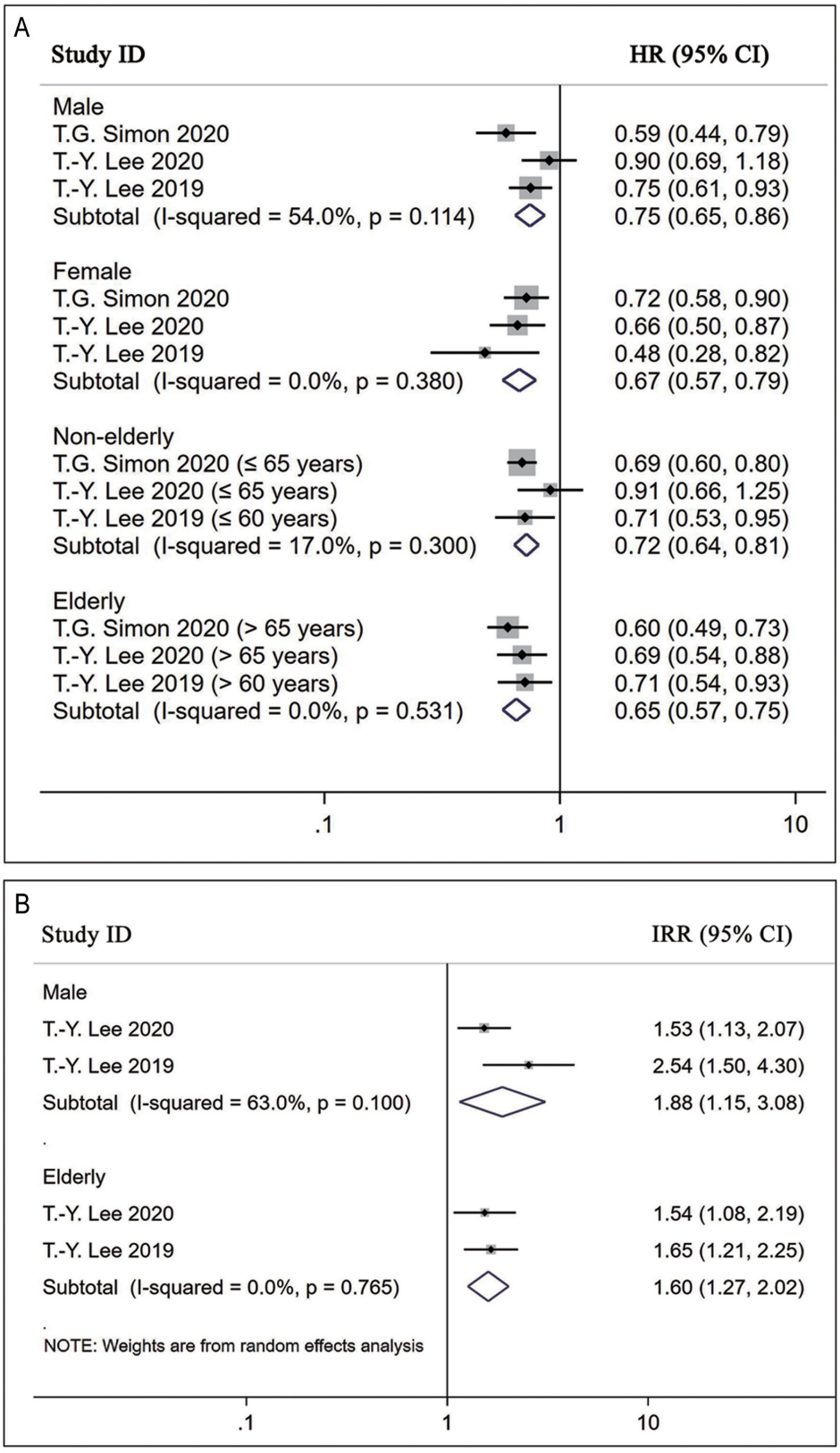

Fig. 2. Forest plots of the association between aspirin use and HCC risk by sex and age. (A) Hazard ratio (HR) for the risk of HCC in men and women and different age groups. (B) Incidence rate ratio (IRR) for the risk of HCC in men and the elderly compared with women and the non-elderly.

