Chapter 7

Non-Steroidal Anti-Inflammatory Drugs and Increased Risk of Sudden Cardiac Death

Soyun M. Hwang, Jennifer E. Gilda, Ziyou Cui and Aldrin V. Gomes

1Department of Neurobiology, Physiology, and Behavior, and
2Department of Physiology and Membrane Biology,
University of California, Davis, CA, US

Introduction

Non-steroidal anti-inflammatory drugs, or NSAIDs, are commonly prescribed, and some are available as over-the-counter (OTC) medications to alleviate pain, inflammation, and fever associated with a diverse array of medical conditions (Table 1, Figure 1) [1]. Each year in the United States alone, 70 million prescriptions are written for patients suffering from painful conditions such as rheumatoid arthritis, osteoarthritis, migraine, ankylosing spondylitis, Reiter’s syndrome, acute gout, menstrual pain, and others [2].

*Corresponding author: Dr. Aldrin V. Gomes, 176 Briggs Hall, One Shields Avenue, Department of Neurobiology, Physiology, and Behavior, University of California, Davis, Davis, CA 95616, US. E-mail: avgomes@ucdavis.edu. Phone: 530-752-3207; Fax: 530-752-5582.
Including the use of OTC NSAIDs, approximately 30 billion dosages are consumed annually [3].

### Table 1. Types of NSAIDs, their common trade names, and typical clinical uses

<table>
<thead>
<tr>
<th>NSAID</th>
<th>Trade names, Other names</th>
<th>Clinical Uses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-prescription</td>
<td></td>
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<tr>
<td>Aspirin</td>
<td>Anacin®, Ascriptin®, Bayer®, Bufferin®, Excedrin®</td>
<td>The prototypical analgesic for mild to moderate pain, inflammation, fever, and minor aches. Also inhibits platelet aggregation and is used in the prevention of arterial and venous thrombosis.</td>
<td>[164]</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Motrin®, Advil®, Nuprin®, Medipren®</td>
<td>Most commonly used for rheumatism and arthritis. Also used for headaches, muscle aches, arthritis, toothaches, backaches, common cold, and menstrual cramps.</td>
<td>[165]</td>
</tr>
<tr>
<td>Naproxen sodium</td>
<td>Aleve®</td>
<td>Used to relieve pain, tenderness, swelling, and stiffness associated with osteoarthritis, rheumatoid arthritis, juvenile arthritis, and ankylosing spondylitis. Can also be used for more general pain from headaches, backaches, fever, cold, toothache, and menstrual pain.</td>
<td>[166]</td>
</tr>
<tr>
<td>Prescription</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Celecoxib</td>
<td>Celebrex®</td>
<td>Prescribed for the treatment of osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms, and to reduce numbers of colon and rectum polyps in patients with familial adenomatous polyposis.</td>
<td>[167]</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>Voltaren®</td>
<td>For relieving pain, tenderness, swelling, and stiffness caused by osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. Also used to treat painful menstrual periods.</td>
<td>[167, 168]</td>
</tr>
<tr>
<td>Diflunisal</td>
<td>Dolobid®</td>
<td>Used for pain, tenderness, swelling, and stiffness caused by osteoarthritis and rheumatoid arthritis.</td>
<td>[169, 170]</td>
</tr>
<tr>
<td>Etodolac</td>
<td>Lodine®</td>
<td>Effective for treating pain associated with osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. Also alleviates postoperative pain.</td>
<td>[171]</td>
</tr>
<tr>
<td>NSAID</td>
<td>Trade names, Other names</td>
<td>Clinical Uses</td>
<td>Reference</td>
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</tr>
<tr>
<td>Fenoprofen</td>
<td>Nalfon®</td>
<td>Effective for osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and in the alleviation of postoperative pain. It is pharmacologically similar to aspirin, but causes less gastrointestinal bleeding.</td>
<td>[172]</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>Ansaid®</td>
<td>Helps reduce bone reabsorption in periodontal disease by inhibiting carbonic anhydrase. Used to relieve pain, tenderness, swelling, and stiffness caused by osteoarthritis and rheumatoid arthritis.</td>
<td>[173]</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Indocin®</td>
<td>Relieves moderate to severe pain, tenderness, swelling, and stiffness caused by osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, bursitis, and tendinitis.</td>
<td>[174]</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Orudis®, Oruvail®, Ketoflam®</td>
<td>An ibuprofen-type anti-inflammatory analgesic and antipyretic used in the treatment of rheumatoid arthritis and osteoarthritis.</td>
<td>[162, 175]</td>
</tr>
<tr>
<td>Meclofenamate sodium</td>
<td>Meclomen®, Ponstel®</td>
<td>Used to relieve pain, tenderness, swelling, and stiffness caused by osteoarthritis and rheumatoid arthritis.</td>
<td>[176]</td>
</tr>
<tr>
<td>Oxaprozin</td>
<td>Daypro®</td>
<td>Used to relieve the inflammation, swelling, stiffness, and joint pain associated with osteoarthritis and rheumatoid arthritis. Also prescribed for juvenile rheumatoid arthritis in children six years of age and older.</td>
<td>[177]</td>
</tr>
<tr>
<td>Phenybutazone</td>
<td>Butazolidin®</td>
<td>A butyl-diphenyl-pyrazolidinedione that has anti-inflammatory, antipyretic, and analgesic activities. It has been used for ankylosing spondylitis, rheumatoid arthritis, and reactive arthritis.</td>
<td>[178]</td>
</tr>
<tr>
<td>Sulindac</td>
<td>Clinoril®</td>
<td>Used to treat osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and shoulder pain caused by bursitis and tendinitis. Also used to relieve gouty arthritis.</td>
<td>[179]</td>
</tr>
<tr>
<td>Tolmetin</td>
<td>Tolectin®</td>
<td>Relieves pain, tenderness, swelling, and stiffness caused by osteoarthritis, rheumatoid arthritis, and juvenile rheumatoid arthritis in children two years of age and older.</td>
<td>[180]</td>
</tr>
<tr>
<td>Salicylate</td>
<td>Trilisate, Disalcid®</td>
<td>Commonly used analgesic, antipyretic, and anti-inflammatory drug. Salicylate is the major drug for the treatment of rheumatic diseases.</td>
<td>[180]</td>
</tr>
</tbody>
</table>
While the majority of NSAIDs commonly ingested have minor toxic effects, with increasing general consumption, more attention is being directed toward their risk profile for gastrointestinal, renal, and cardiovascular side effects [4]. Therefore, an important question regarding NSAID usage is which NSAID has the lowest risks relative to its intended benefits [5].

**NSAIDs Mechanism of Action**

All NSAIDs work through a similar mechanism involving the inhibition of cyclooxygenases (COX) to exert their anti-inflammatory, analgesic and antipyretic effects [6]. Cyclooxygenases are a group of intracellular enzymes that catalyze the conversion of arachidonic acid into prostanoids, which are biologically active lipids that modulate the body’s inflammatory response [6, 7]. Prostanoids are divided into three major groups: prostaglandins (PGs), prostacyclins (PGI₂s), and thromboxanes (TXs), among which prostaglandins are thought to be the main promoter of inflammation and clotting factors [6, 8]. Prostaglandins are also responsible for stimulating secretions of mucin, bicarbonate, and phospholipids by gastric epithelial cells to promote proper gastric function [9]. Two COX isoforms have been investigated closely over the past three decades: COX-1 and COX-2 [10, 11]. COX-1, commonly referred to as the “housekeeping” enzyme, is the most abundant form and is involved in many pathophysiological processes, such as hemostatic integrity, gastric cytoprotection, platelet aggregation, and kidney function [12].

