Aspirin's Effect on Cardiovascular Disease via Cyclooxygenase Inhibition

Forrest Tolman

Copyright May, 2015, Forrest Tolman and Koni Stone

Cardiovascular disease, also termed heart disease, refers to any disease that affects the cardiovascular system. The three most common forms of cardiovascular disease include: heart attack - when a blood clot forms in the heart, blocking blood flow, a stroke - which is essentially the same thing except occurring in the brain, and heart failure - when the heart fails to pump blood as efficiently as it should. Cardiovascular disease is the most prevalent disease in the U.S., and is consistently the leading cause of death amongst the population. In 2013 alone, cardiovascular disease resulted in the recorded deaths of roughly 750,000 people. For comparison, that's roughly 25% more than cancer.\(^{10}\)

Here's where aspirin comes into play. Acetylsalicylic acid (aspirin) is classified as a nonsteroidal anti-inflammatory drug (NSAID), and is commonly used to relieve fever and pain. Beginning in the 1990s however, doctors, (and eventually the FDA), began suggesting daily low-doses (~81mg) of aspirin as a preventative to combat cardiovascular disease.\(^3\) Currently, the U.S. Preventive Task Force suggests for males aged 45-79 and females aged 55-79 to take aspirin daily as a cardiovascular disease preventative.\(^4\)

In order to understand aspirin's role in cardiovascular health, it is crucial to first understand the causation of cardiovascular disease at the molecular level within the body.

Arachidonic acid (AA) is a polyunsaturated omega-6 fatty acid that is present amongst the phospholipids in the membranes of the body's cells, and is an important precursor to numerous bodily functions. Cyclooxygenase-1 (COX-1) and
cyclooxygenase-2 (COX-2) are some of many enzymes found throughout the body that react with AA. When COX-1 reacts with AA, it initiates a biological pathway that results in an increased production of thromboxane-A2 (TxA2), as well as prostaglandins E2, D2, and F2α. The primary role of TxA2, in addition to being a vasoconstrictor, is to promote platelet aggregation (the thickening of the blood). The prostaglandins are largely responsible for the synthesis and regulation of gastric mucosa, which is the layer of mucus lining the stomach walls that allow it to safely hold acid. When COX-2 reacts with AA, it initiates a biological pathway that results in an increased production of prostacyclin I2 (PGI2). PGI2, in addition to being a factor responsible for the sensation of pain, is also a vasodilator and thins the blood. Essentially, PGI2 can be viewed as the biological opposite of TxA2.

Aspirin is an inhibitor of both COX-1 and COX-2, but inhibits the former roughly 170 times more than the latter, making it a COX-1 selective NSAID. Aspirin inhibits COX-1 by irreversibly acetylating the serine located at residue 529. In doing this, it
prevents AA from having access to the tyrosine located at residue 385 by blocking the narrow hydrophobic channel that AA must pass through to reach the tyrosine. As a result, the enzyme is not activated and the corresponding prostaglandins and thromboxane associated with COX-1 are not synthesized. Because aspirin covalently modifies the enzyme, it becomes permanently deactivated. As a result, normal platelet function will not return for up to a week, (the time it takes for the body to create new platelets with new COX enzymes). Although this inhibition has desirable effects, such as the thinning of the blood which decreases the chances of cardiovascular disease, it also has undesirable traits including: weakening of the gastric mucosa, (which increases chance of ulcers), as well as an increased bleed time from trauma to the flesh (as a result of the thinning of the blood).

Many other common NSAIDs, such as ibuprofen (Advil/Motrin), are non-selective COX inhibitors, meaning they inhibit both COX isozymes equally. As opposed to aspirin, they reversibly inhibit COX-1, and only inhibit the enzyme for a few hours because they do not covalently modify the cyclooxygenase enzyme like aspirin does. Because aspirin permanently disables COX-1 instead merely inhibiting it for a few hours, it is the superior NSAID when taken for the intent of improving cardiovascular health.