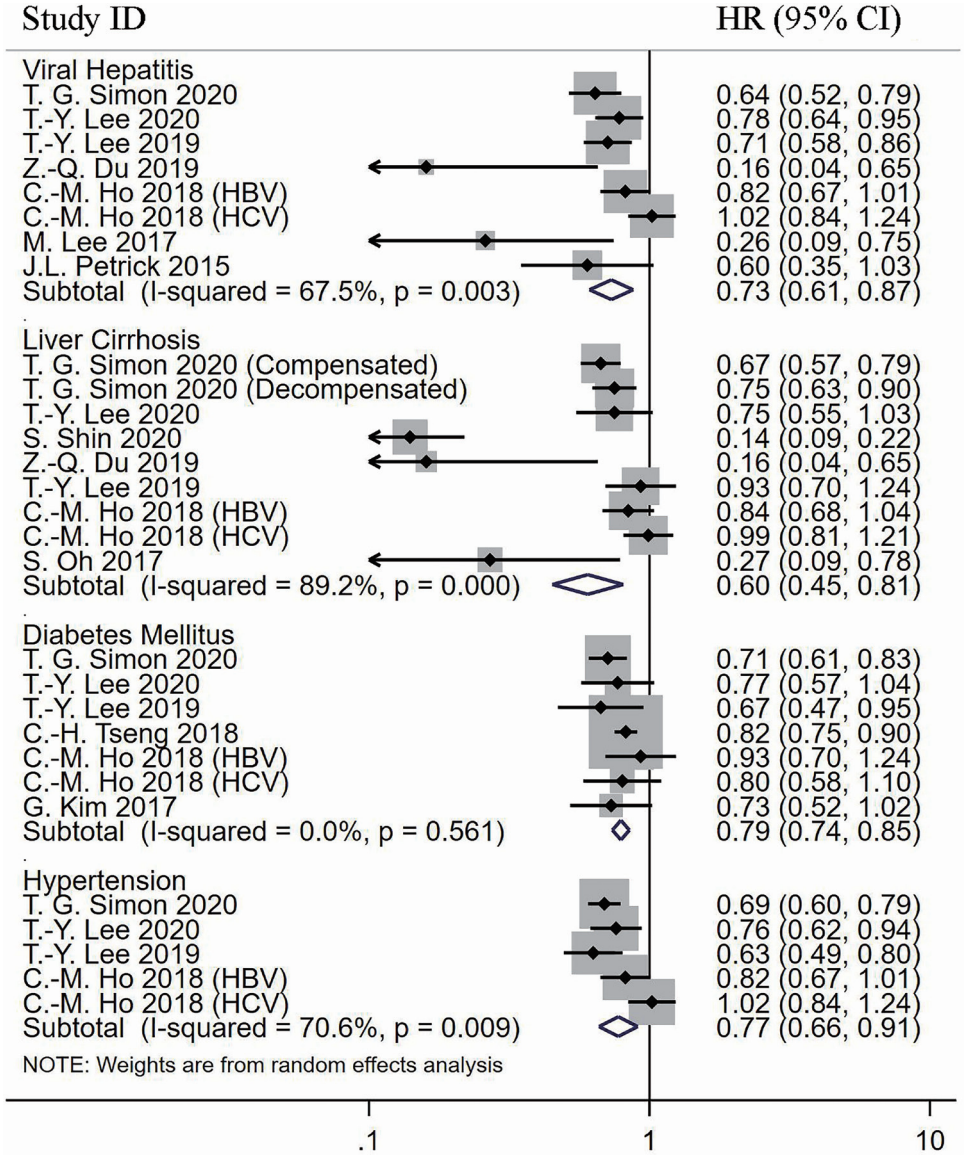

Fig. 3. Forest plots of the association between aspirin use and HCC risk by comorbidity.

oping HCC was significantly higher in men and in elderly patients. The IRR of the risk of HCC in men compared with women aspirin users was 1.88 (95% CI: 1.15–3.08) and that in the elderly compared with the non-elderly group was 1.60 (95% CI: 1.27–2.02). The result implied that women and the non-elderly may benefit more from aspirin use for HCC prevention (Fig. 2B).

