New Topics in Aspirin Therapy

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Abstract: Aspirin (acetylsalicylic acid, ASA), which was initially developed as an analgesic anti-inflammatory agent, has come to be the basis of antiplatelet therapy, and firm evidence supporting its usefulness has continued to accumulate. ASA irreversibly inhibits platelet function by acetylating cyclooxygenase (COX), which is involved in the production of a potent platelet stimulator, thromboxane A₂. There are two types of COX, one that is constitutively expressed in platelets (COX-1) and another that is induced in other tissues, including vascular endothelial cells (COX-2). Although ASA inhibits COX-1 more selectively, it also exerts an inhibitory effect on COX-2, the mechanism of which is considered partly a result of salicylation. The inhibition of COX-2 by ASA forms the basis of its anticipated role in the prevention of colorectal cancer and Alzheimer's disease and the inhibition of the progression of these diseases. It has been pointed out that the incidence of cardiovascular events tends to be high among patients who are not responsive to ASA (aspirin-resistant patients), but the reason for this increased incidence remains unclear. Interesting discussion in regard to ASA is likely to emerge in the future.

Key words: Aspirin; COX-1; NSAID; Antiplatelet therapy

Introduction

Aspirin, or acetylsalicylic acid, is a long-established drug with a history of more than 100 years since its synthesis in 1899 by Hoffman of Bayer Co., Ltd., of Germany. Aspirin, an over-the-counter drug that can be taken easily, was produced by enhancing the efficacy of salicylic acid, an active component of herbal medicines, including willow leaves. These herbs have been known to have analgesic properties since the days of Hippocrates. Although aspirin is the trade name of the acetylsalicylic acid manufactured by Bayer, it is now commonly used as a generic term, even in the scientific literature.

Although aspirin was used as an analgesic anti-inflammatory agent for five decades, in the 1960s it gradually became apparent that an adverse effect, namely, bleeding, is a by-
product of its inhibitory effect on platelet aggregation. At about the same time, through rapidly progressing studies on prostaglandins, it became apparent that aspirin inhibits biosynthesis of the prostaglandin system, particularly of thromboxane A₂ (TXA₂), a potent platelet agonist. This is the mechanism of aspirin’s antiplatelet effect. In fact, it had long been noted that ischemic vascular disorders such as myocardial infarction and cerebral infarction were less frequent among regular aspirin users. As evidence accumulated, aspirin became recognized as an anti-thrombotic agent, rather than solely an anti-inflammatory drug.

In 1979, the US Food and Drug Administration (FDA) approved aspirin as a preventive therapy for recurrent stroke, and its indications were extended to the prevention of recurrent myocardial infarction in 1985. Thereafter, clinical data continued to accumulate, and statistical data from a study by an international study group, the Antiplatelet Trialists’ Collaboration (APT) study, were published in 1994, helping to establish antiplatelet therapy including aspirin as a first-line measure for the treatment and prevention of recurrence of arterial thrombosis. Japan, which was slower to follow this trend, approved the use of aspirin for antiplatelet therapy in 2000.

This paper outlines recent topics on the efficacy of aspirin from the clinical viewpoint, while tracing the basic characteristics of the drug.

## Pharmacology of Aspirin

When platelets are activated at the site of thrombus formation, arachidonic acid is cleaved from membrane phospholipids as the intracellular calcium concentration increases. This fatty acid then is converted to the very unstable prostaglandin G₂/H₂ by the action of cyclooxygenase (COX). TXA₂ then is produced by thromboxane synthetase, which is specific to platelets. TXA₂ has a potent platelet-activating effect, and induces stability of platelet aggregates by promoting further activation of platelets themselves. Aspirin inhibits the function of COX, the rate-limiting enzyme for this pathway, and thereby inhibits the stabilization of platelet aggregates and formation of platelet thrombi.

Aspirin exerts its action by causing acetylation of the enzyme with the acetyl group of its molecule at the 529th amino acid serine, which lies in the vicinity of the active center of the enzyme’s configuration. The important point is that this change is irreversible. Platelets are cells devoid of nuclei. Once its function has been irreversibly suppressed by aspirin, the enzyme in the cell exists as it is without replacement by a new enzyme until the life of the cell ends. In other words, the pharmacologic effect of aspirin lasts for more than a week, the life span of a platelet.

### 1. COX-1 and COX-2

COX, the target of aspirin, is present in all tissues. In vascular endothelial cells, for example, it induces the production of prostacyclin (or prostaglandin I₂), the physiological action of which is opposed to TXA₂, i.e., it has an antiplatelet action. Therefore, a problem theoretically can arise here. Aspirin also inhibits prostacyclin, which has an antiplatelet action, and the possibility exists that the antiplatelet effect resulting from the inhibition of TXA₂ production is attenuated or eliminated. This is the so-called aspirin dilemma.