Activation of COX-1 produces prostacyclin, which prevents thrombosis by inhibiting platelet activation [13] and is found to be upregulated in patients with polypectomy-induced ulcers for gastric healing and protective effects [14].
Non-Steroidal Anti-Inflammatory Drugs...

In contrast, COX-2 is usually undetectable in most tissues unless induced by pro-inflammatory cytokines or growth factors to produce prostaglandins. However, COX-2 is constitutively expressed in the mammalian brain and has diverse functions in the central nervous system [15, 16]. Overexpression of COX-2 has been linked to neuro-inflammation, the inflammatory demyelinating effects of multiple sclerosis, Alzheimer’s disease, dementia, and other brain injuries or trauma [17, 18]. Therefore, COX-2 is thought to be largely responsible for the production of messenger molecules involved in fever, pain, and inflammation [19]. COX-3, a splice variant of the COX-1 gene, is the most recently discovered isoform [20]. It is expressed in the central nervous system and may be inhibited by acetaminophen, but its involvement in the regulation of prostanoids to maintain hemostatic, gastric, and renal homeostasis is still being investigated. However, COX-3 seems to have little relevance in humans as this isoform does not demonstrate COX activity [21].
Types of NSAIDs

Since each COX isoform functions in different pathophysiological responses, understanding the specific inhibitory effects of NSAIDs on COX may explain the related benefits and toxicity of NSAID usage [22, 23]. There are two main types of NSAIDs: non-selective and COX-2 selective (also known as coxibs) [24]. Non-selective NSAIDs, which inhibit both COX-1 and COX-2 isoforms, are also called the traditional NSAIDs, because the use of non-selective NSAIDs predates the development of coxibs [25]. Examples of commonly used NSAIDs are summarized in Table 1. Common examples of non-selective NSAIDs include aspirin, ibuprofen, naproxen, sulindac, diclofenac, and ketoprofen [25]. NSAIDs may also be classified into two groups based upon their intracellular half-lives after oral administration [26]. NSAIDs with short half-lives (<6hrs, such as ibuprofen, diclofenac and ketoprofen) need to be taken more often than NSAIDs with long half-lives (>6hrs, such as naproxen and celecoxib). Naproxen is usually taken 1-2 times a day while ibuprofen is taken every 6-8 hours to maintain its effect.

Because COX-1 is a constitutive enzyme for gastric cytoprotection and COX-2 is a prostaglandin synthase for pro-inflammatory responses, traditional NSAIDs can successfully reduce inflammation by inhibiting COX-2, but they can also cause gastric or renal irritation, toxicity, and other complications by disrupting COX-1 action in gastrointestinal (GI) homeostasis [27].

For instance, upper GI complications like dyspepsia occur in 15-60% of persons taking non-selective NSAIDs, which is twice the rate seen in non-users [28]. Also, risks for upper gastrointestinal bleeding or perforation are 3.8 times higher for patients taking NSAIDs [29]. Such severe gastric toxicity of NSAIDs was reflected by a case study of peptic ulcer surgical therapy, which revealed that 54% of its patients were NSAID users [30]. Furthermore, prostaglandins in the kidneys dilate the vasculature to facilitate renal vascular flow and proper organ perfusion and to mediate the effects of diuretics on sodium and water retention [25]. Inhibition of prostaglandin production by traditional NSAIDs may cause vasoconstriction, thereby significantly reducing renal blood flow and glomerular filtration rate and increasing the risk of renal failure [31]. However, while fluid retention occurs in almost all NSAID users, severe renal conditions such as hyperkalemia and nephritic syndrome occur in only about 1-5% of patients taking NSAIDs [32]. To reduce toxic gastrointestinal and renal side effects while maintaining the benefits of NSAIDs, selective NSAIDs, or COX-2 inhibitors (coxibs), were designed to inhibit only COX-2, the main pro-inflammatory regulator [33].
In various clinical trials and meta-analyses, coxibs significantly reduced gastrointestinal risks in comparison to traditional NSAIDs and had 50% fewer incidences of perforations, ulcers, and gastrointestinal bleeds [34, 35]. Celecoxib, a selective NSAID commonly used for osteoarthritis and rheumatoid arthritis, was also shown to decrease the risk of complicated ulcers by 20-30% [36]. However, the cardiovascular risks of coxibs seem to outweigh the gastrointestinal benefits; the number of hospitalizations over a 3-year period for acute myocardial infarction in patients taking rofecoxib (a coxib) was increased by 30% [37]. Rofecoxib (Vioxx) was approved by the Food and Drug Administration (FDA) in 1999 and was voluntarily withdrawn by Merck in 2004 after more than 88,000 cases of serious heart disease were reported in patients taking this drug. Rofecoxib was one of the most widely used drugs to be withdrawn from the pharmaceutical market [38]. The deleterious cardiovascular effects of rofecoxib were evident in a study in which it was used for Alzheimer’s disease treatment, but these cardiovascular events were under-reported at the time. Recent analyses of the three studies conducted with rofecoxib as a potential treatment of Alzheimer’s disease showed that rofecoxib increased the risk of cardiovascular related death by >3.5-fold [38].

Interestingly, this recent analysis suggest that the increased risk of cardiovascular-related death was statistically significant in June 2001, which meant that rofecoxib was on the market 40 months longer than it should have been [38].

The severe side effects of rofecoxib may be due to the fact that aside from promoting inflammation, COX-2 has other functions in mediating vasodilation and platelet activation for cardiovascular homeostasis [33]. In a knockout mouse model, selective inhibition of COX-2 causes an imbalance in hemostatic regulators, such as thromboxane A₂ and prostacyclin I₂, and increases the risks of thrombosis and platelet aggregation [39]. Also, while the NSAID-associated side effects of edema and sodium retention in the kidneys may have negligible effects on healthy individuals, they may adversely influence the hemostatic balance for patients with a prior history of heart failure [2]. Such disruption of systemic regulatory functions may explain why NSAIDs have a high cardiovascular risk profile, especially for patients with pre-existing heart conditions [2].

However, due to a general lack of wide-scale clinical trials investigating the cardiotoxicity of NSAIDs, there are still many molecular mechanisms to be investigated in order to explain the wide array of cardiovascular risks associated with NSAIDs [19].
NSAIDs and Cardiotoxicity

Over the last decade, COX-2 selective NSAIDs have become increasingly associated with increased risk of cardiovascular diseases (Table 2). The first research trial that attracted the media and researchers’ attention to the cardiovascular risk profile of NSAID usage was the Vioxx Gastrointestinal Outcomes Research, or VIGOR, in 2001 [34].