Comorbidity

To evaluate the preventive effect of aspirin in patients with concomitant comorbidities, another four meta-analyses were conducted. HRs for the risk of HCC in aspirin users among studies that provided data for various comorbidities (Fig. 3) were: 0.73 for viral hepatitis (95% CI: 0.61–0.87, I^2=67.5%, p=0.003); 0.60 for liver cirrhosis (95% CI: 0.45–0.81, I^2=89.2%, p<0.001); 0.79 for diabetes mellitus (95% CI: 0.74–0.85, I^2=0.0%, p=0.561); 0.77 for hypertension (95% CI: 0.66–0.91, I^2=70.6%, p=0.009). Aspirin use in patients with the four comorbidities was found to notably reduce the risk of HCC. Patients with liver cirrhosis had the most benefit, with a substantial 40% reduction in risk. Except for studies that accounted for diabetes mellitus, substantial heterogeneity was detected in the other three meta-analyses. Given the limited number of included studies, further subgroup analyses were not performed.

Duration and frequency

Studies that reported HCC risk in aspirin users according to the duration or frequency of aspirin use were identified and then we performed two additional meta-analyses to explore a more advantageous regimen of aspirin use for HCC prevention. Given the varied cutoff values for the aspirin exposure time, we took 3 years as the dividing line and set up two groups. The HR for the risk of HCC was 0.79 (95%