Fortunately, unlike the platelet, continuous protein synthesis in endothelial cells renews the nonacetylated enzyme. In addition, the cyclooxygenase present in platelets (COX-1) is more sensitive to aspirin than that present in endothelial cells (COX-2). Therefore, it is possible to overcome this problem by lowering the dose of aspirin. This is the basis of low-dose aspirin in anti-thrombotic therapy. Since aspirin inhibits the production of prostaglandin E₂, which plays an important role in maintaining the homeostasis of gastric mucosal cells, it is speculated that the use of aspirin may be asso-
associated with adverse reactions such as gastrointestinal symptoms and gastric ulcer.

In addition to aspirin, non-steroidal anti-inflammatory drugs (NSAIDs) also exert anti-inflammatory actions by similarly inhibiting COX. However, their actions are reversible, unlike those of aspirin, although NSAIDs are more potent than aspirin. The antiplatelet effects of NSAIDs attenuate as their blood concentrations decrease.

In recent years, it has become apparent that there are two COX isozymes with different types of gene regulation: COX-1, which is distributed widely and constantly over platelets and gastric mucosal cells, and COX-2, which is induced in cells through stimulation by inflammatory cytokines and growth factor. Therefore, the chronic inflammatory reaction depends on COX-2. Characteristically, the affinity of NSAIDs, including aspirin, for these two COX isozymes varies among different drugs. While many NSAIDs are capable of inhibiting both isozymes to some extent, the inhibitory action of aspirin is more specific to COX-1. Anti-inflammatory drugs selectively acting on COX-2 have recently become available for practical use. As characteristic features, these drugs are associated with hardly any gastrointestinal problems resulting from the inhibition of COX-1 or bleeding symptoms induced by antiplatelet effects.

2. Acetylation and salicylation

After its absorption from the intestinal tract, aspirin rapidly inactivates COX-1 in platelets by acetylation during the enterohepatic circulation of the drug, and is immediately deacetylated into salicylic acid by esterase in the liver. Aspirin is eliminated from the systemic circulation following its peak concentration, which is achieved only about 15 minutes after aspirin administration, and decreases gradually thereafter at a rate of about 10% per day in proportion to the lifespan of platelets. It is preferable to increase the dose if an acute antiplatelet effect is desired.

Aspirin is absorbed from the intestinal tract and converted to salicylic acid by rapid deacetylation with esterase in the liver. Aspirin reaches its peak blood concentration at about 15 minutes, and is then eliminated from the systemic circulation in 2 hours. In contrast, salicylic acid is present in the blood for up to 12 hours or more. The inhibitory effect on platelet aggregation reaches its maximum level 4 hours after aspirin administration, and decreases gradually thereafter at a rate of about 10% per day in proportion to the lifespan of platelets. It is preferable to increase the dose if an acute antiplatelet effect is desired.

Aspirin (acetylsalicylic acid) exerts its pharmacologic effects by acetylation and salicylation of the target molecule. Antiplatelet effects are achieved by selective acetylation of COX-1, and anti-inflammatory, anti-proliferative, and anti-oxidative effects are achieved by salicylation of COX-2 and various signal enzymes (*) related to inflammation and cell proliferation.
In actuality, a number of basic experiments have suggested the possibility that the action of the metabolite salicylic acid is also involved (Fig. 2).5)

More specifically, it has become apparent that salicylic acid may exert anti-proliferative and anti-inflammatory actions in a dose-dependent manner by inhibiting the proliferation of smooth muscle cells constituting the vascular wall and by inhibiting the inflammation-stimulated increase in the adhesiveness of macrophages. The targets are considered to be the key transcription factors NFκB and AP1 and various enzymes in the Ras/MAP kinase system and the PI3 kinase system, which are major signal transmission pathways for inducing these cell responses.

It has previously been noted that salicylic acid may selectively inhibit COX-2. The current view of the pathogenesis of arterial thrombosis is that arterial thrombosis originates from atherosclerosis, the basis being chronic inflammation of the vascular wall. Therefore, it is possible that salicylation by aspirin, or acetylsalicylic acid, exerts a protective effect against the progression of atherosclerotic lesions. To achieve this protection, it may be necessary to obtain higher blood concentrations of salicylic acid by increasing the dose of aspirin, as in cases of anti-rheumatic therapy. On the other hand, dose increase is far from acceptable as an anti-thrombotic medication in view of the aspirin dilemma, and it is obvious that it would be associated with a higher incidence of side effects such as gastrointestinal disorders.