Table 2. Effects of Different NSAIDs on Cardiovascular Safety

<table>
<thead>
<tr>
<th>NSAID</th>
<th>Subjects and Conditions</th>
<th>Cardiovascular Safety Profile</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>1,031 patients with cardiac risk treated with 100mg aspirin vs. 4,495 nonusers</td>
<td>Reduced risk of cardiac events (myocardial infarction, stroke, and cardiac death) by 10%.</td>
<td>[181]</td>
</tr>
<tr>
<td></td>
<td>Six randomized studies of 9,853 patients, 50% treated with 50-325mg of aspirin vs. 50% with placebo</td>
<td>Reduced risk of cardiovascular death by 21%, risk of nonfatal myocardial infarction by 26%, risk of stroke by 25%. Increased risk of severe bleeding by 2.2-fold.</td>
<td>[182]</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1,228 patients treated with &lt;1600mg ibuprofen vs. 1,188 with 1000mg naproxen</td>
<td>Increased risk of serious coronary heart disease by a factor of 1.34 and cardiovascular death by 1.25.</td>
<td>[49]</td>
</tr>
<tr>
<td></td>
<td>389 new users of 200mg ibuprofen vs. 2,660 nonusers</td>
<td>Increased risk of atrial fibrillation by 40%.</td>
<td>[183]</td>
</tr>
<tr>
<td></td>
<td>Meta-analysis of 31 trials involving 116,429 patients with 117,218 years of follow-up</td>
<td>Increased risk of myocardial infarction by a factor of 1.61, stroke by 3.36, and cardiac death by 2.39.</td>
<td>[50]</td>
</tr>
<tr>
<td>Naproxen</td>
<td>719 Alzheimer’s patients treated with 220mg naproxen vs. 1,083 treated with placebo</td>
<td>Increased risk of cardiac events (myocardial infarction, stroke, cardiac death) by a factor of 1.63.</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>Meta-analysis of 31 trials involving 116,429 patients with 117,218 years of follow-up</td>
<td>Increased the risk of stroke by a factor of 1.76, but did not affect the risks of cardiac death or myocardial infarction.</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>Meta-analysis of 24 case-control studies involving 24,468 patients</td>
<td>Naproxen was risk-neutral at all doses for cardiovascular conditions compared to other NSAIDs.</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>804 patients treated with 150mg diclofenac vs. 1,188 with 1000mg naproxen</td>
<td>Increased risk of serious coronary heart disease by a factor of 1.44 and cardiovascular death by 1.52.</td>
<td>[49]</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>172,362 patients treated with 100mg or higher dosage diclofenac vs. 568,525 nonusers</td>
<td>Two-fold increased risk of cardiovascular death.</td>
<td>[184]</td>
</tr>
<tr>
<td></td>
<td>292 new users of 200mg diclofenac vs. 1,647 nonusers</td>
<td>Increased the risk of atrial fibrillation by 70%.</td>
<td>[183]</td>
</tr>
<tr>
<td>Subjects and Conditions</td>
<td>Cardiovascular Safety Profile</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>Etoricoxib</td>
<td></td>
<td>[185]</td>
<td></td>
</tr>
<tr>
<td>11,459 patients treated with 60 or 90mg etoricoxib vs. 11,212 with 150mg diclofenac</td>
<td>No significant increase in cardiovascular or thrombotic events (risk factor range of 0.9-1.08).</td>
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</tr>
<tr>
<td>Celecoxib</td>
<td></td>
<td>[52]</td>
<td></td>
</tr>
<tr>
<td>3,987 patients treated with 400mg celecoxib vs. 3,981 with 2,400 mg ibuprofen or 75mg diclofenac</td>
<td>No serious cardiovascular event in comparison to non-selective NSAIDs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased the risk of cardiovascular death, myocardial infarction, stroke, and other cardiac events by a factor of 1.10.</td>
<td>[51]</td>
<td></td>
</tr>
<tr>
<td>726 Alzheimer’s patients treated with 220mg naproxen vs. 1,083 treated with placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>685 patients treated with 400mg celecoxib vs. 671 with 800mg celecoxib vs. 679 with placebo</td>
<td></td>
<td>[53]</td>
<td></td>
</tr>
<tr>
<td>Rofecoxib</td>
<td></td>
<td>[34, 40]</td>
<td></td>
</tr>
<tr>
<td>4,047 patients treated with 50mg rofecoxib (Vioxx) vs. 4,029 with 1,000mg naproxen</td>
<td>Reduced gastrointestinal events by 50% compared to naproxen, but increased thrombotic cardiovascular events by a factor of 2.37.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>122 patients treated with 25mg rofecoxib vs. 118 with 220mg naproxen vs. 111 with placebo</td>
<td>1% of patients taking either rofecoxib or naproxen developed stroke or transient ischemic attack. 1% of rofecoxib users developed myocardial infarction.</td>
<td>[186]</td>
<td></td>
</tr>
<tr>
<td>1,287 patients treated with 25mg rofecoxib vs. 1,299 with placebo</td>
<td>Increased the risk of cardiac events (myocardial infarction, sudden death) by a factor of 2.80 and stroke by 2.32.</td>
<td>[41]</td>
<td></td>
</tr>
<tr>
<td>Valdecoxib</td>
<td></td>
<td>[43]</td>
<td></td>
</tr>
<tr>
<td>CABG patients: 555 treated with parecoxib and valdecoxib (Bextra) vs. 556 with valdecoxib only vs. 560 with placebo</td>
<td>Risk of cardiac events (myocardial infarction, cardiac arrest, and stroke) increased by a factor of 3.7 for parecoxib and valdecoxib group and by 2.0 for valdecoxib only group compared to placebo group.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In a randomized controlled trial involving more than 8,000 patients, one group of patients received 50mg of rofecoxib (Vioxx) once a day and another group of patients received 500mg of naproxen twice a day [34].

Initially, this trial was intended to investigate the gastrointestinal benefits of a coxib in comparison to a regular NSAID; rofecoxib reduced gastrointestinal events by 50% in comparison to naproxen [40]. However, rofecoxib doubled the risks of cardiac events, including sudden cardiac death, ischemic injury, chest pain, and cardiac thrombosis [40]. However, in a systematic review analyzing the risks and benefits of selective COX-2 inhibitors, Chaaimmuay et al. argued that the seemingly adverse cardiovascular effects of rofecoxib in the VIGOR study could be due to possibility that they were compared to the cardioprotective effects of naproxen [19]. To address
this concern, a randomized trial of 2,586 patients who were treated with either 25mg rofecoxib or a dose of placebo each day, was subsequently carried out [41].

This study showed that patients using rofecoxib for 18 months or more were 2.5 times more likely to undergo cardiac events, including myocardial infarction and sudden cardiac death [41]. These clinical results as well as others resulted in the withdrawal of rofecoxib from the worldwide market in 2004 [40]. Valdecoxib (Bextra), was another selective COX-2 inhibitor to be withdrawn from the US market in 2005 due to its cardiovascular toxicity [42]. In a study involving 1,600 patients who had undergone a coronary artery bypass graft surgery (CABG), three groups received either valdecoxib and its intravenous prodrug paracoxib, valdecoxib only, or placebo only [43]. The risks of cardiac arrest, stroke, and myocardial infarction were doubled for the valdecoxib only group and almost quadrupled for the valdecoxib and paracoxib group [43, 44].

However, an integrated analysis of the use of valdecoxib and paracoxib in non-cardiac disease patients later showed that neither coxib increased the risk of cardiovascular events [45]. In CABG patients, valdecoxib could have aggravated the stress-induced platelet aggregation already present from the pump-assisted extracorporeal circulation, thus leading to an increased risk of cardiac death [46].

Therefore, the administration of coxibs for certain patients remains controversial and the proper use of coxibs to balance their intended benefits and cardiovascular risks must be investigated further [47].