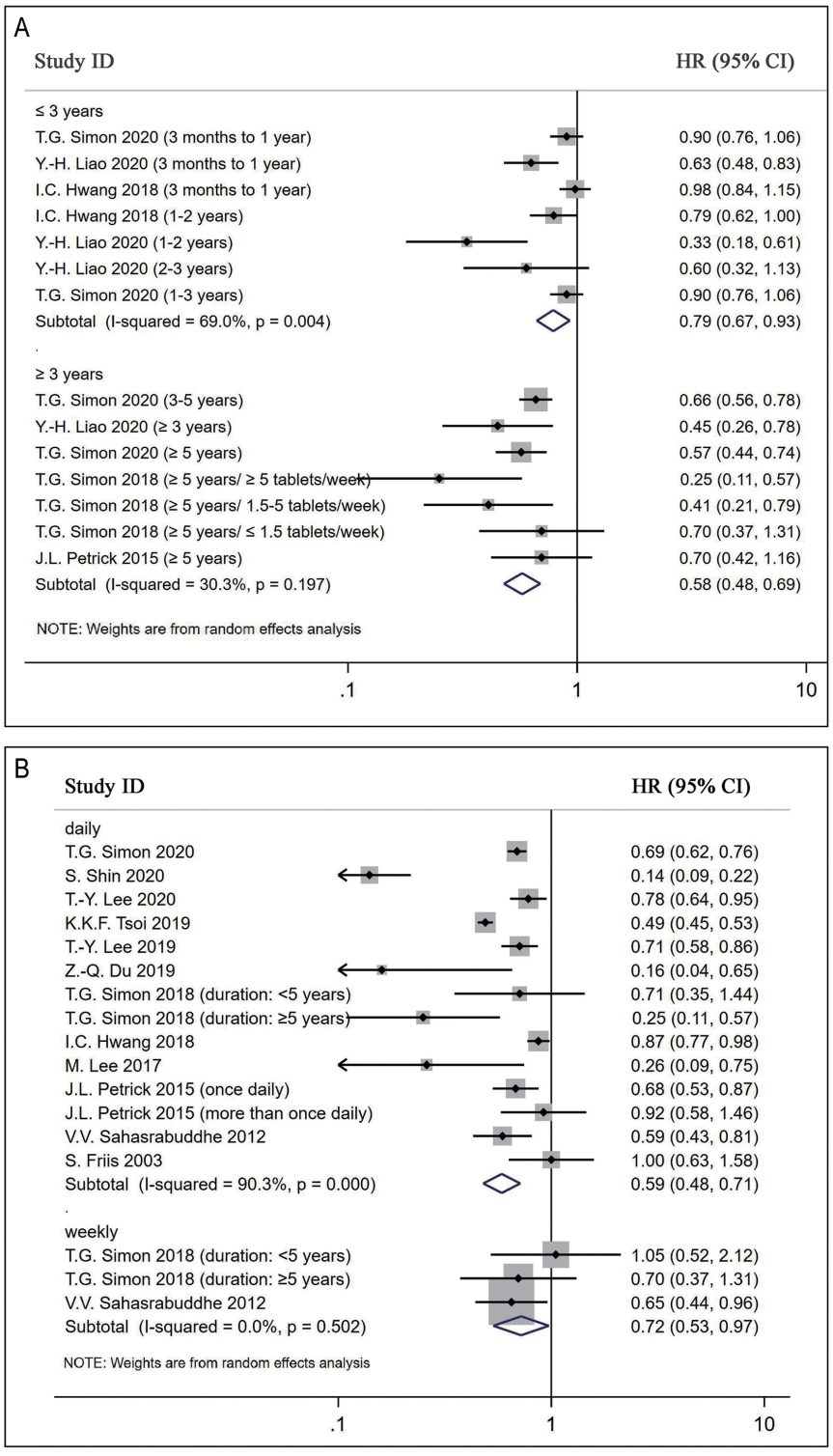

Fig. 4. Forest plots showing the association between the (A) duration and (B) frequency of aspirin use and HCC risk.

CI: 0.67–0.93, I^2=69.0%, p=0.004) in those taking aspirin for less than 3 years. However, for those taking aspirin for more than 3 years (Fig. 4A), aspirin use was associated with a significant 42% reduced risk of HCC (HR=0.58, 95% CI: 0.48–0.69, I^2=30.3%, p=0.197). We also set up two groups based on the frequency of aspirin use (i.e., daily versus weekly) to compare the risk of HCC (Fig. 4B). Akin to previous results, aspirin was consistently associated with lower risk of HCC. HRs for the risk of HCC were 0.59 (95% CI: 0.48–0.71, I^2=90.3%, p<0.001) in those taking aspirin

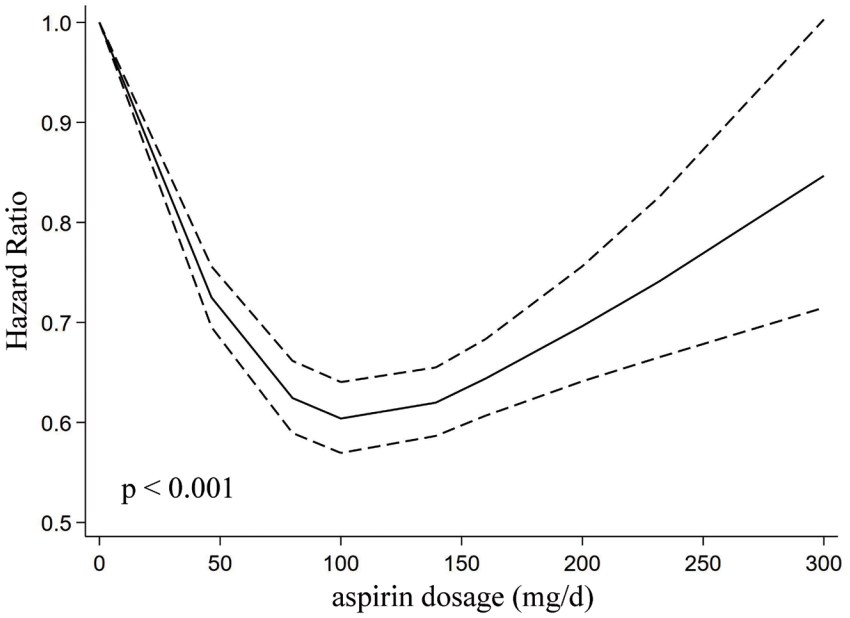

Fig. 5. Dose-response analysis showing a nonlinear association between the aspirin dosage and HCC risk.

daily and 0.72 (95% CI: 0.53–0.97, I^2=0.0%, p=0.502) in those taking aspirin weekly.