3. Inhibitory action of NSAIDs on the effects of aspirin

Aspirin has very high selectivity for COX-1. Among other NSAIDs, COX-1/COX-2 selectivity varies according to the drug. Importantly, some of these NSAIDs have a COX-1 inhibitory action that is antagonistic to that of aspirin. For the substrate arachidonic acid to undergo enzymatic processing, it is necessary that it reach the active center of COX-1, which is located deep inside the hydrophobic pocket, which has a narrow entrance. Aspirin and other NSAIDs inhibit access of the substrate by, respectively, acetylating the 529th amino acid, i.e., a serine residue lying near the active center, and by directly binding to the active center. Therefore, if an NSAID, which is a macro-molecule, binds to COX-1 in advance, no effects can be expected from aspirin.3)

When an 81 mg dose of aspirin and 400 mg dose of highly COX-1-selective ibuprofen were administered at a 2-hour interval in the morning on 6 consecutive days, the order of the two doses was critical. When ibuprofen was administered prior to aspirin, only the reversible inhibition of COX-1 was noted, whereas the stable antiplatelet effects of aspirin occurred when the two drugs were administered in the reverse order.6) In addition, when continuous use of enteric-coated aspirin was combined with ibuprofen, 3 times a day, the stable antiplatelet effects of aspirin were suppressed. On the other hand, no such aspirin antagonism as observed with ibuprofen occurred when a selective COX-2 inhibitor, rofecoxib, and diclofenac or acetaminophen, which have high selectivity for COX-2, were used. It is also known that indomethacin acts similarly to ibuprofen.

Although clinical corroboration is lacking, physicians may have to be prudent in using NSAIDs for patients on low-dose aspirin therapy in whom stable antiplatelet effects are desired. At least, NSAIDs with high sensitivity to COX-2 (diclofenac, etodolac, meloxicam) are preferable when using NSAIDs, given that selective COX-2 inhibitors are not commercially available in Japan. On the other hand, it has been noted that selective COX-2 inhibitors may induce thrombosis by inhibiting prostacyclin production in vascular endothelial cells. In any case, this issue needs to be addressed in a large-scale clinical trial.

4. Aspirin resistance

It has been reported that 10–60% of patients
on aspirin therapy are resistant to aspirin, and such patients are at risk of developing cardiovascular events.\(^7\) Although there may be a number of reasons for this, one that has attracted recent attention is that of increased expression of COX-2, which is hardly present in platelets under normal circumstances. It is speculated that COX-2 shows resistance to aspirin in low-dose aspirin therapy because COX-2 has very low sensitivity to aspirin. In addition, COX genes have more than 100 one-base substitutions, and it is suggested that some of them are associated with structural changes for which aspirin cannot be sufficiently effective. Differences between individuals present an important issue in clinical pharmacology.

**Clinical Efficacy of Aspirin**

1. **Antiplatelet therapy and the aspirin dilemma**

A number of clinical trials have been carried out to examine the efficacy of antiplatelet therapy with aspirin or other antiplatelet drugs in the prevention of recurrence and treatment of ischemic vascular injury. Data from the high-quality randomized prospective studies among these trials were analyzed comprehensively using meta-analysis, and the results were published in 1994, as the APT data analysis mentioned at the beginning of this paper. The efficacy of antiplatelet drugs, particularly aspirin, was established by this analysis. Accumulation of data from clinical trials continued, and the results of analysis limited to antiplatelet therapy, including data for the 8 years after APT, were published at the beginning of 2002 by the Antithrombotic Trialists’ Collaboration as a revised edition of the APT data.\(^8\)

Additional findings on aspirin and other antiplatelet drugs include the following: (1) aspirin significantly prevents cardiovascular events in high-risk patients who have stable angina, intermittent claudication, or atrial fibrillation; (2) prompt use of aspirin in patients with acute myocardial infarction or cerebral infarction is useful, and the preferable acute loading dose is 150–300 mg; and (3) low doses (75–150 mg/day) exert sufficient anti-thrombotic effects and show no marked difference from moderate (160–325 mg/day) or high (500–1,500 mg/day) doses. On the other hand, very low doses of less than 75 mg, used in consideration of aspirin dilemma, failed to produce a stable effect. However, the number of very-low-dose cases was too small to deny aspirin dilemma.