While it is unclear if many or all NSAIDs behave similarly to rofecoxib or valdecoxib, their withdrawal from the market urged the investigation of the widespread use of NSAIDs [5].

There have been several meta-analyses of NSAID usage that compiled years of clinical trials, and they seem to agree that naproxen has the least harmful effects on cardiovascular health when compared to other NSAIDs [48-50].

In a report by Trelle et al., with the exception of naproxen, diclofenac, and etoricoxib, it was shown that all other NSAIDs increased the risk of myocardial infarction by a factor of 1.3-2.8 (Table 2) [50]. Naproxen did show a 1.76-fold increase in the risk of stroke, but in comparison to ibuprofen, celecoxib, and other non-selective and selective NSAIDs, naproxen exhibited an insignificant or much lower probability of risk for cardiovascular events [50].
Ray et al. also reported that naproxen had insignificant, if not cardioprotective, effects in comparison to other NSAIDs [49].

However, in the Alzheimer’s Disease Anti-Inflammatory Prevention Trial (ADAPT), naproxen treatment for early onset of dementia was terminated early because of an increase in cardiovascular and cerebrovascular events [51]. When compared to a placebo treatment, naproxen increased the risk of cardiac events such as myocardial infarction, stroke, and cardiac death by a factor of 1.63 [51].

Most randomized trials used different placebos or control groups to compare against the administration of NSAIDs, which makes it difficult to determine their validity and compare the cardiotoxicity ratios [50].

In the Celecoxib Long-term Arthritis Safety Study (CLASS), celecoxib’s cardiovascular effects were deemed insignificant in comparison to other NSAIDs such as ibuprofen and diclofenac [52]. The CLASS trial also reported that celecoxib had less upper GI irritation, hepatotoxicity, and renal toxicity.

On the other hand, a randomized trial for Adenoma Prevention with Celecoxib (APC) treated the patients with a dose of placebo or 400mg of celecoxib, both administered twice daily for a 3-year period, revealed a significant increase in cardiovascular risk with celecoxib [53]. Celecoxib was associated with increased risk of sudden cardiac death, cardiovascular diseases, and nonfatal myocardial infarction.

Other NSAIDs also demonstrated increased cardiotoxic effects in patients who had prior heart conditions [53]. However, at least two studies with celecoxib suggest that the cardiovascular risk of this NSAID is similar to other non-selective NSAIDs or placebo [54, 55]. When administered to patients with prior myocardial infarction, within weeks of treatment, diclofenac increased the risk of cardiac death by an average factor of 2.56, ibuprofen by 1.47, and celecoxib by 1.65 [56].

Overall, all NSAIDs significantly increased the risks of recurrent myocardial infarction and cardiac death for these patients by a risk factor range of 1.34 to 1.86 [56].

Aside from using various controls to compare NSAID usage against, choosing different dosages of NSAIDs also complicated the cross-analyses of many randomized trials [57]. In 2011, an American Heart Association study showed that ibuprofen, even at an average dosage of 1600mg per day, increased the risk of a recurrent heart attack by 50% in patients with previous record of myocardial infarction [56].

Around the same time, a systematic review of observational studies by McGettigan et al. reported that a low-dosage use of ibuprofen at 1200mg per
day or less had no significant effect on cardiovascular risk, even in the high risk patient population [48].

In this systematic review, it was found that ibuprofen at a low dosage was comparable to naproxen in its low risk profile for cardiotoxicity. Furthermore, in a handful of other randomized trials previously mentioned, such as APC and CLASS, NSAIDs were used at higher dosages than normal since they were used to treat specific clinical symptoms [48, 58].

Consequently, the cardiotoxicity profile of NSAIDs used in specialized trials may not be applicable to the general public using NSAIDs purchased over the counter [48].

Such disparity in systematic reports indicates the need for more clinical trials to investigate the effects of NSAIDs at varying dosages, comparison to different controls, patient population, and other variables to develop a more suitable risk profile for the general use of NSAIDs.

**Molecular Mechanisms Involved in NSAID-Induced Cardiotoxicity**

As previously described, both selective and non-selective NSAIDs have adverse cardiovascular effects. The investigations of the mechanisms through which NSAIDs cause adverse cardiovascular events demonstrate that NSAIDs have multiple targets and mechanisms by which they cause adverse cardiovascular events, including COX-dependent and COX-independent mechanisms (Figure 2) [59, 60].

The main mechanisms known to be involved in NSAID-induced cardiotoxicity are discussed below.

**Shift Towards a Prothrombotic State**

Metabolism of arachidonic acid produces multiple metabolites which have both pro- and antithrombotic effects. COX inhibition by NSAIDs creates an imbalance of arachidonic acid metabolites, shifting the balance toward a prothrombotic state in which the blood has a higher tendency to coagulate, increasing the risk of adverse cardiovascular events [61].
**Decreased Prostacyclin Production**

It was originally believed that the main mechanism by which COX-2 inhibitors caused adverse cardiovascular events was the selective inhibition of COX-2 [60].

Selective inhibition of COX-2 leads to a decrease in the production of antithrombotic prostacyclin without a reduction in prothrombotic thromboxane levels, favoring a prothrombotic state which can result in clot formation and increased risk of adverse cardiovascular effects [60, 61].

**Increased 20-HETE Levels**

There are multiple enzymes that process arachidonic acid to produce a variety of metabolites, called eicosanoids [62]. 20-hydroxyeicosatetraenoic
acid (20-HETE) is synthesized from arachidonic acid by the enzyme cytochrome P450 [63].

In a study aimed at evaluating the mechanisms behind the adverse cardiovascular effects of the selective NSAID rofecoxib, it was found that levels of 20-HETE were increased greater than 120-fold in mice treated with rofecoxib, which correlated with decreased tail bleeding and blood clotting time and increased platelet aggregability [61].

These parameters serve as a possible readout for cardiac risk, since bleeding time correlates to platelet aggregability, which is related to myocardial infarction and stroke [61].

Additionally, increased platelet aggregability and decreased bleeding time has been observed in patients with myocardial infarction [61]. The increase in 20-HETE levels was also seen for the selective COX-1 inhibitor SC-560 and the non-selective COX inhibitor indomethacin, which both differ structurally from rofecoxib [61].

One possible explanation of this effect is that inhibition of the cyclooxygenases leaves more arachidonic acid available for conversion into other metabolites. However, if this was the case, one would expect an increase in other arachidonic acid metabolites, a phenomenon which is not observed [61]. An alternative explanation is that rofecoxib induces expression of other enzymes involved in 20-HETE synthesis; however, increased expression of these enzymes was not observed [61].

The most likely explanation for the increase in 20-HETE levels is that inhibition of COX blocks the metabolism of 20-HETE, giving rise to increased 20-HETE levels, and therefore increased platelet aggregability and adverse cardiovascular outcomes [64]. Because COX inhibition is a general property of NSAIDs, and increased 20-HETE levels were seen for both selective and non-selective COX inhibitors, an increase in 20-HETE levels is likely to be a general effect of NSAIDs.

