Mechanistic view into the therapeutic effect, anticancer potential and the adverse effect of aspirin

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Abstract. In this research, the working mechanism behind the therapeutic effect of aspirin will be discussed in detail. Aspirin will also be assessed as a promising drug to treat cancerous cells in the future. Despite being demonstrated that aspirin has excellent efficacy in CVD treatment, the application of aspirin still faces numerous challenges, including aspirin resistance and the bleeding effect induced by the administration of aspirin. The chemistry behind the resistance against aspirin will be discussed and analysed. To cope with aspirin resistance, some diagnosing methods for aspirin resistance are introduced and some other alternative treatment schemes will also be discussed and compared with the traditional aspirin treatment. Bleeding risk is another severe adverse effect that might occur when long-term low-dose aspirin is applied. Through a large number of current studies, the bleeding risks that the administration of aspirin imposing on the upper gastrointestinal tract and the lower gastrointestinal tract are analysed. The relevance among the dose of aspirin, the frequency of the aspirin dose, the duration of the dosing period of aspirin administration and the bleeding risk induced by aspirin is deduced by analysing the experimental observations extracted from several clinical trials focusing on these factors.

1 Introduction

Acetylsalicylic acid (ASA) or aspirin is probably one of the most essential drugs in human's pharmacology history, known as its excellent effects in treating cardiovascular-related diseases. Aspirin is one of the most essential drugs for treating cardiovascular diseases, which is one of the most widely used and successful drugs in the history of pharmacology. Aspirin mainly targets at the enzyme COX-1 and disrupts its normal function. The therapeutic effect of aspirin is caused by its subsequent metabolism inducing the failure in production of thromboxane A2 and the occurrence of platelet aggregation. The antithrombotic effect of aspirin has given researchers insight into diverse promising applications of aspirin and also causes a few adverse side-effects during its administration. Aspirin was also found very effective in relieving pain, inflammation, high temperature without inducing any unpleasant side effects. The first successful synthesis of aspirin was carried out by a young chemist

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called Felix Hoffman from Bayer pharmaceutical company in 1897, who obtained aspirin by reacting salicylic acid and acetic anhydride under reflux [1].

Being as one of the oldest synthetic drugs, aspirin was studied for various medical application throughout the years. Apart from the typical anti-inflammatory effect, many studies have shown the antiplatelet effect of aspirin. It is suggested that the antiplatelet effect of aspirin is due to its disruption of the normal function of monotropic integral enzymes COX-1 and COX-2 to synthesise thromboxane [2]. It is found out that aspirin also have promising anticancer potential by several other research groups [3]. Clear evidence has shown that regular daily low dosage of aspirin, the incidence of colorectal cancer and its mortality rate can be significantly reduced [4]. Moreover, the potential therapeutic effect of aspirin on mental and neurobiological diseases is discovered. Aspirin might be capable of preventing neuroinflammation, and hence oxidative stress development and Alzheimer's disease symptoms because of its excellent anti-inflammatory effect [2]. Several in vitro studies have also indicated that aspirin might have antiviral effect on various types of viruses. However, the inhibitory effect was observed to be the least on respiratory syncytical virus [5].

Despite having versatile application potentials, the application of aspirin still faces a few challenges. Through years of trials, aspirin has proved to be very effective in the secondary prevention of cardiovascular diseases (CVD) due to its preventive effect on arterial thrombosis. Nevertheless, drawbacks related to the secondary prevention of CVD by aspirin exist. The application of aspirin can possibly induce increased gastrointestinal bleeding risks. In addition, the feasibility of using aspirin for primary prevention of CVD is still unclear and relatively controversial and the risk of bleeding is observed to be unchanging [6]. Therefore, there are still a lot of potentials and improvements that needs to be done in the field of aspirin, and to understand these, this research focuses on the investigation and discussion on the pharmacokinetics and pharmacodynamics of aspirin.

2 Mechanism of the therapeutic effects of aspirin on CVD

The common targets for aspirin are cyclooxygenases (COXs), a membrane-bound glycoprotein, or prostaglandin endoperoxidase synthase [6]. COX is an enzyme family including 3 isoforms (COX-1, -2, and -3) and it is believed that the therapeutic effects of aspirin are closely relevant to the first two isoforms (COX-1 and COX-2) [7].