Dose-response analysis

Nine studies were included in the dose-response analysis.[14–20,38,39] The combined results (Fig. 5) showed a significant U-shaped nonlinear association between aspirin dosage and the risk of developing HCC (p<0.001), which implied that aspirin use at a dose of 100 mg per day was associated with the lowest risk of HCC. Aspirin use at doses higher than 100 mg per day were also associated with a significant preventive effect, the magnitude of the benefit was smaller.

Bleeding risk

Six studies provided data on the risk of bleeding during the follow-up period.[14,15,21,36,39,40] In five studies, bleeding events were not significantly more common among aspirin users than nonusers during the follow-up period. Surprisingly, the pooled HR for the risk of bleeding in the six cohorts was 1.14 (95% CI: 1.02–1.27, I^2=37.1%, p=0.159), which implies that aspirin use for HCC prevention may increase the risk of bleeding (Fig. 6). In Lee 2017[21] and Shin

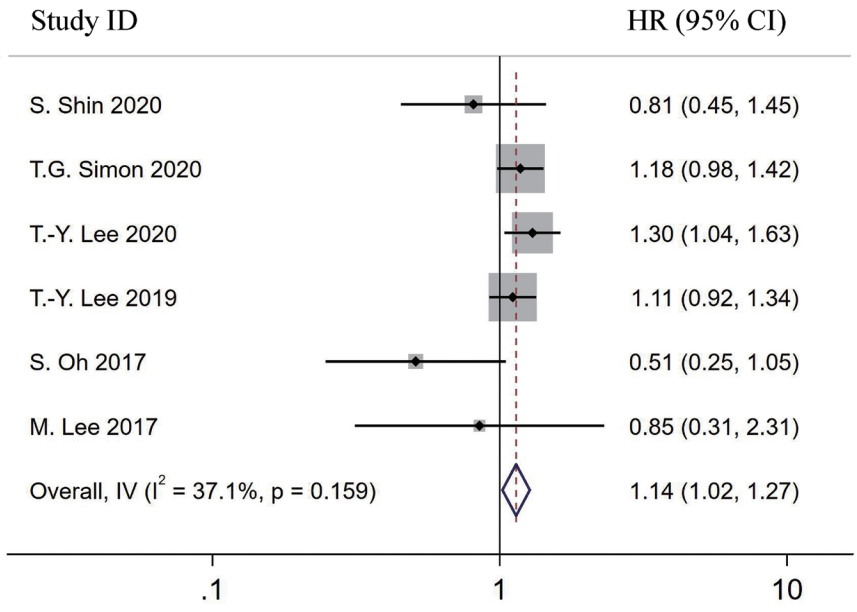

Fig. 6. Forest plots of the association between aspirin use and risk of bleeding in the included studies.