**Induction of Apoptosis**

Increased cardiomyocyte apoptosis is associated with adverse cardiovascular outcomes [65, 66]. Multiple groups have reported elevated levels of apoptosis in patients suffering from dilated cardiomyopathy, a condition which precedes heart failure and is characterized by enlargement of the heart chambers and contractile dysfunction [67-69]. Others have reported increased rates of apoptosis in hearts that have undergone myocardial
infarction [65, 66, 70]. Additionally, induction of apoptosis in transgenic mice is sufficient to cause lethal cardiomyopathy, while inhibition of cardiomyocyte death prevents heart failure [71]. Together, these reports suggest that the increases in the rate of cardiomyocyte apoptosis have a significant effect on heart function and identify cardiomyocyte apoptosis as an essential step in the progression to heart failure. Several reports have demonstrated that various NSAIDs induce apoptosis [72-79].

Inhibition of Prostaglandin Signaling

Prostaglandins, the metabolites of arachidonic acid, bind to prostaglandin receptors called EP receptors, which apart from regulating processes involved in pain and inflammation, also control apoptosis and cell survival [80]. Blocking prostaglandin signaling by preventing prostaglandin formation may play a role in NSAID-induced apoptosis. Binding of prostaglandin E2 (PGE2) to the PGE2 receptors EP2 and EP4 activates cAMP-dependent protein kinase (PKA), which leads to phosphorylation of glycogen synthase kinase-3 (GSK3) [81]. This alters APC/β-catenin/TCF signaling, which under normal conditions negatively regulates apoptosis and promotes cell proliferation [80]. PGE2 can also activate the epidermal growth factor (EGF) receptor, inhibiting phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB)/Akt signaling that would normally activate peroxisome proliferator-activated receptor γ (PPARγ). PPARγ is a ligand-binding transcription factor that regulates cell proliferation, differentiation, and apoptosis, and alterations in its activity could play a role in NSAID-induced apoptosis [82-85]. Interestingly, NSAIDs have also been shown to act as direct PPARγ ligands at high concentrations [86]. The effects of NSAIDs on PPARγ activity suggest another mechanism by which NSAIDs induce apoptosis.

Inhibition of Akt Signaling

Akt signaling is one of the most important pathways in promoting cell survival and suppressing apoptosis [87]. As discussed in the previous section, Akt signaling is controlled by PGE2 and EGF receptor signaling, which is activated by PGE2. Consequently, inhibition of Akt signaling via inhibition of COX-mediated prostaglandin formation may play a role in inducing apoptosis [80]. In addition to this COX-dependent mechanism, a COX-2-independent mechanism of altering Akt signaling has been demonstrated [79]. Kulp et al. used DMC, a celecoxib analogue lacking COX-2 inhibitory activity, to investigate non-COX-2 targets of celecoxib in a human prostate cancer cell line. Celecoxib and DMC blocked Akt activation by inhibiting...
phosphoinositide-dependent kinase-1 (PDK-1), a kinase which phosphorylates Akt, resulting in its activation. Inhibition of Akt signaling by celecoxib and DMC caused G1 cell cycle arrest and apoptosis. These data indicate that Akt inhibition can occur independently of COX-2 inhibitory action [79]. Additionally, the NSAIDs NS-398 and indomethacin prevent Akt activation by activating phosphatase and tensin homolog (PTEN) [87].

**Increased Expression of EGR-1**

Early growth response gene 1 (EGR-1) is a zinc finger transcription factor which is induced by stress, injury, and growth factors [88]. NSAIDs increase expression of EGR-1, which has pro-apoptotic functions through its control of p53 [89]. *NSAID*-activated gene-1 (NAG-1) [90], and PTEN [91], whose roles in NSAID-induced apoptosis are further discussed in subsequent sections [80].

**Activation of PTEN**

The main functions of the tumor suppressor phosphatase and tensin homolog (PTEN) involve its lipid phosphatase activity but it could also function in a phosphatase-independent manner [92]. PTEN regulates several intracellular pathways including pathways involving the extracellular signal-regulated kinase (ERK)1/2, phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB/AKT), janus kinase (JAK)/signal transducers and activators of transcription (STAT) [92]. The NSAIDs NS-398 and indomethacin were shown to activate PTEN in human colon cancer cells [87]. Virolle et al. showed that exposing cells to ultraviolet light induced the transcription factor EGR-1, which caused an increase in the levels of PTEN messenger RNA and protein and induced apoptosis. In cells lacking EGR-1, PTEN was not induced, and cells were resistant to apoptosis, demonstrating that EGR-1 directly activates PTEN, which has pro-apoptotic effects [91].

PTEN dephosphorylates phosphatidylinositol (PtdIns), which prevents activation of PDK-1, and thus prevents activation of Akt, which may contribute to induction of apoptosis [87].

**Upregulation of NAG-1/GDF 15**

NSAIDs have been shown to induce the expression of NSAID-activated gene-1 (NAG-1)/growth differentiation factor 15 (GDF 15), resulting in induction of apoptosis [93, 94]. The traditional NSAIDs sulindac sulfide and indomethacin, and the selective COX-1 inhibitor SC-560 induce the expression of the EGR-1 transcription factor, which leads to up-regulation of
NAG-1. NAG-1 is a target of EGR-1, and EGR-1 may be required for NSAID-induced up-regulation in NAG-1. It is likely that NSAIDs induce NAG-1 expression through upregulation of EGR-1 [90, 95].

**Altered p53 Signaling**

NSAIDs have been shown to increase levels of the protein p53, a transcription factor that regulates anti-proliferative processes and apoptosis [96]. NSAID-induced apoptosis correlates to the p53 gene status: in a gastric cancer cell line, cells expressing p53 are more susceptible to aspirin- and indomethacin-induced apoptosis [77, 97, 98]. This is associated with upregulation of Bax [77, 97] and Bak [77] and downregulation of Bcl-2 [97]. NSAIDs also appear to regulate p53-dependent apoptosis through increased expression 15-lipoxygenase-1 (15-LOX-1), an enzyme that metabolizes arachidonic acid, producing mainly the metabolite 15-HETE [62]. Shureiqi et al. demonstrated that in colon cancer cells, the NSAIDs sulindac sulfone and NS-398 induce expression of 15-LOX-1, which precedes apoptosis, and overexpression of 15-LOX-1 caused phosphorylation of p53, which increased expression of p53 target genes and resulted in growth inhibitory effects. This was observed in the absence of COX-2, and therefore is likely independent of COX inhibition [80, 99]. Additionally, a p53 binding site has been identified in the promoter of NAG-1, a protein with pro-apoptotic effects [90].

**Altered NF-κB Signaling**

The transcription factor nuclear factor kappa B (NF-κB) regulates genes which promote cell survival, including FLIP, TRAFs and c-IAPs, which prevent the activation of caspases involved in apoptosis [100, 101]. Activation of NF-κB promotes cell proliferation and protects against apoptosis, while inhibition of NF-κB decreases anti-apoptotic signaling in the cell, causing a shift in favor of apoptosis. Reports on the effects of NSAIDs on NF-κB signaling are conflicting.

A study conducted by Smartt et al. reported that the COX-2 inhibitor NS-398, which induced apoptosis in colorectal carcinoma cells, increased binding of NF-κB to DNA, but did not increase transcriptional activity. Inhibition of NF-κB binding to DNA did not further enhance apoptosis induction, indicating that NF-κB was not transcriptionally active [102]. However, others have reported that NSAIDs inhibit NF-κB activation. Aspirin has been shown to inhibit NF-κB activation by inhibiting the proteasome [96]. NF-κB activation is regulated by IκB, the inhibitor of NF-κB. Degradation of IκB by the proteasome causes translocation of NF-κB into the nucleus where it initiates
transcription of target genes [96]. Proteasome inhibition results in decreased degradation of IκB, and therefore decreased NF-κB activity. It is likely that some NSAIDs induce apoptosis partially through altered NF-κB signaling, though these mechanisms remain to be clarified and may differ between NSAIDs.