The core chemical reaction between aspirin and COX-1 is a selective acetylation of the hydroxyl group from a serine residue 70 amino acids away from the C terminus of COX-1, resulting in an irreversible inhibition of COX-1 [6]. This could prevent the substrate of COX-1, arachidonic acid (AA) from binding to the enzyme. The subsequent production of thromboxane A2 is consequently unable to proceed. Thromboxane A2 is an essential substance involving in stimulating platelet aggregation and vasoconstriction and the absence of thromboxane A2 can cause failure in platelet aggregation and vasoconstriction, and low level of adenosine diphosphate, whose release is highly dependent on platelet aggregation. Aspirin is thought to be antithrombotic through these serial chemical reactions and thus prevent the blood vessels from over-vasoconstriction, which can significantly have a pain-relief effect [8].

Aspirin is normally metabolised via the route illustrated above at regularly low dosage. Nevertheless, it is surprisingly discovered that at relatively higher dosage, aspirin will tend to inhibit COX-2 instead which is mainly found in inflammatory cells, responsible for generation of prostaglandin E2 during pathophysiological processes such as hyperalgesia and inflammatory reactions. The function of COX-1 also secrets prostaglandins, but they are mainly involved in physiological processes related to platelet aggregation. As research progresses, chemists start to realise that platelets also play an important role in various

inflammatory, immune responses and the maintenance of blood vessels so the inhibition of COX-1 by aspirin can result in anti-inflammatory effects in fact.

3 Aspirin in prevention and treatment of cancers

The most significant efficacy of aspirin is to inhibit the normal function of the enzyme cyclooxygenases (COXs), which regulate the generation of a lipid family called prostanoids that are found to be essential in most of the cancer signalling pathways. As aspirin is found to be involving in the disruption of numerous biological pathways regulating the proliferation of cancerous cells, this intrigues a huge research interest into the anti-cancer potential of aspirin [9].

Aspirin is shown to be effective in restricting the metastatic cancer spread in a *in vivo* trial. Since then, a large number of clinical trials were carried out aiming to testify the hypothetic therapeutic effect of aspirin on different types of cancers. The application of aspirin can have a beneficial effect on cancer at doses of 75 mg upwards, but no increasing therapeutic effect was observed as the doses continue to increase [10]. In one of the most well-known clinical trials, known as the Colorectal Adenoma Prevention Program (CAPP), patients with familial adenomatous polyposis (CAPP1) or Lynch syndrome (CAPP2) were exposed to aspirin dosed at 600 mg daily, 30 g of resistant starch and placebo in 2×2 factorial design. CAPP1 research has shown that the application of aspirin did not have significant effect in reduction of the colonic polyp number. However, decrease in the size of the largest polyps was indeed observed. CAPP2 mainly focuses on patients with Lynch syndrome, which is an uncommon dominant genetic disorder relevant to the risk of development of several different cancers. Aspirin might be capable of reducing the expression of MCM6 and RRM2, both playing an important role in DNA repair mechanisms. In CAPP2, the therapeutic effect on cancers by aspirin was also thought to be insignificant at first and it was confirmed until 2020, when extended clinical trials were carried out and aspirin was demonstrated to have positive effect in decreased incidence of colorectal cancer (CRC). As a result, it is very rare to prescribe aspirin as the remedy for cancers in many countries worldwide at current stage because of the indirect correlation between the primary working mechanism of aspirin and the cancer-inducing pathways and the possible danger of causing massive internal bleeding in the body, which is a major problem with aspirin and will be mentioned later. Nevertheless, patients suffering from Lynch Syndrome seem to be exceptions [11].

4 The clinical failure and adverse effects of aspirin

4.1 Aspirin resistance

Although aspirin has been proven to be one of the most effective drugs of treating cardiovascular diseases, the situation of non-responsiveness towards the application of aspirin was observed and reported from several clinical trials. This therapeutic failure can be reflected as platelet non-responsiveness and a reduction in the antiplatelet effect of aspirin mentioned before and is proposed to be caused by multiple factors involving both pharmacokinetic and pharmacological pathways [12].

The aspirin resistance has drawn great research interest to figure out its possibly viable mechanisms. It is suggested that aspirin resistance can be mainly subdivided into two types, pharmacokinetic resistance and pharmacodynamic resistance [13]. Pharmacokinetic resistance, usually COX-1 irrelevant, occurs when a sufficient concentration of aspirin is unable to be built up in the blood stream. This is mainly because of the inefficient absorption towards aspirin in the gut or the degradation of aspirin by the digestive fluid in most common