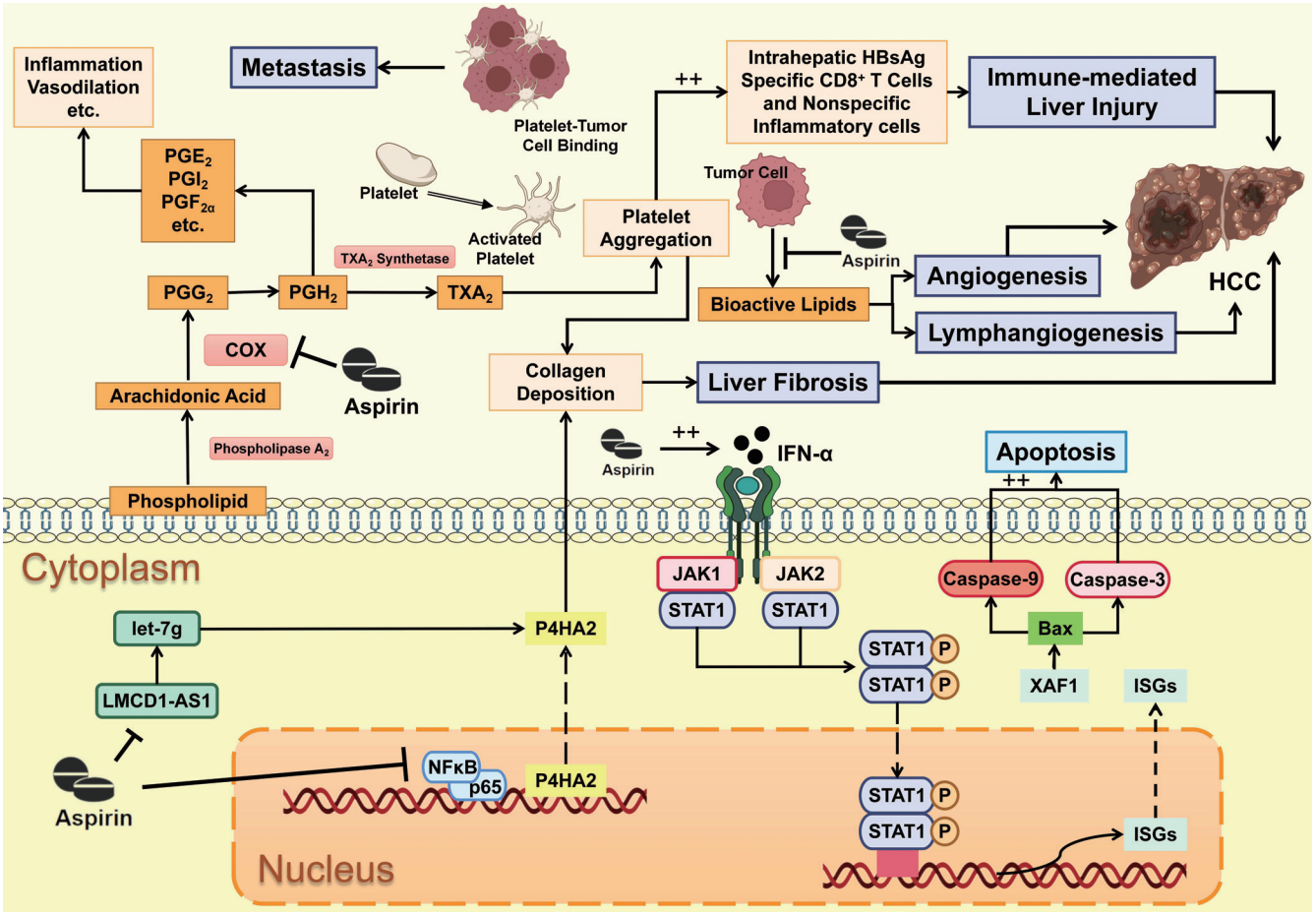

Fig. 7. Mechanisms and pathways of the chemoprotective effect of aspirin in HCC.

2020,[40] the aspirin group included patients treated with aspirin 100 mg/day, but the four other studies did not provide enough information to evaluate the dosage of aspirin. Considering the limited information provided in the original studies, further dose-response analysis was not possible.

Discussion

Overall, akin to previous meta-analyses, our study also revealed an association between aspirin use and a decreased risk of HCC. However, there was substantial heterogeneity among the included studies. Meta-regression analysis and subgroup analysis identified comorbidities of participants as the major contributor to heterogeneity. We also performed sensitivity analyses that indicated Tsoi *et al*. 2018[38] contributed to the excess heterogeneity, and persisting between-study variation may be partially explained by the fact that the studies were conducted in different countries, in study populations that differed in genetic background, lifestyle, and dietary habits. All the studies were published in English, had a high quality assessment, and in some original studies the results were adjusted for a wide range of potential confounders.