**Increased p27Kip1 Expression**

The protein encoded by p27Kip1 controls cell cycle progression by preventing activation of CDK-cyclin complexes and is involved in regulating apoptosis. Huang et al. demonstrated that in human lung cancer cells, the NSAIDs NS-398 and indomethacin increase levels of p27 by downregulating expression of proteasome subunits, leading to decreased degradation of p27, which has an antiproliferative effect [103].

**Upregulation of Bax and Bak and Downregulation of Bcl-2**

B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax) play opposing roles in signaling apoptosis; Bax promotes cell death, while Bcl-2 promotes cell survival. Bcl-2 can heterodimerize with multiple proteins, which can have pro- or anti-apoptotic effects, and evidence indicates that the Bax and Bcl-2 proteins form a dimer which is important in controlling apoptosis. The primary function of Bcl-2 and Bax is to control the integrity of the mitochondrial membrane to influence the release of proteins from the mitochondria which can have pro- or anti-apoptotic effects [65]. Bcl-2 homologous antagonist killer (Bak) is another member of the Bcl-2 gene family that is involved in initiating apoptosis [104, 105]. NSAIDs have been shown to upregulate Bax and Bak and downregulate Bcl-2. The protein Bax is thought to be controlled by p53, presenting a possible explanation for its upregulation; however NSAIDs have been shown to induce expression of Bax in p53 mutants, indicating that increased expression induced by NSAIDs is independent of p53 [97].

Zhou et al. reported that aspirin and indomethacin increased levels of Bax and Bak, but did not affect Bcl-2 levels [77]. Dikshit et al. demonstrated that aspirin induces apoptosis in mouse Neuro 2a cells through inhibition of the proteasome. Apoptosis was induced due to various proteasome substrates failing to be degraded, including Bax and p53 [96]. Proteasome inhibition could partially explain the increased levels of Bax which contribute to NSAID-induced apoptosis.
Downregulation of Specificity Proteins

NSAIDs have been shown to affect the activity and degradation of Specificity proteins (Sps), which are transcription factors that control expression of housekeeping genes and other genes, including manganese superoxide dismutase (MnSOD). MnSOD is an antioxidant which is important for decreasing oxidative stress caused by reactive oxygen species. Significantly, overexpression of MnSOD has been shown to have myocardial protective effects; endogenous overexpression of MnSOD in rats led to an increased myocardial tolerance to ischemia-reperfusion [106]. Abdelrahim et al., demonstrated that the COX-2 inhibitors celecoxib, nimesulfide, and NS-398 enhanced the degradation of Sp1 and Sp4. [107]. This increased degradation was accompanied by increased levels of ubiquitination and was blocked by the proteasome inhibitor gliotoxin, suggesting that these COX-2 inhibitors increased degradation in a proteasome-dependent manner. In addition, celecoxib has been shown to decrease phosphorylation of Sp1. Decreased phosphorylation and increased degradation of Sp proteins are important for the NSAID-induced downregulation of vascular endothelial growth factor (VEGF) [104] [80], a protein that has anti-apoptotic effects and promotes angiogenesis [108-110]. The NSAIDs mentioned here likely induce apoptosis partially through their effects on the levels and activity of Specificity proteins.

Generation of Reactive Oxygen Species

Induction of apoptosis by various drugs is accompanied by the generation of reactive oxygen species (ROS), indicating that oxidative stress caused by ROS generation may play a role in inducing apoptosis [111, 112]. Many different NSAIDs have been shown to generate ROS. Kusuhara et al. examined the role of reactive oxygen species in NSAID-induced apoptosis in cultured rat gastric cells and found that multiple NSAIDs, including indomethacin and diclofenac, generate ROS and induce apoptosis [113]. Treatment with antioxidants suppressed apoptotic DNA fragmentation induced by indomethacin and flubiprofen, demonstrating an important link between NSAID-induced apoptosis and the generation of ROS [113]. It has also been shown that diclofenac affects mitochondrial permeability transition, which results in the generation of ROS [114]. Generation of ROS increases levels of oxidative stress, which causes mitochondrial permeability transition (MPT) pores to open. The opening of the MPT pores allows the release of cytochrome c from mitochondria, activating caspase-9 and caspase-3, inducing apoptosis via the intrinsic/mitochondrial pathway [65].
Proteasome Inhibition

Proteasome inhibition has many implications in the heart. Dikshit et al. demonstrated that aspirin inhibited the proteasome in mouse Neuro 2A cells, and induced apoptosis [96]. As previously mentioned, proteasome inhibition led to decreased degradation of several proteasome substrates, including IkB, p53, Bax, and p27, and induced apoptosis. Huang et al. showed that NSAIDs decreased expression of the proteasome catalytic subunits β5, β1i (LMP2), and β5i (LMP7), which led to increased levels of p27, and had an anti-proliferative effect [103].

Additionally, ubiquitin-proteasome system (UPS) dysfunction plays a role in cardiac proteinopathy, a cardiac disease in which proteins form harmful aggregates in the cell, disrupting cellular homeostasis and playing a role in progression to heart failure [115]. The proteasome is largely responsible for degrading damaged proteins such as oxidized proteins and preventing harmful protein aggregates from forming in the cell. As mentioned in the previous section, NSAIDs have been shown to generate reactive oxygen species (ROS), which can cause oxidative stress and damage proteins. NSAIDs may have multiple effects on the proteasome including indirect inhibition of the proteasome (possibly by oxidizing some of the proteasomal subunits) as well as increased levels of oxidized proteins, which may overload the proteasome. Although the cause of NSAID-induced proteasome dysfunction is not known, inhibition of the proteasome contributes to proteasome functional insufficiency and consequently cardiac proteinopathy [115]. High levels of proteasome inhibition result in cell death [116] and proteasome inhibitors are currently used in cancer treatments [117]. Interestingly, an important side effect of these anti-cancer proteasome inhibitors is increased cardiotoxicity [118].

This may be due to differences in the susceptibility of proteasomes from different tissues to proteasome inhibitors. Purified proteasomes from murine cardiac tissue were more susceptible to proteasome inhibitors than proteasomes from murine liver tissue [119]. It is possible that cardiac proteasomes are more susceptible to inhibition by NSAIDs than proteasomes in other tissues.

Inadequate Supply of ATP

As previously described, NSAID-induced mitochondrial dysfunction plays a role in apoptosis. Additionally, mitochondrial dysfunction caused by NSAIDs affects ATP production. The heart requires a constant supply of ATP,
and mitochondrial dysfunction leading to an inadequate supply of ATP causes cardiac contractile dysfunction which can lead to heart failure if severe [120, 121]. Mitochondria account for more than a third of the myocardial mass of the adult heart, highlighting the importance of mitochondria in supplying the heart with ATP [122].