cases. As shown in Fig. 1, the normal therapeutic pathway of aspirin including the inhibition of generation of thromboxane A2 (TXA2), and subsequent platelet aggregation is failed to proceed, eventually resulting in aspirin resistance. Pharmacodynamic resistance on the other hand, occurs when a sufficient concentration of aspirin has already been present in the bloodstream. This type of aspirin is usually COX-1 related and genetically caused in most cases. Polymorphism in the gene regulating the synthesis of COX-1 enzyme or the distortion in the conformation of platelet itself can make aspirin no longer capable of inhibiting COX-1 enzyme and the following platelet aggregation. Other reports have also shown that the genetic polymorphism in COX-1 enzyme may raise the incidence of an efflux anion plump Multidrug Resistance Protein 4 (MRP4), which is another contributor towards the reduced inhibition of COX-1 enzyme functionality in human body [14]. Aspirin resistance is quite a severe treatment failure with a high incidence of almost 20-30% of the patients in some research studies and these studies have also shown that aspirin resistance may even aggravate the adverse cardiovascular symptoms by almost 3-fold in some reported extreme cases.

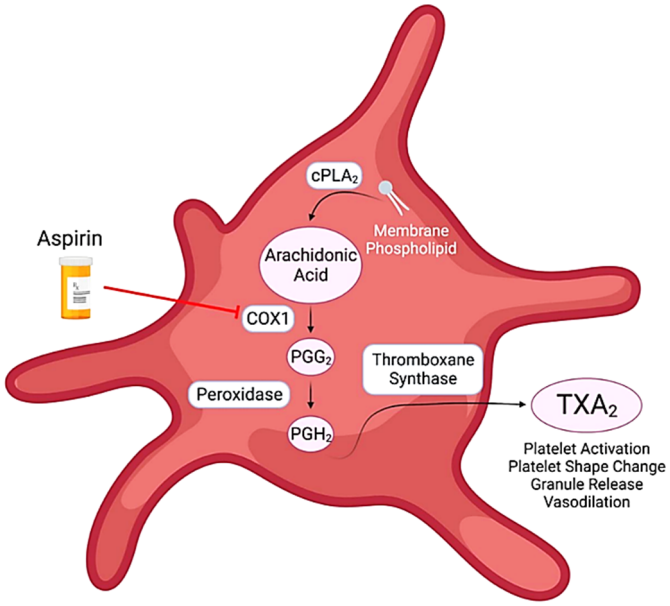

Fig. 1. The pharmacological reaction after aspirin is absorbed and the process how inhibition of enzyme COX-1 results in platelet aggregation and failure in the generation of TXA2 [13].

Figuring out the promising ways of diagnosing, treating and curing aspirin resistance has always been a heated research topic. As shown in Fig. 2, numerous powerful diagnosis techniques for aspirin resistance have been developed, including *Light Transmission Aggregometry* (LTA), which measures the change in the light transmittance to indicate the activity of COX-1 enzyme, *Thromboxane B2* (TXB2) Testing, which measures the blood level of COX-1 enzyme activity indicator molecule TXB2, *Thromboelastogrphy*, which provide a real-time measurement of viscoelastic clot strength in blood serum by forming a hemoplastic plug on a torsion wire suspended on a needle [13]. As for the treatment of aspirin resistance, it is proven to be a huge challenge and there are no effective therapeutic remedies to fully overcome the non-responsiveness towards aspirin in patients taken in low doses of aspirin at current stage. It is reported that through an *in vitro* experiment that by increasing the dose of aspirin or using omega-3 fatty acids along with the aspirin, the resistance against the efficacy of aspirin might be relieved, but detailed data supporting this observation remain quite limited [15]. Research looking into diverse different alternative treatment schemes for

aspirin are also carried out to avoid aspirin resistance. Well-known replacement for aspirin includes P2Y(12) receptor inhibitors *clopidogrel* and *ticagrelor*, which also work by inhibiting platelet aggregation, especially the ADP-induced platelet activation and in some clinical trials it was shown to have even stronger efficacy in preventing extremely adverse and fatal cardiovascular events than aspirin. Another effective alternative drug for aspirin is called *rivaroxaban*. However, the drawback of the administration of this drug is that it will cause massive bleeding so in general it is usually used along with aspirin [13]. Several other studies have also pointed out the potential of using Chinese herbal medicine as the alternative treatment for aspirin. They discovered that some types of herbal medicine can also have significant antiplatelet effect [16].