**Table 1 Results of Analysis of the Preventive Effect of Cerebral Vascular Accident in Relation to Aspirin Dose in ATT (Adapted from a figure in reference 8.)**

The table shows the results of meta-analysis of controlled studies comparing the incidence rates of cerebral vascular accident for aspirin therapy and placebo control groups of high-risk patients (present or previous arterial thrombosis, stable angina, transient cerebral ischemia, atrial fibrillation). Aspirin at low doses of 75–150 mg showed an adequate preventive effect. Higher doses were also expected to be effective. In contrast, very low doses of less than 75 mg, used in consideration of aspirin dilemma, failed to produce a stable effect. However, the number of very-low-dose cases was too small to deny aspirin dilemma.

\[\text{Table 1 Results of Analysis of the Preventive Effect of Cerebral Vascular Accident in Relation to Aspirin Dose in ATT (Adapted from a figure in reference 8.)}\]

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>No. of trials with data</th>
<th>Incidence of vascular events (%)</th>
<th>Relative risk</th>
<th>% Odds reduction (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500–1,500</td>
<td>34</td>
<td>14.5</td>
<td>17.2</td>
<td>19 (3)</td>
</tr>
<tr>
<td>160–325</td>
<td>19</td>
<td>11.5</td>
<td>14.8</td>
<td>26 (3)</td>
</tr>
<tr>
<td>75–150</td>
<td>12</td>
<td>10.9</td>
<td>15.2</td>
<td>32 (6)</td>
</tr>
<tr>
<td>&lt;75</td>
<td>3</td>
<td>17.3</td>
<td>19.4</td>
<td>13 (8)</td>
</tr>
<tr>
<td>Any aspirin</td>
<td>65(^*)</td>
<td>12.9</td>
<td>16.0</td>
<td>23 (2)</td>
</tr>
</tbody>
</table>

*Some trials contributed to more than one comparison.
2. Anti-tumor therapy

Based on the fact that the activity of prostaglandin E\textsubscript{2} is increased in colorectal cancer tissues, an inhibitory effect of NSAIDs on the proliferation of colorectal cancer has been suggested. Indeed, epidemiologic data that the incidence of colorectal cancer was lower in aspirin users than in the general population were reported by several researchers.\textsuperscript{9} Among these data, the Melbourne Colorectal Cancer Study in 1988 revealed that the incidence of colorectal cancer was 40–50% lower in regular aspirin users than in controls. In addition, a large-scale study performed in 1991 covering 600,000 people showed that the rates of colorectal cancer were 40% and 52% lower in men and women, respectively, who took 16 doses of aspirin per month than in controls.

It became apparent that COX-2 expression is increased in patients with colorectal cancer or familial polyposis. The ability to prevent proliferation and cause regression of colorectal cancer/familial polyposis was also found in other NSAIDs, in addition to aspirin, and it has been considered that such effects are at least partly attributable to COX-2 inhibition by these drugs. In fact, several randomized studies in patients with familial polyposis, who are at high risk of developing colorectal cancer, led to accreditation by the World Health Organization (WHO) of the colorectal cancer-inhibiting agents sulindac, an NSAID with high selective COX-2 inhibitory activity, and selecoxib, a selective COX-2 inhibitor.

In contrast, in the well-known Physicians’ Health Study, in which the frequencies of cardiovascular events in male physicians who took aspirin (325 mg every other day) or a placebo for 5 years were followed for 12 years, sub-analysis showed no difference in the incidence of colorectal cancer between the two groups. Currently, a number of clinical trials examining the inhibitory effect of aspirin at a daily dose of 80–600 mg on the development and progression of colorectal cancer in patients with familial polyposis, a high-risk group, are underway, in addition to studies of the efficacy of other NSAIDs. It is expected that the efficacy of aspirin will be better defined within a few years.\textsuperscript{9}

In addition, the epidemiologic finding that Alzheimer’s disease is less frequent in rheumatic patients who are on prolonged aspirin therapy suggests the diverse potential of the clinical efficacy of aspirin. This may be related to the observed increase of COX-2 expression in microglia cells in the areas surrounding lesions.\textsuperscript{10}

Conclusion

Because of its antiplatelet activity for the prevention and treatment of thrombosis, aspirin is one of the most commonly used drugs in the world. However, it has been reported that only one-fourth of all patients with coronary artery disease amenable to aspirin therapy currently use it. If the administration of aspirin to suitable patients is increasingly promoted as recognition by general clinicians is enhanced, aspirin will undoubtedly become the most frequently used drug in the world. No other drug is so inexpensive and has such abundant scientific evidence of its clinical efficacy. However, new questions are arising as the understanding of aspirin deepens, and the potential of its diverse efficacy is staggering. Aspirin, therefore, is not only a well-established therapeutic agent but also an exciting new drug.

REFERENCES