**Mitochondrial Dysfunction Leading to Defective Oxidative Phosphorylation**

Many NSAIDs have been shown to induce opening of mitochondrial permeability transition (MPT) pores *in vitro*, which induces membrane depolarization. In addition to the role MPT pores play in apoptosis, prolonged opening of MPT pores leads to mitochondrial dysfunction and defective oxidative phosphorylation, resulting in insufficient levels of ATP [122]. Additionally, various NSAIDs have also been shown to inhibit mitochondrial complex I, which may play a role in NSAID-induced mitochondrial dysfunction [120].

**Altered Cardiac Electrical Activity**

Cardiac electrical activity depends on the generation of action potentials in cardiomyocytes, which is based on the opening and closing of ion channels. Ion channel dysfunction has been implicated in inherited and acquired heart diseases, indicating the importance of ion channels in normal heart rhythm [123]. Mutations in genes encoding cardiac ion channels are also associated with cardiac arrhythmias and sudden cardiac death [124]. NSAIDs have been shown to inhibit multiple different ion channels via direct and indirect mechanisms, offering another explanation for the mechanism of NSAIDs in causing heart failure.

**Inhibition of Voltage-Gated Potassium Channels**

Potassium channels play an important role in heart function. Heart failure is associated with altered potassium currents, in particular Ca\(^{2+}\)-independent transient outward K\(^+\) (I\(_{to}\)) and inward rectifier K\(^+\) current (I\(_{KI}\)).

I\(_{to}\) currents are due to voltage-gated K\(^+\) (Kv) channels [125]. The selective NSAID celecoxib has been shown to inhibit rat, *Drosophila*, and human voltage-activated delayed rectifier K\(^+\) (Kv2) channels and lead to arrhythmia in *Drosophila* hearts and rat heart cells.
The fact that *Drosophila* lack cyclooxygenase enzymes suggests that the mechanism of Kv2 inhibition is independent of the COX inhibitory action of celecoxib [59]. Additionally, treatment with aspirin failed to reproduce these results, suggesting that Kv2 inhibition is not a general property of NSAIDs. In another study, celecoxib was shown to inhibit Kv1.5, Kv4.3, and Kv7.1 channels while rofecoxib were shown to inhibit Kv4.3 channels. Celecoxib prolonged the duration of the action potential in mouse cardiomyocytes and had the opposite effect in guinea pig cardiomyocytes, again demonstrating that Kv channel inhibition has significant effects on the heart [126]. It is likely that inhibition of Kv2 channels contributes to the cardiotoxicity of some NSAIDs.

**Inhibition of Voltage-Gated Calcium and Sodium Channels**

Celecoxib, but not rofecoxib or diclofenac, was found to dramatically suppress L-type voltage-sensitive calcium currents in A7r5 rat aortic smooth muscle cells and freshly isolated rat mesenteric artery myocytes [127]. Activation of L-type Ca\(^{2+}\) currents is correlated with heart contractions, and data suggest that differences in L-type voltage-dependent Ca\(^{2+}\) channel expression and activity may play a role in heart failure [128]. A celecoxib analog, 2,5-dimethyl-celecoxib, which is unable to inhibit COX-2, mimicked celecoxib in its enhancement of vascular KCNQ5 currents and suppression of L-type calcium currents, suggesting that these effects are independent of its COX-2 inhibitory actions [127]. These effects of celecoxib, rofecoxib, and diclofenac may partially explain the differential risk of cardiovascular events in patients taking different drugs of this class [127, 129]. Additionally, sodium channels are highly important for normal cardiac electrical activity, and changes in sodium channel function have been associated with myocardial ischemia, ventricular arrhythmias, and heart failure [130]. At low micromolar concentrations celecoxib suppressed both tetrodotoxin-sensitive and tetrodotoxin-resistant Na\(^+\) channel currents in a dose- and frequency-dependent manner in rat dorsal root ganglion neurons [131]. Celecoxib seems to bind to both inactivated and resting Na\(^+\) channels.

**Molecular Mechanisms Involved in NSAID-Induced Sudden Cardiac Death**

Sudden cardiac death (SCD) as the name suggests, is a sudden, unexpected natural death due to loss of cardiac function, which occurs within
one hour of the onset of symptoms [132]. SCD is the leading cause of natural deaths in adults in the US, accounting for over 450,000 adult deaths (>35 years old) each year [132]. Men are at a greater risk of SCD in comparison to women [133]. A significant proportion of all cardiac deaths are classified as SCD. Sudden cardiac death occurs when the electrical system of the heart becomes irregular and causes arrhythmia. While many patients are likely to have anatomical and/or functional cardiac abnormalities that predispose them to acquire ventricular arrhythmias, only a small percentage actually develop SCD [134]. As shown in Figures 2 and 3, several pathways are likely involved in the increased risk of NSAID-induced SCD. The most direct method by which NSAIDs are suggested to cause cardiac death is by altering cardiac electrical activity and inducing arrhythmias [135-137]. Arrhythmia has been associated with increased mortality due to increased risk of cardiac arrest, stroke, heart failure, sudden arrhythmic death syndrome, and sudden cardiac death (Figure 3).

The most common life-threatening arrhythmia is ventricular fibrillation, the uncoordinated contraction of the ventricles [135]. Ventricular fibrillation prevents the heart chambers from pumping blood throughout the body and if left untreated can result in loss of consciousness, as blood flow to the brain is decreased, and subsequent death can occur within minutes. The importance of ventricular arrhythmia in SCD prompted the United States Food and Drug Administration (FDA) to approve Microvolt T-wave alternans (MTWA) measurements as a non-invasive predictor of the risk of potentially fatal ventricular arrhythmia. MTWA refers to an alternating pattern (beat-to-beat variability) in the amplitude or shape of the T-wave portion of an electrocardiogram. MTWA is able to detect small irregular waveforms which routine electrocardiogram (ECG) does not detect. However, the predictive value of MTWA varies, depending on the type of patients investigated [138, 139].

Other likely possibilities for inducing sudden cardiac death include NSAID-induced contractile dysfunction, increased blood clotting, and significant increases in cardiac myocyte apoptosis. Studies suggest that defects in mitochondrial oxidative pathways can also contribute to SCD.

Tissues such as kidney, cardiac, skeletal, or smooth muscle, which are highly dependent on oxygen, are more sensitive to defective mitochondrial oxidative phosphorylation. Several NSAIDs have been shown to inhibit or uncouple oxidative phosphorylation, resulting in reduced cell viability [54]. This reduction in oxidative phosphorylation results in significantly decreased ATP production. Some research suggests that the decreased oxidative
phosphorylation caused by NSAIDs may be due to inadequate mitochondrial inner membrane potential for ATP synthesis by the F0F1-ATPase.

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Figure 3. Possible alterations in cardiac molecular pathways that lead to sudden cardiac death.

Evidence also suggests that NSAIDs (such as diclofenac sodium and mefenamic acid) induce mitochondrial permeability transition (MPT) pores to open which leads to both reduction in oxidative phosphorylation (due to membrane depolarization) as well as initiation of apoptosis [55]. The consequences of an inadequate supply of ATP are numerous, but the most likely adverse effect related to increased risk of SCD is contractile dysfunction. Muscle contraction requires a significant amount of ATP for myosin to bring about interaction and movement relative to the actin thin filament [140, 141]. Patients with severe contractile dysfunction have been shown to be at high risk for SCD [142, 143]. Contractile dysfunction is likely to affect cardiomyocyte Ca^{2+} homeostasis, as observed in hearts containing mutant myofilament proteins associated with cardiomyopathies [144]. This
abnormal Ca\textsuperscript{2+} homeostasis can lead to ventricular arrhythmias. Impairment of mitochondrial function affecting mitochondrial membrane potential or the electron transport chain has been shown to affect cardiac Ca\textsuperscript{2+} cycling, leading to proarrhythmic Ca\textsuperscript{2+} alternans [145].