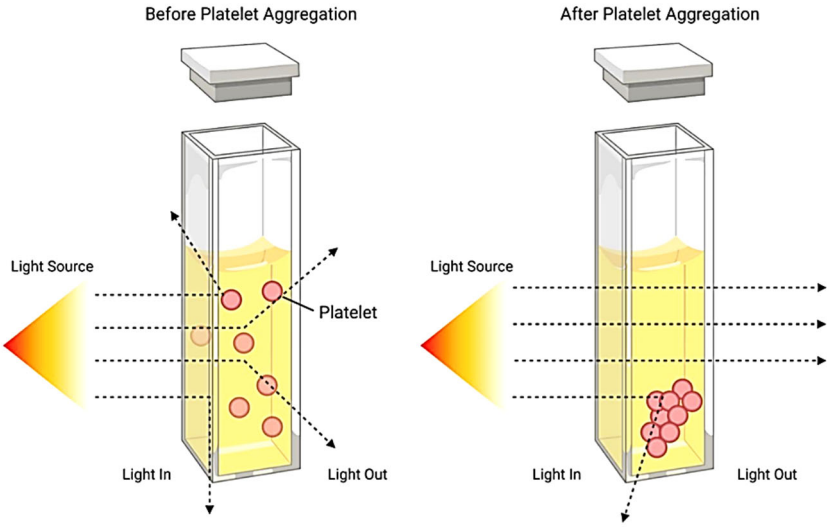

Fig. 2. The working mechanism of LTA. The dotted lines represent the light beams emitted from the light source [13].

4.2 Bleeding effect caused by aspirin

Although aspirin is proven to be effective in prevention of various cardiovascular diseases at low doses, it is also known for its notorious gastrointestinal bleeding effect after a long-term administration [17]. The bleeding risk is mainly due to the topical mucosal injury caused by aspirin. Another essential factor of the bleeding risk is that the COX-1 enzyme inhibition induced by the application of aspirin can cause significant reduction in the concentration of prostaglandin in the bloodstream [6].

There have been a large number of research studies providing clinical data on the upper gastrointestinal bleeding (UGIB) and lower gastrointestinal bleeding (LGIB) caused by the administration of aspirin. The risk of inducing UGIB might be relatively higher when aspirin is used as CVD secondary prevention agent than that in the primary prevention cohort, although critics argue that this observation is not convincing enough as the patients studied on were mostly old and have a history of ulcers so the baseline of the risk of UGIB in these patients is higher than regular level. The risk of UGIB might be relevant to the frequency of aspirin doses given and higher dosing frequency tend to induce more severe UGIB risk [18]. Two clinical trials that last for up to 10 years or 20 years point out that the dose of aspirin administered has very subtle impact on the RR of UGIB. In addition, it was also observed that the most significant risk of inducing UGIB generally occurs during the first 2 months of the therapy and RR of UGIB normally declines with increasing treatment period length [19].

Investigations focusing on the possible effect of aspirin on the RR of lower gastrointestinal bleeding were also carried out. However, the research outcome obtained turned out to be not very consistent from research programs to others. The administration of aspirin seems to significantly enhance the RR of LGIB in some studies, but the reported situation is completely on the contrary in some other studies. Nevertheless, the results collected from the majority of the studies undergone show that the use of aspirin in fact impose very little or no significant danger on inducing LGIB [17].

5 Conclusion

Aspirin works as one of the most historic, widely used, effective remedies for treatment of cardiovascular diseases. It mainly undergoes a mechanism via enzyme COX-1 inhibition, disrupting the normal process of platelet aggregation and causing significant antithrombotic effect. The antithrombotic property of aspirin intrigues the research interest into the anticancer potential of aspirin. Though aspirin was observed to show some suppressive effect on the metastatic growth of cancerous cell sample collected from patients with Lynch syndrome in a few clinical trials, there is no direct pharmacological and clinical evidence to show that the efficacy of aspirin is closely related to the cancer development pathways. Further investigations are required to fully explore the anticancer potential of aspirin. The application of aspirin also faces challenges, including aspirin resistance and the possible bleeding effect caused by aspirin. The aspirin resistance can be either pharmacokinetic and pharmacodynamic, aroused by poor absorption of aspirin and genetic polymorphism respectively. Research studies have suggested possible ways to diagnose the aspirin resistance and substitution for aspirin treatment. Nevertheless, further investigations are required to confirm whether the efficacy of the treatment can be maintained while reducing the body resistance towards the drug administered. As for the bleeding risk caused by the administration of aspirin, aspirin can mainly cause the bleeding in the upper gastrointestinal tract. To avoid such low-dose aspirin UGIB risk, the frequency of the use of aspirin should be as reasonably low as possible and the administration of aspirin should be ideally in long-term because administration of aspirin as long as up to more than 10 years the UGIB risk could be minimised.

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