Subgroup analysis found that the preventive effect of aspirin on HCC was robust regardless of the geographical region area (i.e., Western or Asian countries). Our updated study further explored sex and age differences in the preventive effect of aspirin. Aspirin use was associated with lower risk of HCC in all subgroups, but women and the nonelderly were found to benefit more from aspirin for HCC prevention. Additionally, we evaluated if the association was robust among aspirin users with different comorbid conditions. We found that aspirin use reduced the incidence of HCC in patients with chronic hepatitis, liver cirrhosis, diabetes mellitus, and hypertension. Chronic hepatitis, liver cirrhosis, and diabetes mellitus are known risk factors for HCC. Exploring feasible approaches to prevent HCC in specific populations, especially those at high risk, is vital to reduce the cancer burden. Our study indicated that aspirin may be useful in reducing the risk of HCC in at-risk populations.

With respect to the optimal duration and frequency of aspirin use for HCC prevention, use of aspirin for more than 3 years and daily use of aspirin were more effective in preventing HCC than short duration and irregular use. Furthermore, on dose-response analysis, aspirin 100 mg per day was associated with the greatest benefits for HCC prevention, and higher doses were associated with lower preventive efficacy. Low-dose aspirin can irreversibly prevent platelet aggregation by reducing the synthesis of TXA2, which is believed to contribute to the reduced risk of HCC.[5,6] However, high-dose aspirin exerts anti-inflammatory effects by inhibition of both COX-1 and COX-2, which inhibits prostaglandin I_2 synthesis. That in turn suppresses its antagonistic action against TXA_2, promoting thrombogenesis. Moreover, high-dose aspirin inhibits the formation of prothrombin, which

may cause coagulation disorders and aggravation of bleeding tendency. As the efficacy of aspirin for HCC prevention is somewhat related to increased duration, high doses of aspirin and the subsequent increased risk of adverse events may affect patient compliance and lead to discontinuation of treatment.

Drug safety is a prerequisite for clinical application. Inhibition of TXA_2 synthesis in platelets has an inhibitory effect on platelet aggregation that may increase the risk of hemorrhage. García Rodríguez *et al*.[22] reported an approximately 40% increased risk of all gastrointestinal bleeding with low-dose aspirin. The risk of aspirin-related serious bleeding is a major impediment to the wider use of aspirin for preventing HCC in high-risk populations. In this study, we analyzed data from six studies that reported the risk of bleeding in aspirin users that showed aspirin use did increase the risk of bleeding by 14%. Although use of aspirin for prevention of HCC appears promising, it should be used in specific subsets of patients in whom the risk of serious bleeding does not outweigh the benefits. Whitlock *et al*. focused on the baseline risk of serious bleeding with regular aspirin use as primary prevention for cardiovascular disease and reported various factors that increased baseline risk or enhanced aspirin's effect on bleeding, such as older age, which increased 1.5- to 2-fold in each decade after 50 years of age; male sex, history of gastrointestinal ulcer or bleeding; and combination with other drugs such as NSAIDs or clopidogrel.[23] The condition of patients, especially the coagulation function, blood routine testing, history of medication, and past medical history, especially history of bleeding, should be carefully assessed before administration to assess baseline risk and followed up regularly after administration. Prior to incorporation of aspirin use in the clinical guidelines for HCC prevention, further studies are required to demonstrate its potential hazards across the complete spectrum of liver diseases.