Most NSAIDs are highly lipophilic and are able to be readily transported through the cell membrane and become enriched within the mitochondrion [146]. Although most NSAIDs are acidic, even non-acidic NSAIDs such as nabumetone have been shown to affect the mitochondrial inner membrane potential and reduce ATP synthesis [147].

Interestingly, the COX-2 selective inhibitor celecoxib, which does not seem to affect oxidative phosphorylation, is still associated with an increased risk of SCD. However, coxibs seem to be associated with a lower risk than some other non-selective NSAIDs. Neither nabumetone or celecoxib cause in vitro uncoupling [148].

Even though some NSAIDs themselves do not affect oxidative phosphorylation, their metabolites sometimes do. Salicylate, the main metabolite of aspirin, is able to uncouple oxidative phosphorylation, unlike its parent compound aspirin [146]. The increased risk of SCD associated with celecoxib may be related to this compound’s ability to affect ion channels, ultimately affecting the electrical activity of the cardiomyocytes.

The intrinsic apoptotic pathway initiated by the MPT pore opening is also likely to be detrimental for the cardiomyocyte. Caspases are proteolytic enzymes that carry out the ordered destruction of the cell without induction of inflammation or leakage of cellular components [149]. Adult cardiomyocytes are post-mitotic cells, and therefore show limited capability to respond to acute damage. In the normal myocardium, apoptosis occurs at an extremely low rate (0.001-0.002%), but under pathological conditions such as in the hearts of patients with end-stage dilated cardiomyopathy, apoptosis increases to 0.08–0.25% [67, 68, 149]. This 40-250 fold increase in apoptosis is substantial compared to the absolute amount of cardiomyocyte apoptosis in diseased hearts (<0.3%), and is pathologically important as over-expression of caspase 8 in mice increased apoptosis only 11.5 fold (from approx. 0.002 to 0.023%) but resulted in dilated cardiomyopathy after two months and death within six months [71].

NSAIDs have also been shown to induce a prothrombotic state by inhibition of COX-2, which results in decreased levels of the antithrombotic prostacyclin without reducing prothrombotic thromboxane levels [60, 61]. Persons in a prothrombotic state are at an increased risk for blood clotting and ischemia.
A frequent cause of SCD for persons at risk is pulmonary embolism and acute thrombosis, which leads to the fatal ventricular arrhythmia [150]. Although severe dysfunction of any of these pathways (contractile dysfunction, etc.) is likely to be a main contributor to SCD, combinations of different types of cardiovascular dysfunction would also likely lead to SCD.

Another potential contributor to NSAID-induced SCD is ROS. Several NSAIDs, including indomethacin and diclofenac, are known to generate ROS [111-113]. In a canine post-myocardial infarction (MI) model of SCD Belevych et al. found that redox modification of ryanodine receptors promotes generation of Ca2+ alternans by increasing the steepness of the Ca2+ release–load relationship [151]. Hence, it is possible that increases in ROS may affect the redox status of several important cardiovascular proteins which leads to arrhythmias and then SCD. The oldest and best known NSAID, aspirin, is atypical when compared to other NSAIDs since it has been shown to lower the risk of heart attacks and strokes due to its anticoagulant effect. All currently available evidence suggests that daily use of aspirin is beneficial in men ages 45 to 79 who are at risk of having a heart attack within the next ten years [152]. The benefits of aspirin in preventing heart attacks are only seen in older females (>55 years old) [152, 153]. Unlike other NSAIDs, aspirin does not seem to be associated with SCD.

While many of the cases of SCD associated with NSAIDs are likely due to NSAIDs themselves, it is important to recognize that some of the incidents of SCD in patients taking NSAIDs may be related to undiagnosed genetic mutations in these patients. Mutations associated with several myofibrillar genes, including troponin T, troponin I, and myosin heavy chain, are associated with increased risk of SCD [141, 154].

Some of the SCDS related to NSAIDs were in persons who used NSAIDs with other drugs, making it difficult to discern the NSAID-independent effects of other drug(s). Another important consideration is the interaction of NSAIDs with other drugs. The use of aspirin with other NSAIDs can be a dangerous combination and has been shown to increase the risk of GI bleeding.

Taking aspirin in combination with ibuprofen has been shown by one research group to interfere with aspirin’s beneficial effects while another group found that taking ibuprofen in combination with aspirin reduced the beneficial heart-protective effects of aspirin [155, 156]. Both investigations suggest that this combination of NSAIDs should be avoided. Use of NSAIDs together with quinolones has been shown to increase the risk of quinolones’ adverse central nervous system effects, such as inducing seizures [157].
Conclusion

The evidence undoubtedly suggests that NSAID use is associated with an increased risk of cardiovascular problems and in some cases the risk of adverse cardiovascular events, including SCD, increases with long term exposure to NSAIDs. Taking NSAIDs for long periods should therefore be avoided if possible. Persons who have high blood pressure or kidney disease or who are at risk of heart attack or stroke should be careful when considering taking NSAIDs even for short periods. In such patients, the use of NSAIDs (with the exception of low-dose aspirin) can be associated with as much as a 10-fold or greater increase in heart failure [158], while the risk to the general population is a 2-fold increase. In heart failure patients, NSAIDs increase the mortality risk by approximately 1.2-1.3 fold for naproxen and ibuprofen, 1.7 for celecoxib and rofecoxib, and 2.1 for diclofenac [159]. Current clinical data suggests that patients at risk of cardiovascular problems that require NSAID treatment should use naproxen [142, 143, 160]. SCD is the leading cause of death in young athletes, and screening of athletes by ECG was found to increase the ability to identify athletes at risk [161]. It may be possible to use ECG to screen patients taking NSAIDs long-term for increased risk of SCD.

More extensive and accurate information needs to be provided to doctors, and the number of prescriptions for NSAIDs should be reduced, as greater than 40% of prescriptions were found to be unnecessary [162]. Doctors should be encouraged to advise patients to take the lowest dose of NSAID necessary to reduce pain. Doctors should also encourage patients to take NSAIDs for as short a period of time as necessary. Significant clinical trials are needed to determine the effects of NSAIDs at varying dosage levels and lengths of treatment time on cardiovascular diseases. By doing so, we can determine the dosage and period of time at which NSAIDs become unsafe and the benefits of the NSAIDs are outweighed by the risk.

Additionally, it is unclear if a significant proportion of SCDs associated with NSAIDs occur in patients who are already at risk of SCD due to genetic or physiological factors. Myocarditis, various valvular diseases (such as aortic stenosis), inherited arrhythmia syndromes, and hypertrophic and dilated cardiomyopathy are associated with an increased risk of SCD. The current guidelines to reduce NSAID-associated complications are mainly limited to prevention of cardiovascular and GI related problems and are underutilized [160]. The possible availability of generic versions of celecoxib within the next few years (the patent for celecoxib will expire in 2014) may result in an increased use of this COX-2 selective NSAID [163]. More clinical studies and
evidence-based analyses which take into account gastrointestinal and cardiovascular risks at an individual level are needed so that informed decisions can be made regarding the use of NSAIDs.

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