Albeit far less common due to greater health concerns, a few other NSAIDs are COX-2 selective. Recent understandings of cyclooxygenase have resulted in recalls for the majority of COX-2 selective NSAIDs due to increased harmful cardiovascular events (recall the cardiovascular benefits of PGI₂). Furthermore, studies have shown that, despite popular belief, COX-2 selective NSAIDs are no more efficient in the nullification of pain when compared to other non-selective COX inhibitors. Some recent well-known recalls include: Vioxx and Bextra.
There is much promise regarding the future of NSAIDs. In 2013, Rafael Consolin Chelucci and his team at the Sao Paulo State University attempted to find an improved NSAID that would provide comparable inhibition of platelet aggregation to aspirin, but without the increase in bleed time that is also associated with it. They used five common NSAIDs and attached an n-acyl hydrazone subunit to each of them. These five compounds, in addition to regular aspirin, were then tested on mice to measure how well each NSAID inhibited plate aggregation in relation to bleed time. Bleed time was assessed via a small incision on each mouse's tail, and the resulting blood was soaked up using filter paper, which was monitored until the bleeding halted. The time from incision to the time of the stoppage of bleeding was recorded and labeled as 'bleed time.' Platelet aggregation was monitored using a Chrono-Log aggregometer. Blood was collected from a series of mice and centrifuged. A small amount of the resulting platelet-rich plasma was then collected and mixed with 150μM of one of the NSAID compounds, (or DMSO for the control), and the aggregation curve was determined.⁶

<table>
<thead>
<tr>
<th>NSAID Title</th>
<th>Structure</th>
<th>% Inhibition of AA-Induced Platelet Aggregation</th>
<th>Bleed Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No NSAID)</td>
<td>-</td>
<td>0</td>
<td>343 (±11.5)</td>
</tr>
<tr>
<td>Aspirin</td>
<td></td>
<td>80 (±1.6)</td>
<td>818 (±21.8)</td>
</tr>
<tr>
<td>Compound 1</td>
<td></td>
<td>71.4 (±2.2)</td>
<td>642 (±18.1)</td>
</tr>
<tr>
<td>Compound 2</td>
<td></td>
<td>80.8 (±1.1)</td>
<td>1271 (±24.9)</td>
</tr>
<tr>
<td>Compound 3</td>
<td></td>
<td>66.8 (±1.7)</td>
<td>671 (±16.7)</td>
</tr>
<tr>
<td>Compound 4</td>
<td></td>
<td>67.6 (±2.1)</td>
<td>1178 (±25.3)</td>
</tr>
<tr>
<td>Compound 5</td>
<td></td>
<td>65.9 (±1.3)</td>
<td>653 (±14.1)</td>
</tr>
</tbody>
</table>

Data are expressed as the means ± standard errors of the means. Statistical differences between the experimental and control groups were evaluated by analysis of variance followed by the Tukey test. p < 0.01 vs. the control group; p < 0.01 vs. Aspirin.
It is worth noting that Compounds 1, 3, and 5 resulted in ~20% shorter bleeding times than that of aspirin. However, they also inhibited platelet aggregation by ~15% less. Compound 2 was the only compound that inhibited platelet aggregation by at least the same amount as aspirin, but resulted in ~50% longer bleeding times, (Compound 2 was also a derivative of aspirin, as can be seen from the structure). Although an improved replacement for aspirin wasn't necessarily discovered this experiment, the researchers claim the added n-acyl hydrazone subunits were a success, and the next step would likely be to modify and improve one of Compounds 1, 3, or 5 to inhibit platelet aggregation more, since all three showed reduced bleeding times when compared to aspirin.\(^6\)

Furthermore, a third form of cyclooxygenase, (named COX-3), has recently been uncovered in the early 2000s. The biological roles of this new isozyme are uncertain, but it is hypothesized that it may play a role in sleep, the sensation of pain, and perhaps even in cardiovascular disease. Further investigation in this area will lead not only to a better understanding of the role of COX-3 enzyme in these areas, (or lack thereof), but also to more specific and efficient treatments of ailments involving this enzyme.\(^8\)
References