The chronic inflammatory milieu in the liver leads to a maladaptive reparative reaction and stimulates liver-cell death and regeneration, which is eventually responsible for the development of dysplastic nodules and even cancer.[2,24,25,41] Preclinical studies have demonstrated the overexpression of COX-2 in HCC. In addition, regulation of prostaglandin metabolism by COX-2 has been shown to participate in the pathogenesis of HCC.[42] COX-2 overexpression induces profibrotic and proliferative signaling cascades, including protein kinase 3, mammalian target of rapamycin and nuclear factor kappa-B pathways.[8] That is the rationale for the use of aspirin, a COX-2 inhibitor, for HCC prevention. On the other hand, aspirin functions as an antiplatelet drug. Activated platelets release granular mediators that promote tumor cell growth and migration.[43,44] Another proven mechanism focuses on the binding of platelets and cancer cells. P-selectin expressed on platelets and the sialylated fucosylated carbohydrates on cancer cells have been shown to mediate the binding in numerous types of cancer cells such as breast cancer, melanoma, neuroblastoma, lung cancer, and colon cancer.[45–47] Zhang *et al*.[47] showed that platelets from HCC patients were more activated and they also detected increased platelet-tumor cell binding in poorly differentiated as compared with well-differentiated HCC tissues. Based on those findings, they speculated that the binding may activate intracellular pathways that finally stop the differentiation of hepatoma cells. Aspirin also blocks bioactive lipids such as sphingosine-1-phosphate secreted by cancer cells that induce angiogenesis and lymphangiogenesis and facilitate tumor growth and metastasis formation.[7] Several recent studies have elucidated the underlying molecular mechanisms in mouse models. In a study by Wang *et al*.,[48] aspirin targeted prolyl 4-hydroxylase α2 (P4HA2) to decrease collagen deposition, which inhibited liver tumor growth in an HCC cell line and in a nude mouse xenograft model by dampening the nuclear factor kappa-B/p65 and the lncRNA LMCD1-AS1/let-7g axis. Tumor size, volume and weight were significantly increased in the P4HA2 overexpression group compared with the wild-type xenograft mice, and the increases were inhibited by aspiring treatment. Our previous study also revealed that aspiring treatment enhanced tumor suppression and apoptosis induced by IFN-α in HCC cell lines and in a nude mouse xenograft model. In vitro, IFN-α and aspirin alone promoted a small increase in cell apoptosis, but when combined, apoptosis of HCC cells was significantly increased, which implied a synergetic effect of IFN-α and aspirin. In mouse models, the volume of tumors was reduced after IFN-α treatment compared with in the control group. Though treatment with aspirin alone did not inhibit tumor growth, it significantly improved tumor growth inhibition caused by IFN-α treatment alone. The effect resulted from aspirin-prompted phosphorylation of STAT1, which was activated through phosphorylation of JAK1.[49] Sitia *et al*. found that platelet activation promoted the accumulation of HBV-specific $CD8^+$ T cells and HBV-nonspecific inflammatory cells in the liver in a mouse model of chronic HBV immune-mediated HCC. Aspirin alone or with clopidogrel reduced hepatic inflammation and immune infiltration. Antiplatelet therapy also diminished the severity of liver fibrosis and the development of HCC and improved the overall survival.[50] The potential targets and mechanisms of aspirin in HCC are summarized in Fig. 7.

Some limitations of our study should be acknowledged. First, the findings are mainly based on case-control and cohort studies. Although adjusted for a wide range of potential confounders, the influence of selection bias on our results cannot be ruled out. Second, there was considerable disparity in the definition of drug use among the included studies. Hence, larger multicenter studies are required to examine the preventive efficacy of NSAID use in HCC. Third, other adverse events induced by aspirin except for bleeding such as ulcers were too limited to incorporate because of limitations in study reporting.

Our study overcame some of the limitations of previous meta-analyses concerning aspirin use and the risk of HCC. First, the meta-analysis included a larger number of studies based on extensive literature search. Second, we explored the association between aspirin use and HCC risk in different subsets of patients, providing evidence for administration of aspirin to high-risk patients. Third, we found that an aspirin dosage of 100 mg/day may be a feasible regimen for use in further research on the primary prevention of HCC.

Conclusion

Our study provides more robust evidence of the association of aspirin use with a reduced risk of HCC. Aspirin may be an effective preventive regimen for at-risk populations, as regular and long-term use of aspirin seems to offer increased benefits for HCC prevention. We recommend 100 mg/d aspirin as a feasible dose for further research on primary prevention of HCC, while bleeding risk should receive more attention.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (LJY, TL), acquisition of data (LJY, SYY, HCL), analysis and interpretation of data (LJY, GXM, KXL, ZND), drafting of the manuscript (LJY), critical revision of the manuscript for important intellectual content (ZRD, ZQC, JGH), study supervision (TL).

Data sharing statement

All data are available upon request.

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